ISSN 0370 - 4327

“ REDIA „

GIORNALE DI ZOOLOGIA

PUBLISHED
Consiglio per la ricerca in agricoltura e l’analisi dell’economia agraria
CREA Research Centre for Plant Protection and Certification

Volume CII
TERZA SERIE
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Journal of Zoology

Founded by Antonio Berlese, Firenze, 1903

Published by CREA Research Centre for Plant Protection and Certification
(formerly: Istituto Sperimentale per la Zoologia Agraria, Firenze)
Consiglio per la ricerca in agricoltura e l’analisi dell’economia agraria

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CREA Research Centre for Plant Protection and Certification
via Lanciola 12/a - 50125 Firenze, Italy - www.redia.it

Autorizzazione Tribunale di Firenze
Decreto n. 999 del 9 aprile 1955
Tipografia Coppini - Firenze

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REVIEW OF THE GENUS *DIOCTRIA* MEIGEN, 1803 (DIPTERA ASILIDAE) FROM IRAN, WITH FOUR NEW SPECIES RECORDS FOR THE IRANIAN FAUNA

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Mohammadi R., Talebi A.A., Fathipour Y., Kazerani F., Van Den Broek R. – Review of the genus *Dioctria* Meigen, 1803 (Diptera Asilidae) from Iran, with four new species records for the Iranian fauna.

Based on specimens collected, using sweeping nets and Malaise traps, from the East Azarbaijan, Guilan, Alborz and Mazandaran Provinces of Iran between May 2010 and September 2015, six species of the genus *Dioctria* Meigen, 1803 were collected. They were subsequently identified, and four species: *Dioctria arnoldii* V. Richter, 1964, *D. humeralis* Zeller, 1840, *D. pilithorax* V. Richter, 1980, and *D. speculifrons* Wiedemann, 1820, had not been previously recorded from Iran. Diagnostic characters and geographical distribution of the newly recorded species are provided. An illustrated key to all known Iranian *Dioctria* species is given.

KEY WORDS: *Dioctria*, Asilidae, Key, New records, Iran.

INTRODUCTION

The flies of the genus *Dioctria* Meigen, 1803 (Insecta: Diptera: Asilidae) are rather small to medium sized (6-20 mm), nearly bare and often shiny black. The antennae are elongate, longer than the height of the eye and borne upon a protuberance which may be only slightly developed in some species. The fore tibia lacks an apical claw-like spur, the hind femora without strong bristles and the pulvilli are normally developed. Wings are relatively large, the 1st radial cell, all medial cells and posterior cubital cell are open (RICHTER, 1988). Among Asilidae, only the Dioctriinae possess an arched postmentum (DIKOW, 2009).

The genus *Dioctria* was placed within the tribe Dioctriini by HULL (1962) along with 14 genera of Dasypogoninae in which the 9th tergite of females lacks both acanthophorites and spines. PAPAVERO (1973) transferred the Dioctriini to the subfamily Stenopogoninae. LEHR (2001) lifted the tribe Dioctriini to the subfamily level. The Dioctriinae then comprised three tribes: Dioctriini, Echthodopini and Molobratiini (ADISOEMARTO and WOOD, 1975; LEHR, 2002). DIKOW (2009), using multiple character states, verified that Dioctriini and Echthodopini were indeed Dioctriinae, but Molobratiini was transferred back to the Dasypogoninae. With 90 species, *Dioctria* is the largest genus in the subfamily; 85 of these are known from the Palaearctic region (GELLER-GRIMM *et al.*, 2015). Most of the species of the genus *Dioctria* can be found in the temperate belt and inhabit thick herbaceous grasslands, bushy thickets and wooded areas (LEHR, 1958).

Prior to this study, 11 *Dioctria* species had been reported from Iran. LEHR (1988) and LEHR *et al.* (2007) listed *Dioctria lugens* Loew, 1873, *D. abdominalis* Becker, 1923, *D. atricapilla* Meigen, 1804, *D. rufonigra* Theodor, 1980, *D. striata* Theodor, 1980 and *D. valida* Loew, 1856 from Iran. GHAHARI *et al.* (2007) and HAYAT *et al.* (2008) added *D. dispar* Loew, 1871, *D. gracilis* Meigen, 1820, *D. hyalipennis* (Fabricius, 1794), *D. flavipennis* Meigen, 1820 and *D. linearis* (Fabricius, 1787) to the Iranian fauna.

This study was conducted to provide a comprehensive taxonomic treatment of *Dioctria* species known from Iran.

MATERIALS AND METHODS

Specimens of *Dioctria* were collected using sweeping nets and Malaise traps from May 2010 to September 2015 in East Azarbaijan, Guilan, Alborz and Mazandaran Provinces. The following references were used for identification of specimens and geographical distribution of species: ENGEL (1930); RICHTER (1960, 1964, 1988); LEHR (1988, 2001, 2002); GELLER-GRIMM (2003) and GELLER-GRIMM *et al.* (2015). Photos were taken using an Olympus TM SZX9 stereo-microscope equipped with a Sony TM digital camera. A series of 7-10 captured images were merged into a single in-focus image using the image-stacking software, Helicon Focus 6. The authors used Garmin, geko 301 to take GPS coordinates. The examined specimens are deposited in the Insect Collection of Department of Entomology, Tarbiat Modares University, Tehran, Iran (TMUC).

RESULTS

In this study, six species of the genus *Dioctria* Meigen, 1803 were collected and identified, of which four species including *Dioctria arnoldii* V. Richter, 1964, *D. humeralis* Zeller, 1840, *D. pilithorax* V. Richter, 1980, and *D. speculifrons* Wiedemann, 1820 are recorded from Iran for

– Received 24 May 2018 Accepted 5 February 2019

the first time. In addition, *Dioctria atricapilla* Meigen, 1804 and *Dioctria linearis* (Fabricius, 1787) are reported from the East Azarbaijan Province for the first time.

LIST OF DIOCTRIA SPECIES KNOWN FROM IRAN

Dioctria abdominalis Becker, 1923

Iranian records: Isfahan province (Lehr *et al.*, 2007).

General distribution**:** Turkey (Geller-Grimm *et al.*, 2015).

Dioctria arnoldii V. Richter, 1964

(Fig. I, 1-5)

Material examined: Guilan province (Roodsar, Rahim abad, Ziaz), 36°52′34.44″ N, 50°13′17.40″ E, 537 m, 15 June 2010, 2♂ (photographed specimen); Guilan province (Roodsar, Rahim abad, Ghazichak), 36°45′52.62″ N, 50°20′01.08″ E, 1787m, 5 July 2010, 2♀; Guilan province (Roodsar, Rahim abad, Orkom), 36°45′44.34″ N, 50°18′11.88″ E, 1201 m, 8 June 2010, 1♀.

Iranian records: Guilan province (current study), new record for Iran.

General distribution: Armenia, Azerbaijan, Georgia (Lehr,1988, 2002).

Diagnostic characters: Large black species (12-15 mm) with a black stripe on the face. Facial tomentum only present along eye margins leaving a shiny black stripe on the face between mystax and frontal tubercle (Fig. I, 1); scape about 1.5x to 2x pedicel; mystax black (Fig. I, 3). Thorax black, scutum shiny black without tomentum; stripe of pale tomentum on pleura complete (Fig. I, 2); coxae black. Legs somewhat variable, usually front and middle femora yellowish-red with a black posterior side, hind femur black with a narrow red apex, front and middle tibiae yellowish-red with a black apex, hind tibia black with a red base, all tarsi black (Fig. I, 1). Wings faintly infuscate (Fig. I, 5), darker in basal part, abdomen shiny black (Fig. I, 4).

Dioctria atricapilla Meigen, 1804

(Fig. II, 1-3)

Material examined: East Azarbaijan province, Arasbaran Forests, 46°26'32''N, 44°54'2''E, 1534m, 25 July 2012, 1♀, 3♂ (photographed specimen).

Iranian records: Golestan province (Lehr *et al.*, 2007), East Azarbaijan province (current study).

General distribution: Austria, Belgium, Bulgaria, Republic of Belarus, Corsica, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Kazakhstan, Latvia, Lithuania, Luxembourg, Moldavia, Mongolia, Netherlands, Poland, Romania, Russia, Sweden, Switzerland, Turkey, Ukraine, United Kingdom (Geller-Grimm *et al.*, 2015).

Dioctria dispar Loew, 1871

Iranian records: Guilan province (Hayat *et al.*, 2008).

General distribution: Russia, Kyrgyzstan, Tajikistan, Uzbekistan (Geller-Grimm *et al.*, 2015).

Dioctria flavipennis Meigen, 1820

Iranian records: Provinces: Guilan (Hayat *et al.*, 2008); Isfahan (Ghahari *et al.*, 2007); Mazandaran (Ghahari *et al.*, 2007; Hayat *et al.*, 2008).

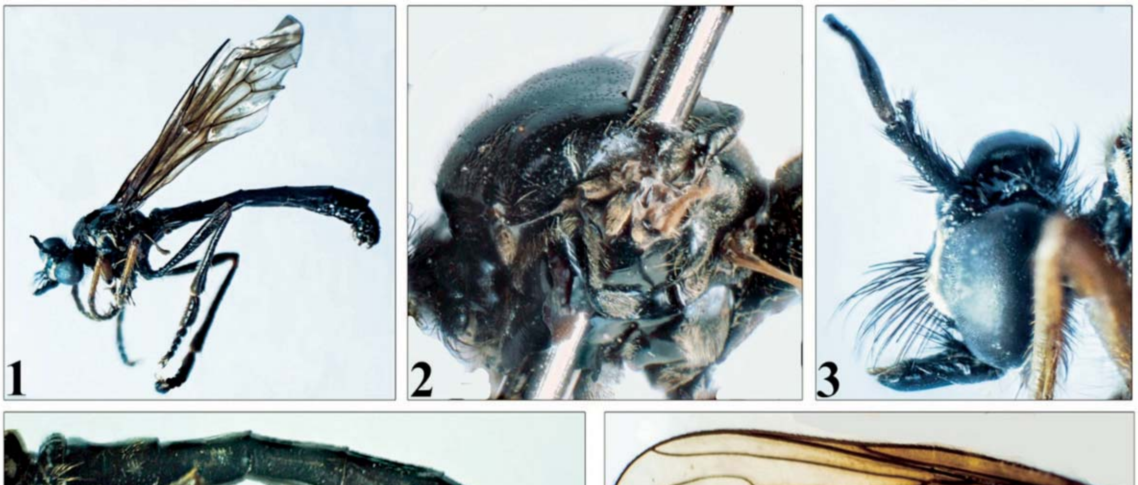

Fig. I – *Dioctria arnoldii* V. Richter, 1964 1-5: male. 1. General habitus, lateral view; 2. Thorax, lateral view; 3. Head, lateral view; 4. Abdomen, lateral view; 5. Wing, dorsal view.

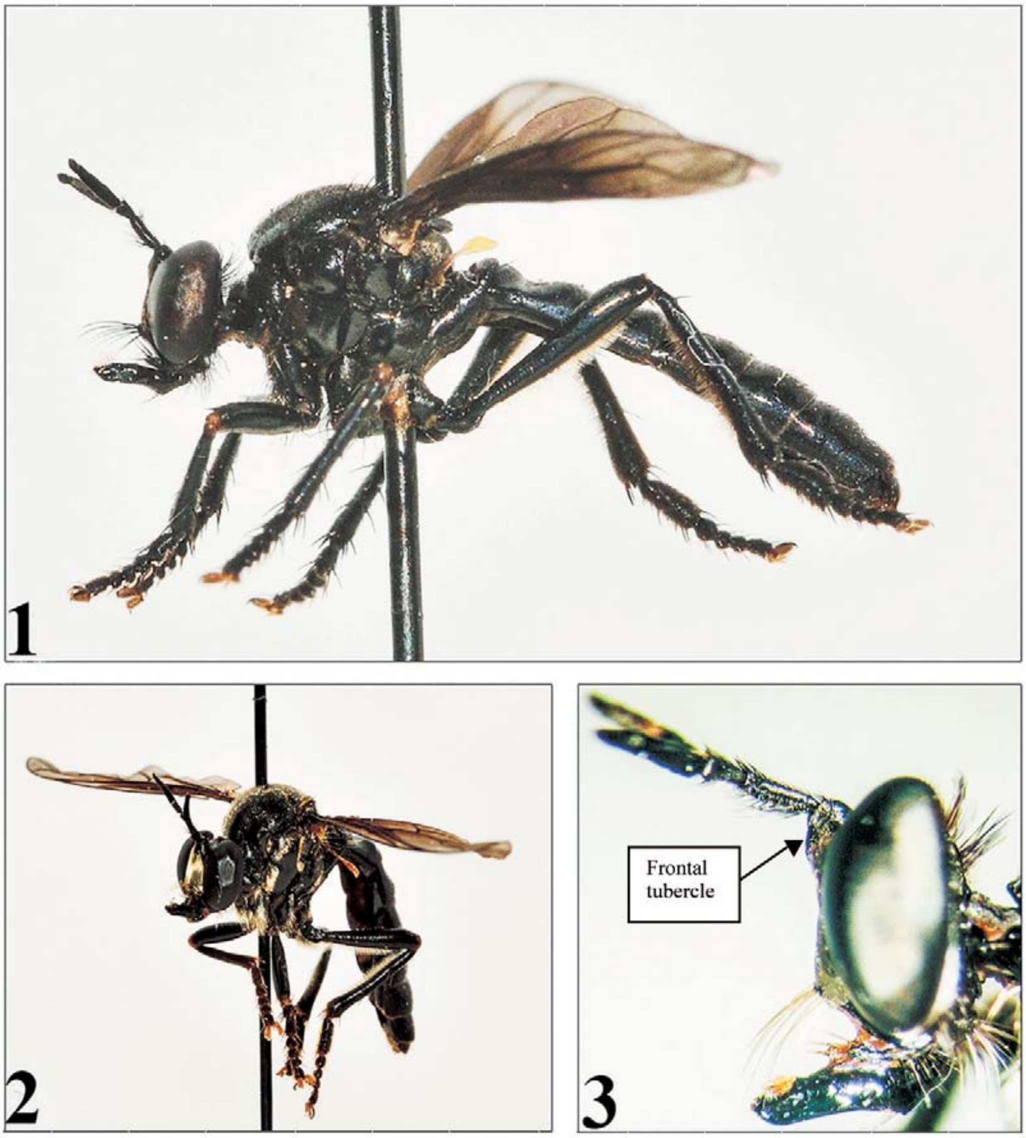

Fig. II – *Dioctria atricapilla* Meigen, 1804 1-3: male. 1. General habitus, lateral view; 2. General habitus, laterofrontal; 3. Head, lateral view.

General distribution: Austria, Bulgaria, Republic of Belarus, China, Czech Republic, Estonia, France, Germany, Great Britain, Hungary, Kazakhstan, Kyrgyzstan, Latvia, Lithuania, Moldavia, Poland, Romania, Russia, Tajikistan, Turkey, Turkmenistan, Ukraine, Uzbekistan, West Siberia (GELLER-GRIMM *et al.*, 2015).

Dioctria gracilis Meigen, 1820

Iranian records: Southern Khorasan province (HAYAT *et al.*, 2008).

General distribution: Algeria, France, Germany, Italy, Sardinia, Switzerland, Turkey (GELLER-GRIMM *et al.*, 2015).

Dioctria humeralis Zeller, 1840

(Fig. III, 1-4)

Material examined: Guilan province (Roodsar, Rahim abad, Ziaz), 36°52′27.18″ N, 50°13′24.78″ E, 490 m, 4 June 2010, 1♀ (photographed specimen); Mazandaran province (Tangheh vaz), 36°21’55” N, 52° 06’10”E, 692 m, 20 July 2012, 2♀.

Iranian records: Guilan and Mazandaran provinces (current study), new record for Iran.

General distribution: Bulgaria, Czech Republic, Germany, France, Hungary, Poland, former Yugoslavia, Kazakhstan, China, Hungary, Slovakia (Lehr, 1988; GELLER-GRIMM *et al.*, 2015).

Diagnostic characters: A small species (7-8 mm) with reddish-brown parts on thorax. Shiny tomentum on face, golden yellow; mystax yellow; scape about 2x pedicel. Thorax black, postpronotal lobes, often postalar calli and part of scutellum reddish-brown (Fig. III, 2). Scutum with the typical pattern of tomentum leaving the two para median stripes and the lateral spots black; stripe of pale tomentum on pleura complete and yellowish in colour; coxae yellow. Legs almost entirely yellow including tarsi. Wings almost hyaline (Fig. III, 1).

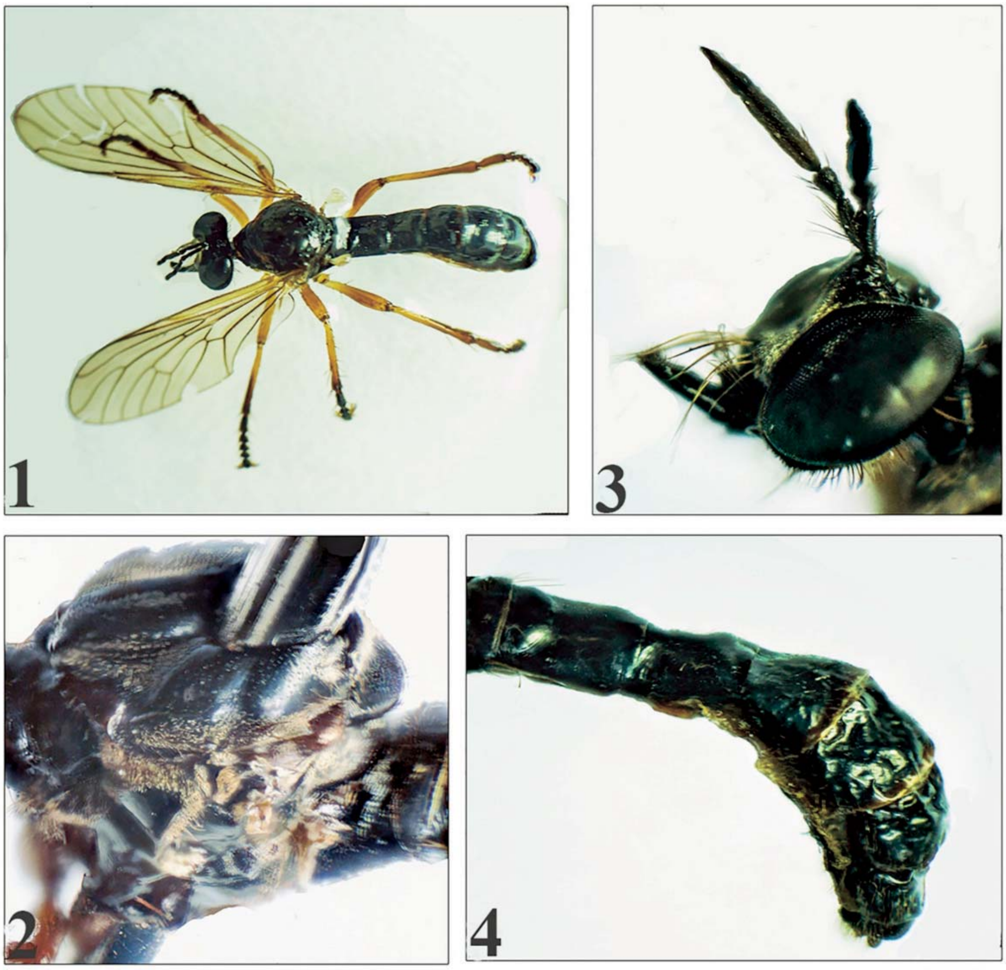

Fig. III – *Dioctria humeralis* Zeller, 1840 1-4: female. 1. General habitus, lateral view; 2. Thorax, lateral view; 3. Head, lateral view; 4. Abdomen, lateral view.

Dioctria hyalipennis (Fabricius, 1794)

Iranian records: Sistan & Baluchestan province (HAYAT *et al.*, 2008).

General distribution: Algeria, Austria, Belgium, Bulgaria, Canada, Czech Republic, Denmark, England, Finland, France, Great Britain, Italy, Luxembourg, Poland, Romania, Russia, Spain, Switzerland, The Netherlands, Tunisia, USA, former Yugoslavia (Geller-Grimm *et al.*, 2015).

Dioctria linearis (Fabricius, 1787)

(Fig. IV, 1-3)

Material examined: East Azarbaijan province, Arasbaran forests, 38°51'42'' N, 46°58'17'' E, 1568 m, 25 May 2013, 1♀ (photographed specimen); Ghurigol 37°55'12'' N, 46°41'31'' E, 1888 m, 22 July 2013, 1♀, 1♂; Hashtroud 37°31'10'' N, 46°51'13'' E, 1682 m, 4 August 2010, 1♀, 1♂.

Iranian records: Guilan province (Hayat *et al.*, 2008), East Azarbaijan province (current study).

General distribution: Austria, Belgium, Bulgaria, Czech Republic, Denmark, England, former Yugoslavia, France, Germany, Hungary, Italy, Luxembourg, The Netherlands, Poland, Romania, Russia, Switzerland (Geller-Grimm *et al.*, 2015).

Dioctria lugens Loew, 1873

Iranian records: Iran (no locality cited) (Lehr, 1988).

General distribution: Turkey (Geller-Grimm *et al.*, 2015).

Dioctria pilithorax Richter, 1960

(Fig. V, 1-3)

Material examined: East Azarbaijan province, Arasbaran forests 38°51'32'' N, 46°59'42'' E, 1783 m, 1 June 2013, 2♀, 1♂ (photographed specimen); Ghurigol 37°55'12'' N, 46°41'31'' E, 1888 m, 29 May 2012, 1♂; Kandovan: 37° 46' N, 46° 15' E, 2341m, 23 June 2015, 2♀.

Iranian records: East Azarbaijan province (Current study), new record for Iran.

General distribution: Azerbaijan (Lehr, 1988).

Diagnostic characters: Rather small, black species (10 mm). Mystax black in both sexes (Fig. V, 2). Frontal tubercle large, as high as length of pedicel; scape about 1.5x pedicel (Fig. V, 2). Thorax black, scutum with remarkably erect black setae (Fig. V, 3); stripe of pale tomentum on pleura dorsally widely interrupted; coxae black. Legs entirely black (Fig. V, 1). Wings largely

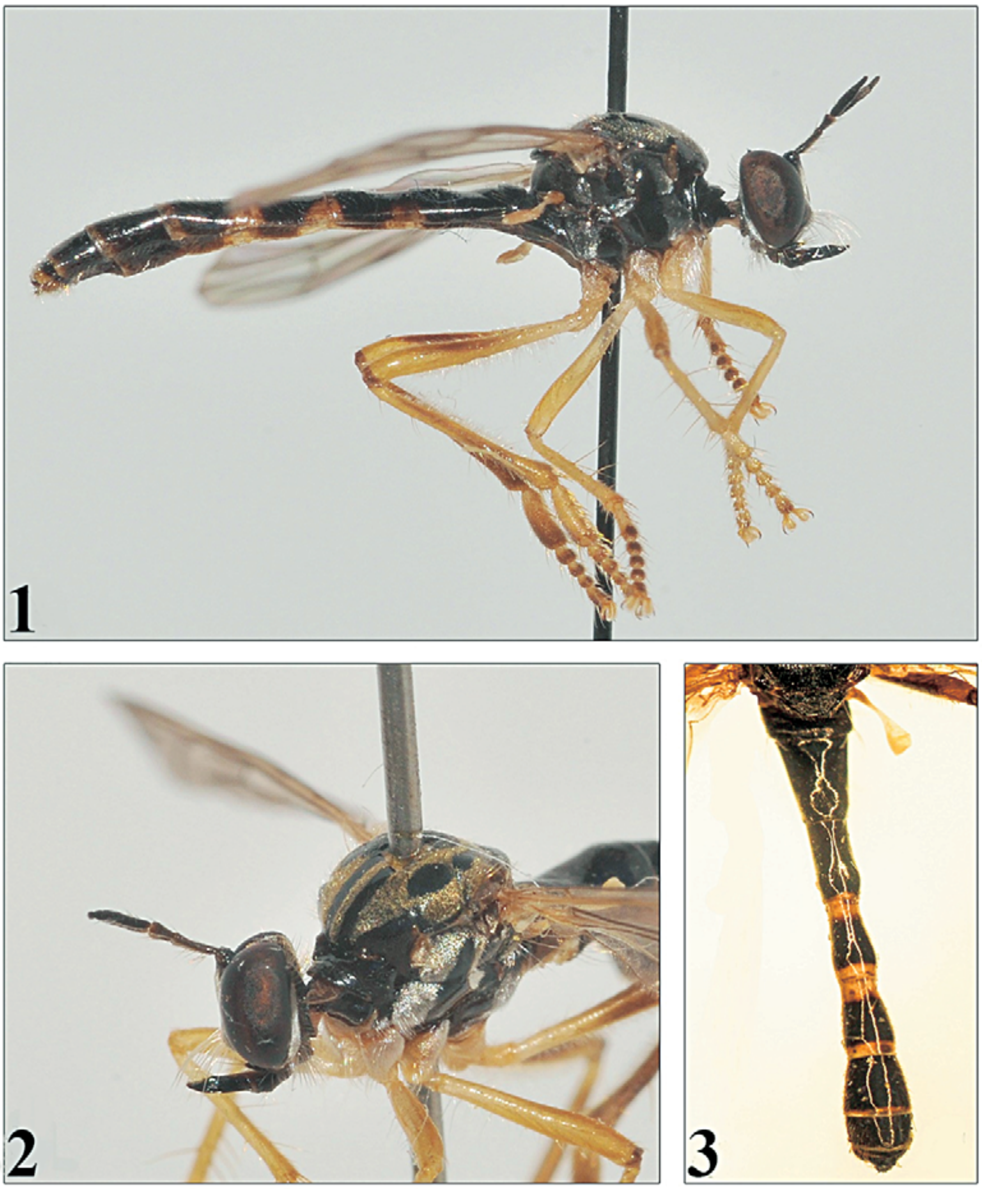

Fig. IV – *Dioctria linearis* (Fabricius, 1787) 1-3: female. 1. General habitus, lateral view; 2. Thorax, laterofrontal; 3. Abdomen, dorsal view.

infused with black microtrichia, apex lighter in males, apical half lighter in females (Fig. V, 1). Abdomen shiny black (Fig. V, 1).

Dioctria rufonigra Theodor, 1980

Iranian records: Mazandaran province (LEHR *et al.*, 2007).

General distribution: Israel, Palestine, Turkey (GELLER-GRIMM *et al.*, 2015).

Dioctria speculifrons Wiedemann, 1820
(Fig. VI, 1-4)

Material examined: Guilan province (Roodsar, Rahim abad, Ghazichak), 36°45′57.54″ N, 50°19′35.22″ E, 1803 m, 3 August 2010, 2♂ (photographed specimen); Guilan province (Roodsar, Rahim abad, Orkom), 36°45′44.34″ N, 50°18′11.88″ E, 1201 m, 16 August 2010, 3♂; Alborz province (Chalous Road), 35°55′07.20″ N, 51°05′09.24″ E, 1891 m, 5 July 2010, 2♂.

Iranian records: Alborz and Guilan provinces (current study), new record for Iran.

General distribution: Armenia, Azerbaijan, Georgia, Spain, Portugal (LEHR, 1988; GELLER-GRIMM *et al.*, 2015).

Diagnostic characters: A smaller sized (7-10 mm), black *Dioctria* species with a dark face. Shiny facial tomentum blackish, mystax black (Fig. VI, 2). Frontal tubercle about half as high as length of pedicel. Thorax black, scutum with distinct pattern of yellowish-brown tomentum leaving the two para median stripes and the lateral spots shiny black; Stripe of pale tomentum on pleura complete and brownish (Fig. VI, 3); coxae black. Legs black, only knees of legs sometimes light coloured (Fig. VI, 1). Wings hyaline (Fig. VI, 1). Abdomen shiny black (Fig. VI, 4).

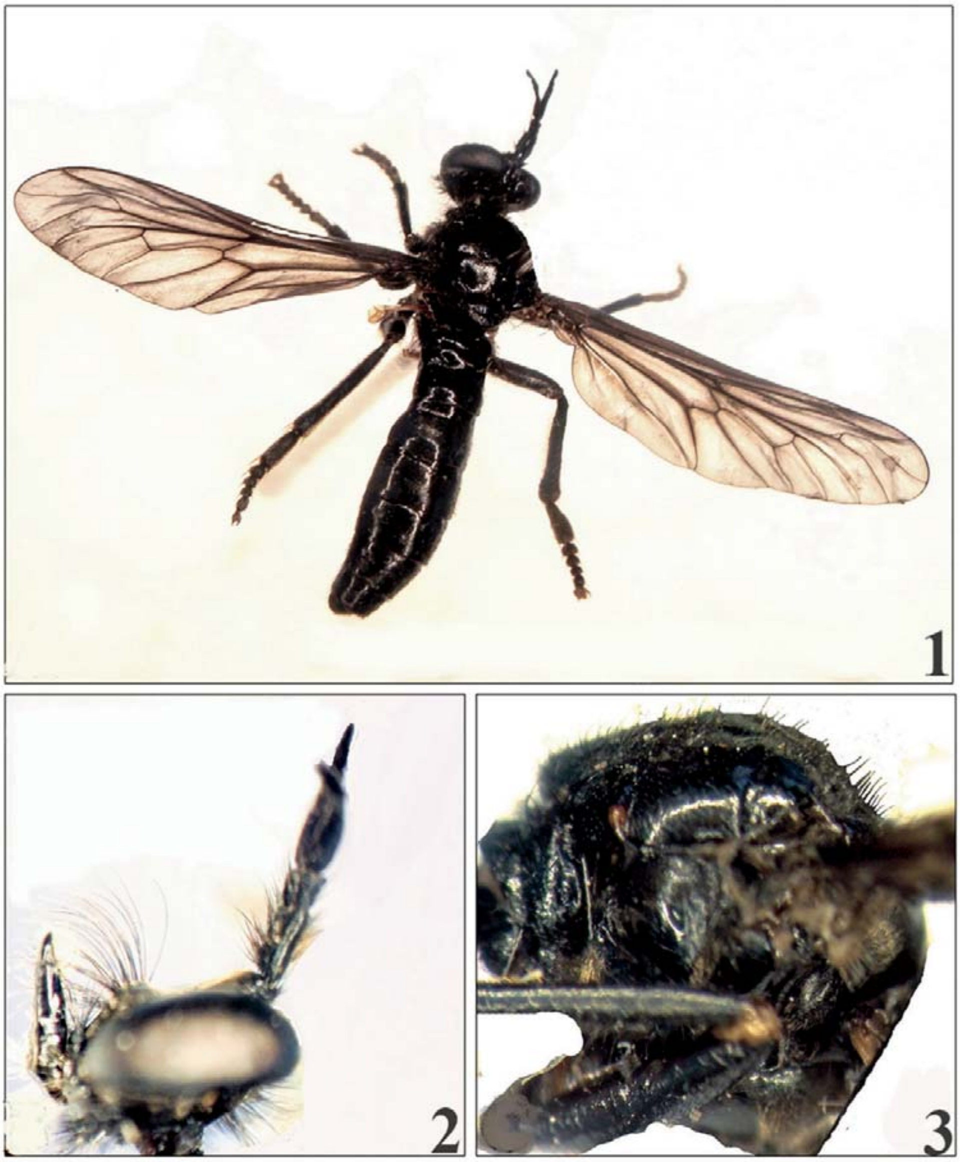

Fig. V – *Dioctria pilithorax* Richter, 1960 1-3: female. 1. General habitus, dorsal view; 2. Head, lateral view; 3. Thorax, lateral view.

Dioctria striata Theodor, 1980

Iranian records: Fars, Kerman (Lehr *et al.*, 2007).

General distribution: Israel, Palestine, Turkey (Geller-Grimm *et al.*, 2015).

Dioctria valida Loew, 1856

Iranian records: Isfahan province (Lehr *et al.*, 2007).

General distribution: Israel, Lebanon, Palestine, Syria, Turkey (Geller-Grimm *et al.*, 2015).

KEY TO THE IRANIAN SPECIES OF THE GENUS *DIOCTRIA*

This present and provisional key is based on Engel (1930), Richter (1988) and Geller-Grimm (2003). Important characters for the identification are the ratio of the antennal segments, especially the scape and the pedicel and a stripe of pale tomentum and pubescence on the side of the thorax. This stripe starts just above the front coxa, up to the dorsal side of the anepisternum and down again to just above the hind coxa (Fig. VII, 4). This stripe can be complete but in some species it is interrupted or partly absent.

1a. Thorax largely red or black with reddish brown parts (Fig. III, 2) **2**

1b. Thorax black, at most a small red spot on humeral callus (Fig. I, 2; Fig. V, 3; Fig. VI, 3) **4**

2a. Thorax largely reddish brown with a black median stripe on the scutum; abdomen black, lateral sides of tergite 2 and tergites 3-4 wholly reddish; wings blackened in anterior basal two thirds .... ***D. rufonigra***

2b. Only humeral and postalar calli red or brownish, sometimes also scutellum wholly or partly red; abdomen black **3**

3a. Only humeral area brownish, rest of scutum shiny black with two broad stripes of tomentum bordering median bare stripe .... ***D. striata***

3b. Both humeral and postalar calli and often part of scutellum

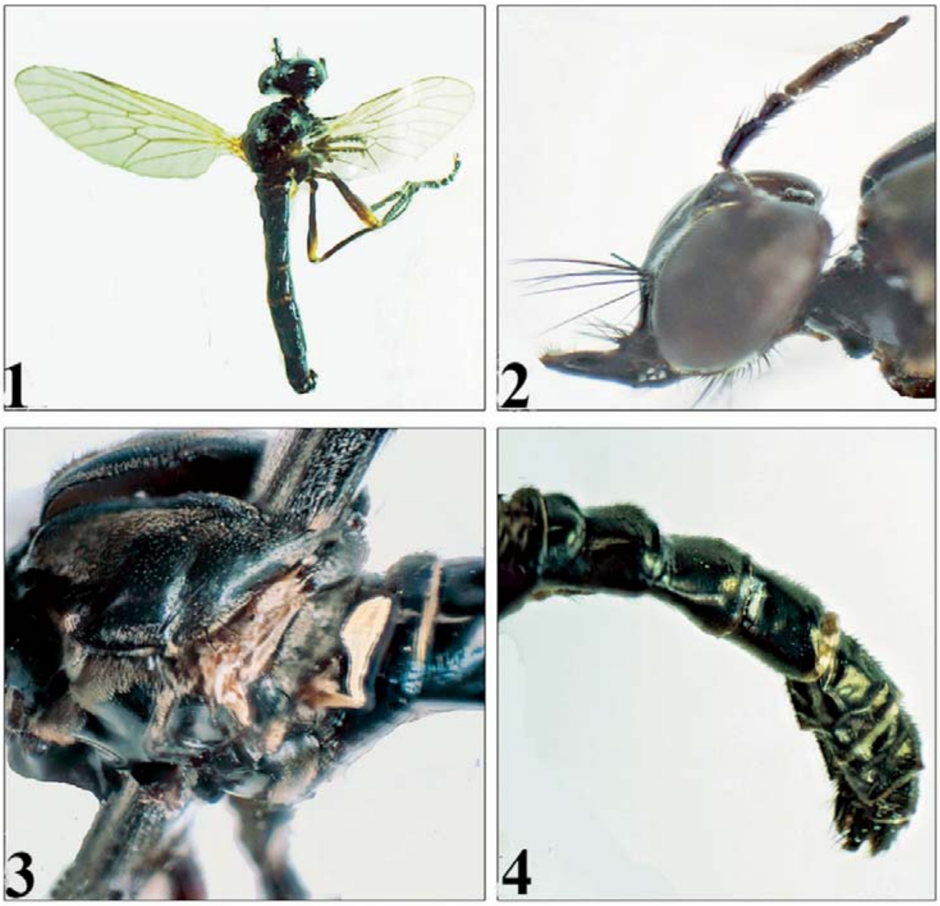

Fig. VI – *Dioctria speculifrons* Wiedemann, 1820 1-4: male. 1. General habitus, lateral view; 2. Head, lateral view; 3. Thorax, lateral view; 4. Abdomen, lateral view.

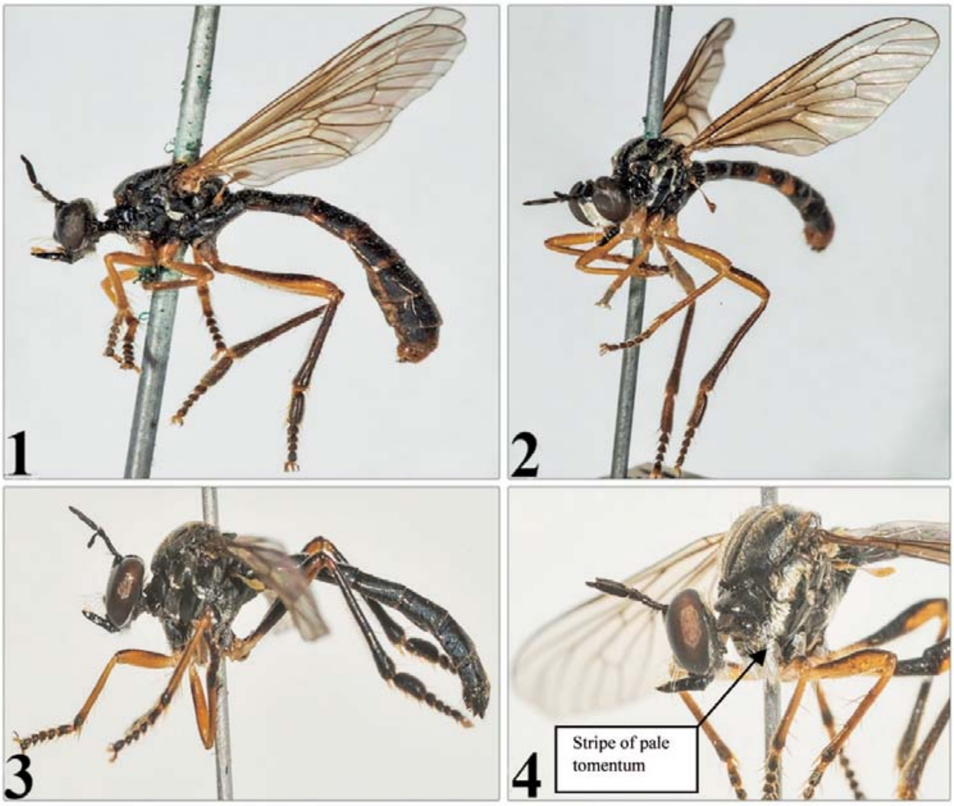

Fig. VII – *Dioctria dispar* Loew, 1871 1-2: male. 1. General habitus, lateral view; 2. General habitus, laterofrontal. *Dioctria hyalipennis* (Fabricius, 1794) 3-4: male: 3. General habitus, lateral view; 4. General habitus, laterofrontal. (Figs. 1 and 2 taken by Dr. Geller-Grimm).

reddish, scutum with yellowish grey tomentum leaving two lines along medium stripe and the two connected lateral spots shiny black .... ***D. humeralis***

4a. Scape equal to, or very slightly longer (1x to 1.2x) than pedicel **5**

4b. Scape clearly longer (more than 1.4 x) than pedicel (Fig. I, 3; Fig. V, 2; Fig. VI, 2) **6**

5a. Coxae yellow, front and middle legs yellow, tarsi darker, hind leg largely black .... ***D. gracilis***

5b. Coxae black, all legs largely reddish yellow, tarsi brownish except last segment always yellow .... ***D. valida***

6a. Legs entirely or largely black (Fig. II, 1, 2) **7**

6b. Legs largely yellow or red (Fig. I, 1; Fig. IV, 1) **10**

7a. Wings hyaline; knees sometimes yellow or red; mystax black in both sexes (Fig. VI, 2) .... ***D. speculifrons***

7b. Wings largely or partly darkened; knees never yellow or red; mystax white or black **8**

8a. Scutum covered with erect long black hairs (Fig. V, 3); mystax black in both sexes (Fig. V, 2) .... ***D. pilithorax***

8b. Scutum with only very short hair in varying colour; mystax not black in both sexes **9**

9a. Stripe of pale tomentum on pleura is brownish white and interrupted on dorsal side of anepisternum; parts of hind legs lighter coloured; mystax white in both sexes .... ***D. lugens***

9b. Brownish or yellowish stripe of pale tomentum on pleura complete (Fig. II, 2); hind legs not with lighter parts; mystax black in males, white in females .... ***D. atricapilla***

10a. Face with only tomentum on the lateral edges leaving a bare, vertical black stripe; mystax black (Fig. I, 3); wings darkened on anterior basal half (Fig. I, 5) .... ***D. arnoldii***

10b. Face covered with shiny tomentum; mystax white or yellow; wings hyaline or anterior base coloured **11**

11a. Coxae black; abdomen black, at most posterior margins of tergites lighter in colour; scutum largely, but rather thinly covered with greyish tomentum leaving lines along median stripe and the lateral spots black; stripe of white tomentum on pleura complete with a thin additional stripe just above the middle coxa (Fig. VII, 3, 4) .... ***D. hyalipennis***

11b. Coxae yellow or brown; scutum with or without tomentum, the yellowish stripe of tomentum on pleura complete but without additional stripe **12**

12a. Scutum somewhat shiny black and without tomentum; tergite 3 with a reddish brown band in the middle, tergite 4 and anterior part of tergite 5 reddish brown .... ***D. abdominalis***

12b. Scutum black with a distinct pattern of tomentum; abdomen black or black with yellow lateral spots **13**

13a. Tergites 3-5 black with light coloured posterior margins and yellow spots on the lateral sides (Fig. IV, 3); hind femur yellow with a dark spot on the anterior side that can be variable in size (Fig. IV, 1) .... ***D. linearis***

13b. Abdomen black, at most posterior margins lighter in colour; hind femur different coloured **14**

14a. All legs reddish yellow; base of wing somewhat brownish infuscate in male, yellowish in female .... ***D. flavipennis***

14b. Legs largely reddish yellow, femur of the hind legs dorsally black, front tibia with darkened apex, middle tibia darkened with a lighter base, hind tibia black; basal half of wing in male darkened (Fig. VII, 2), in female wings almost hyaline (Fig. VII, 1) .... ***D. dispar***

DISCUSSION

The Asilidae are thermophilic predacious flies that mainly found in arid and semiarid areas of the planet (Hull, 1962). Iran is located on the crossroad of three ecological zones, the Palearctic, Afrotropical and the Oriental (Ghahari *et al.,* 2014). Consequently, Iran harbors a rich vegetation as well as a rich insect fauna, including many species of robber flies (Diptera: Asilidae). Between 2008 and 2014 the number of species, including subspecies, of Asilidae recorded from Iran almost doubled from 156 (Hayat *et al.*, 2008) to 289 (Ghahari *et al.*, 2014). In many recent studies (Mohammadi and Khaghaninia, 2015, 2016; Mohammadi *et al.*, 2017) including this study, more species are added. It seems that the study of Asilidae has long been neglected in Iran and many species can be expected in future studies in different parts of the country. With four new records of this study, the number of species of the genus *Dioctria* in Iran has increased to 15. However, as many areas of Iran remain unexplored, we expect that the *Dioctria* fauna of Iran will be substantially increased.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Dmitry Astakhov (Volgograd State University, Russia) and Dr. Jarsalov Bosak (Ecological Consulting a.s., Na Střelnici, Czech Republic) for their valuable help with the identification of the species and also we would like to thank Dr. Geller-Grimm (Museum Wiesbaden Natural History; Germany) for his help in producing photos of species previously reported from Iran but to which that we did not have access.

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REDIA, 102, 2019: 13-21 http://dx.doi.org/10.19263/REDIA-102.19.02

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MOSQUITOES (DIPTERA CULICIDAE) OF FRIULI VENEZIA GIULIA (NORTH-EASTERN ITALY): ANNOTATED CHECKLIST, GEOGRAPHIC DISTRIBUTION AND HABITATS OF PRE-IMAGINAL STAGES

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Zamburlini R., Cargnus E., Zandigiacomo P. − Mosquitoes (Diptera Culicidae) of Friuli Venezia Giulia (north-eastern Italy): annotated Checklist, geographic distribution and habitats of pre-imaginal stages.

The updated Checklist of the Diptera Culicidae recorded in Friuli Venezia Giulia (north-eastern Italy) is reported. In addition to already published data since 1920s, recent and original data are provided. The list includes a total of 37 mosquito species (out of 66 species known for Italy) of which 31 currently present. The occurrence of *Anopheles hyrcanus, Culex martinii, Culiseta subochrea* and *Uranotaenia unguiculata* needs to be confirmed; *Ae. dorsalis* is of uncertain identification; *An. sacharovi,* the main past malaria vector, is presumably locally extinct. The list includes three exotic species, *Aedes albopictus* widespread in plain and hilly urban areas, *Ae. japonicus* and *Ae. koreicus*, both recently detected in some hilly and mountain areas. Data on the geographic distribution in the region and on the ecology of the pre-imaginal stages are provided. Many of the recorded species are of health concern as potential vectors of pathogens. Further studies, conducted mainly in the less investigated mountain areas, will probably enrich the regional mosquito fauna.

KEY WORDS: larval habitat, vectors, exotic species, biodiversity.

INTRODUCTION

The family of Culicidae (Diptera) comprises more than 3550 species distributed worldwide (HARBACH, 2016) of which about a hundred occur in Europe (BECKER *et al*., 2010; Schaffner F., pers. comm.). The Italian inventory of Culicidae is currently composed of 66 species (SEVERINI *et al*., 2009; CAPELLI *et al*., 2011; SEIDEL *et al*., 2016).

First investigations on Culicidae of the Friuli Venezia Giulia region (FVG) (north-eastern Italy) were carried out in the early decades of 1900 by the "Istituto Interprovinciale per la Lotta Antimalarica nelle Venezie" (Interprovincial Institute for the Fight against Malaria in the Venetian regions) on malaria vectors when the Adriatic Sea coastal area was affected by endemic malaria.

From 1927 to 1939 five species, at that time considered as different "races" of *Anopheles maculipennis* Meigen, were identified: *An. sacharovi* Favr (sub *An. elutus* Edwards, 1921), which was the main malaria vector, *An. atroparvus* Van Thiel*, An. maculipennis* (named "*typicus*"), *An. messeae* Falleroni and *An. claviger* (Meigen) [sub *An. bifurcatus* (Linnaeus, 1758)] (DE FAVERI, 1939; SEPULCRI, 1963). In the same period, within a study on the aquatic fauna of the Timavo River mouth (Trieste district), eight species were recognized: *An. maculipennis, Aedes caspius* (Pallas), *Ae. dorsalis* (Meigen) (mentioned as doubtful by the Author), *Ae. vexans* (Meigen), *Culex modestus* Ficalbi, *Cx. pipiens* Linnaeus, *Cx. territans* Walker (sub *Cx. apicalis* Adams, 1903) and *Coquillettidia richiardii* (Ficalbi) (sub *Mansonia richiardii*) (STAMMER, 1932).

Malaria was eradicated in the late 1940s mainly through extended reclamations of the brackish swamps which were the breeding habitat of the primary malaria vector *An. sacharovi* (SEPULCRI, 1963; ZAMBURLINI, 2000).

Investigations conducted in the northern Adriatic littoral area during 1950s and 1960s, a period of further strong reduction of natural habitats caused by increasing urbanization and agriculture expansion, showed the disappearance of *An. sacharovi* together with the persistence of the other previously recorded species of the *An. maculipennis* complex (SEPULCRI, 1963). During this time, renewed attention was paid to mosquito fauna mainly because of its economic impact on expanding tourism activities along the Adriatic coast. In this respect, in FVG 11 species were recorded: *Ae. caspius, Ae. cinereus* Meigen*, Ae. detritus* (Haliday)*, Ae. vexans, Cx. modestus, Cx. pipiens, Cx. territans, Cx. theileri* Theobald*, Culiseta annulata* (Schrank)*, Cs. morsitans* (Theobald) and *Cs. subochrea* (Edwards) (DECHIGI *et al*., 1967).

Starting from 1980s 10 more species were firstly detected in FVG: *An. hyrcanus* (Pallas), *An. plumbeus* Stephens, *Ae. cantans* Meigen, *Ae. geniculatus* (Olivier), *Ae. pullatus* (Coquillett)*, Ae. sticticus* (Meigen), *Cx. hortensis* (Ficalbi) and *Uranotaenia unguiculata* Edwards (ZAMBURLINI, 1996b), and *Ae. cataphylla* Dyar and *Ae. communis* (De Geer) (CARGNUS & ZAMBURLINI, 1999). Moreover, two records were the first for the whole Italian mosquito fauna: *Ae. annulipes* (Meigen) (ZAMBURLINI, 1996a) and *Ae. geminus* Peus (ZAMBURLINI & CARGNUS, 1998a).

Regarding the *An. maculipennis* complex, the absence of *An. sacharovi* in the coastal area was confirmed (ZAMBURLINI & CARGNUS, 1998b). In addition, a morphological study on the regional populations of *An. claviger* complex allowed to assign all individuals examined to the nominal species (ZAMBURLINI & CARGNUS, 1998c).

In 1995 the exotic *Ae. albopictus* (Skuse), or Asian tiger mosquito, was detected (ZAMBURLINI, 1995) and soon it became the most widespread and annoying mosquito

– Received 6 August 2018 Accepted 11 February 2019

throughout plain and hilly urban areas of the region (ZAMBURLINI & FRILLI, 2006).

From 2000s, the known composition of the regional mosquito fauna was not only confirmed (ZAMBURLINI & CARGNUS, 2009; TOMA *et al*., 2013; MANCINI *et al*., 2017) but also increased with some new records, such as the three native species *Ae. punctor* Kirby (ZAMBURLINI & CARGNUS, 2009), *Cx. impudicus* Ficalbi and *Cx. martinii* Medschid (TOMA *et al*., 2013), and the two exotic species *Ae. koreicus* (Edwards) (MONTARSI *et al*., 2015; ZAMBURLINI & CARGNUS, 2015), presumably introduced from the adjacent Veneto region where it was first detected (CAPELLI *et al*., 2011), and *Ae. japonicus* (Theobald) (SEIDEL *et al*., 2016).

The aim of this paper is to update the Checklist of the Culicidae of the FVG region, gathering all available data since the 1920s to 2017, and to provide information on the mosquito geographic distribution and pre-imaginal ecology, useful for conservation and medical purposes.

MATERIALS AND METHODS

STUDY AREA

The FVG region (7845 km²) from north to south comprises mountains (Carnic and Julian Pre-Alps and Alps), plains (Friulian plains and Morainic hills), sloping towards the northern Adriatic Sea coast, and the Karst plateau in the South-East. FVG has a moderately humid continental climate with a wide variability across the territory. The annual average temperature ranges from a minimum of 2.9 °C in the mountains to 14.5-15.5 °C along the coast. Annual average rainfall varies from 900 mm in the coast to 1200-1800 mm in the plain, hilly and mountain areas, and up to 3200 mm in some portions of the Julian Pre-Alps, with most rainfall occurring during the late spring and autumn (ARPA FVG - OSMER, 2014). For its climate and geomorphology, the region has a rich variety of aquatic ecosystems and a large array of natural and artificial stagnant water bodies where the immature stages of mosquitoes can find suitable development habitats.

COLLECTION OF MOSQUITOES

Mosquito collections have been carried out in numerous sites distributed in the four districts of FVG (Pordenone, PN; Udine, UD; Gorizia, GO; Trieste, TS) (Table 1). Sites N. 1-83 were visited from 1983 to 2017 by the Authors of this paper (original and/or already published data), while data related to sites N. 84-88 are taken from literature. The sites were included in six ecosystems, five natural and one urbanized, representative of the environmental diversity of the study territory (Table 2). The site with rice fields was located a few kilometers beyond the regional border in the adjacent Veneto region (N. 83, Bibione, Venezia district, VE). For the urban areas data were reported only for a few sites representative of the whole study territory.

Larvae and pupae were collected by a standard dipper in different types of stagnant water bodies. Eggs of *Ae. albopictus* were sampled by means of ovitraps (ZAMBURLINI & FRILLI, 2006). Adults were caught by a manual electric aspirator. Gravid females of the *An. maculipennis* complex were captured in cattle farms located only in former malarial areas of the low Friulian plains.

SPECIES IDENTIFICATION AND NOMENCLATURE

The species identification of individuals, preserved in 70% ethanol (larvae and pupae), dried (adults) or slide mounted in Faure's liquid (4th-5th instar larvae, male genitalia), was undertaken by morphological criteria based on the keys of GUTSEVICH *et al.* (1974), TANAKA *et al.* (1979), ROMI *et al.* (1997), SEVERINI *et al.* (2009) and BECKER *et al.* (2010). The larvae and pupae of *An. maculipennis* complex, morphologically not identifiable to species level, were indicated as *An. maculipennis* s.l. The collected females of *An. maculipennis* complex were individually identified to species after laying eggs in laboratory, on the basis of the egg colour pattern as described by ANGELUCCI (1955). For some species, collected immatures were reared in laboratory up to the adult stage in order to observe the diagnostic characters. Most of the material studied by the Authors of this paper is stored in the Culicidae collection of the Department of Agricultural, Food, Environmental and Animal Science - Entomology, by the University of Udine (Italy).

The nomenclature adopted in this paper follows those of KNIGHT & STONE (1977), KNIGHT (1978) and WARD (1984, 1992), while for the tribe Aedini the genera and subgenera refer to WILKERSON *et al*. (2015).

RESULTS AND DISCUSSION

RECORDED SPECIES

The updated Checklist of Culicidae of FVG comprises 37 species belonging to six genera. Thirty-two species were collected by the Authors of this paper (of which 22 were already recorded by other Authors) and other five (*An. sacharovi*, *Ae. dorsalis*, *Cx. impudicus*, *Cx. martinii* and *Cs. subochrea*) were collected by other Authors and reported in literature (Table 3). Presently, the occurrence of 31 species is to be considered as certain. It remains to confirm the occurrence of four species, up today singly reported: *An. hyrcanus* and *U. unguiculata* [mentioned in ZAMBURLINI (1995)] both detected only as larvae, and *Cs. subochrea* (DECHIGI *et al*., 1967) and *Cx. martinii* Medschid both observed as single adult, the latter as a female (TOMA *et al*., 2013; L. TOMA pers. comm.). In addition, the presence of *Ae. dorsalis* is to be confirmed as the identity of the collected larvae was mentioned as doubtful by the Author being they difficult to distinguish from those of *Ae. caspiu*s (STAMMER, 1932). Finally, the malaria vector *An. sacharovi* is to be considered as locally extinct because it has never been observed since the late 1940s (SEPULCRI, 1963; ZAMBURLINI & CARGNUS, 1998b).

SPECIES IDENTIFICATION

Among the 32 species collected by the Authors of this paper, 24 were identified both as immatures and adults, four species only as larvae (*Ae. detritus, An. hyrcanus, Cx. theileri* and *U. unguiculata*), three species of the *An. maculipennis* complex on the basis of egg features (*An. atroparvus, An. maculipennis* s.s. and *An. messeae*) and one species (*Cq. richiardii*) only as adult females.

GEOGRAPHIC DISTRIBUTION OF SPECIES IN FVG

Most of the taxa (30) are reported from the northern Adriatic Sea coast and Friulian plains (sites between 0-280 m a.s.l.) (urban areas excluded) (Table 3 and Table 4 in Supplementary material). In particular, *Ae. caspius*, *Ae. detritus*, *An. hyrcanus* and *U. unguiculata* have been detected only in sites placed at lower elevations (0-3 m a.s.l.), as well as the three identified species of the *An. maculipennis* complex: *An. atroparvus*, *An. maculipennis* s.s. and *An. messeae*. Moreover, the five species not collected by the Authors of this paper, i.e. *Cx. impudicus* and

Table 1 – Sites of collection of Culicidae in Friuli Venezia Giulia (north-eastern Italy). The sites indicated in bold (N. 84-89) refer to species not collected by the Authors of this paper but reported in literature. One site (N. 83) is from the nearby Veneto region.

Site N.	Municipality (District acronym)	Site of collection	Coordinates	Altitude m a.s.l.
1	Aviano (PN)	Cimitery of Marsure	46°05’28.56”N, 12°35’32.17”E	226
2	Chions (PN)	Bosco di Torrate	45°53’37.28”N, 12°48’01.64”E	26
3	Cordenons (PN)	Risorgive fiume Meduna	45°58’46.61”N, 12°42’17.66”E	35
4	Montereale Valcellina (PN)	Cimitery	46°09’59.20”N, 12°39’39.12”E	331
5	Morsano al Tagliamento (PN)	Bosco di golena del Torreano	45°51’39.11”N, 12°58’18.53”E	10
6	Polcenigo (PN)	Risorgive fiume Livenza - Santissima	46°01’17.56”N, 12°28’43.53”E	30
7	Pravisdomini (PN)	Palude di Barco	45°49’34.87”N, 12°40’05.20”E	4
8	San Vito al Tagliamento (PN)	Grave fiume Tagliamento, loc. Rosa	45°55’25.78”N, 12°54’32.52”E	12
9	San Vito al Tagliamento (PN)	Urban area	45°55’04.73”N, 12°51’26.26”E	32
10	Sequals (PN)	Torbiera	46°10’34.74”N, 12°51’35.33”E	207
11	Valvasone (PN)	Grave fiume Tagliamento	46°00’42.70”N, 12°54’25.98”E	68
12	Amaro (UD)	Urban area	46°22’22.57”N, 13°05’32.19”E	295
13	Ampezzo (UD)	Palude di Cima Corso	46°23’43.76”N, 12°44’40.56”E	850
14	Aquileia (UD)	Bonifica IV Partita	45°46’05.40”N, 13°21’39.40”E	2
15	Attimis (UD)	Area umida ex polveriera Racchiuso	46°09’54.30”N, 13°19’42.49”E	180
16	Carlino (UD)	Bosco Bolderatis	45°47’15.10”N, 13°12’27.50”E	5
17	Carlino (UD)	Bosco Sacile	45°47’23.49”N, 13°11’11.09”E	5
18	Carlino (UD)	Valle Zellina, strada Chiamana	45°47’44.08”N, 13°11’48.41”E	1
19	Cassacco (UD)	Torbiera Chichinot, Raspano	46°12’04.55”N, 13°10’30.26”E	196
20	Castions di Strada (UD)	Bosco Boscat	45°50’00.19”N, 13°10’02.99”E	20
21	Castions di Strada (UD)	Palude Moretto	45°53’48.60”N, 13°09’42.06”E	19
22	Castions di Strada (UD)	Palude Selvote	45°53’12.58”N, 13°11’33.99”E	15
23	Cavazzo Carnico (UD)	Palude Vuarbis	46°21’30.62”N, 13°03’06.71”E	278
24	Cervignano del Friuli (UD)	Bosco Pradiziolo	45°49’18.95”N, 13°18’52.89”E	19
25	Fagagna (UD)	Prati umidi Quadris	46°08’04.88”N, 13°04’27.25”E	172
26	Forni di Sopra (UD)	Urban area	46°25’18.54”N, 12°34’57.18”E	907
27	Forni di Sotto (UD)	Urban area	46°23’33.62”N, 12°40’10.90”E	751
28	Lignano Sabbiadoro (UD)	Valle del Pantanel	45°41’13.26”N, 13°06’05.05”E	0
29	Lignano Sabbiadoro (UD)	Valle via Tagliamento	45°42’55.60”N, 13°04’21.32”E	1
30	Lignano Sabbiadoro (UD)	Urban area	45°40’06.80”N, 13°06’14.71”E	3
31	Lignano Sabbiadoro (UD)	Crop fields	45°39’51.15”N, 13°05’57.99”E	3
32	Majano (UD)	Torbiera di Casasola	46°11’44.05”N, 13°04’35.66”E	162
33	Marano Lagunare (UD)	Le Favole	45°46’32.31”N, 13°08’59.15”E	1
34	Marano Lagunare (UD)	Isola di Sant’Andrea	45°42’51.63”N, 13°12’59.57”E	0
35	Moruzzo (UD)	Torbiera Borgo Pegoraro	46°07’31.53”N, 13°06’53.32”E	186
36	Muzzana del Turgnano (UD)	Bosco Baredi - Selva di Arvonchi	45°47’22.34”N, 13°07’00.34”E	10
37	Muzzana del Turgnano (UD)	Bosco Coda di Manin	45°47’59.59”N, 13°08’41.21”E	10
38	Osoppo (UD)	Risorgive dei Bars	46°13’37.62”N, 13°02’47.37”E	150
39	Pagnacco (UD)	Torbiera di Lazzacco	46°07’56.38”N, 13°09’35.77”E	189
40	Palazzo Stella (UD)	Casali Marianis	45°46’14.43”N, 13°05’34.73”E	0
41	Paluzza (UD)	Pal Piccolo	46°36’02.00”N, 12°57’00.00”E	1613
42	Paluzza (UD)	Torbiera Val Pudia	46°32’12.36”N, 13°01’32.34”E	760
43	Paluzza (UD)	Torre Moscarda	46°33’17.30”N, 13°00’57.07”E	815
44	Paularo (UD)	Torbiera Zuc Guardia, Cason Lanza	46°33’43.28”N, 13°10’18.24”E	1570
45	Pontebba (UD)	Urban area	46°30’20.34”N, 13°18’07.34”E	589
46	Precenicco (UD)	Bosco Bando	45°46’45.05”N, 13°03’46.40”E	15
47	Precenicco (UD)	Titiano	45°44’41.71”N, 13°04’18.71”E	1
48	San Daniele del Friuli (UD)	Bosco di Soprapaludo	46°09’53.70”N, 13°01’49.30”E	151
49	San Daniele del Friuli (UD)	Urban area	46°09’27.17”N, 13°00’36.25”E	235
50	San Giorgio di Nogaro (UD)	Porto Nogaro	45°45’32.20”N, 13°14’24.54”E	1

(continued)

Continued Table 1

51	Talmassons - Bertiolo (UD)	Risorgive F. Stella, Flambro	45°55’48.53”N, 13°04’30.26”E	10
52	Tarvisio (UD)	Torbiera Scicchizza, Fusine in Valromana	46°29’28.67”N, 13°40’32.89”E	845
53	Timau (UD)	Bosco ripario torrente But, Muse	46°34’56.98”N, 13°00’03.31”E	840
54	Tolmezzo (UD)	Torbiera Curiedi, Fusea	46°25’33.47”N, 12°58’49.85”E	849
55	Torreano di Cividale (UD)	Cava di Togliano	46°07’25.21”N, 13°22’43.61”E	125
56	Torviscosa (UD)	Bosco Ronchi di Sass	45°48’23.39”N, 13°14’22.99”E	10
57	Varmo (UD)	Grave fiume Tagliamento, Madrisio	45°51’59.45”N, 12°58’27.12”E	11
58	Grado (GO)	Boscat	45°43’49.97”N, 13°25’25.07”E	1
59	Grado (GO)	Bosco “la Rotta”	45°41’26.25”N, 13°26’49.64”E	1
60	Grado (GO)	Fossalon di Grado	45°43’44.63”N, 13°29’37.15”E	1
61	Grado (GO)	Punta Sdobba	45°43’31.60”N, 13°32’32.81”E	0
62	Grado (GO)	Valle Cavarera	45°41’21.83”N, 13°25’20.07”E	1
63	Monfalcone (GO)	Marina Julia	45°46’47.37”N, 13°31’59.34”E	0
64	Monfalcone (GO)	Palude Sablici	45°48’30.20”N, 13°33’59.05”E	15
65	Pieris (GO)	Grave fiume Isonzo	45°48’47.99”N, 13°25’46.04”E	5
66	Ronchi dei Legionari (GO)	Stagni di Selz, Mucille	45°49’18.67”N, 13°31’30.43”E	13
67	San Floriano del Collio (GO)	Palude di Preval	45°57’42.66”N, 13°31’39.51”E	59
68	San Floriano del Collio (GO)	Urban area	45°58’56.64”N, 13°35’14.82”E	262
69	Staranzano (GO)	Isola della Cona	45°43’48.56”N, 13°33’14.60”E	0
70	Versa (GO)	Grave fiume Judrio	45°53’54.98”N, 13°25’24.33”E	25
71	Duino Aurisina (TS)	Stagno di Slivia N. 34	45°46’20.01”N, 13°39’39.09”E	125
72	Duino Aurisina (TS)	Villaggio del Pescatore	45°46’41.90”N, 13°34’38.55”E	1
73	Monrupino (TS)	Campi solcati Colognatti	45°42’10.66”N, 13°48’34.18”E	308
74	Monrupino (TS)	Stagno Zolla di Monrupino, N. 50	45°43’01.10”N, 13°48’03.00”E	367
75	Monrupino (TS)	Stagno di Rupingrande N. 7	45°43’14.40”N, 13°47’03.60”E	292
76	Opicina (TS)	Cisterna romana, Ovcjak, N. 44	45°42’05.92”N, 13°47’38.11”E	271
77	Opicina (TS)	Stagno di Percedol, N. 103	45°42’19.71”N, 13°48’12.26”E	270
78	Sgonico (TS)	Campi solcati Borgo Grotta Gigante	45°42’26.35”N, 13°45’29.79”E	256
79	Sgonico (TS)	Stagno di Sagrado, N. 46	45°43’34.10”N, 13°46’20.00”E	343
80	Sgonico (TS)	Urban area	45°44’12.39”N, 13°44’56.43”E	275
81	Trieste	Urban area (Querceto di Bovedo)	45°40’39.92”N, 13°45’45.62”E	99
82	Trieste	Stagno di Conconello, N. 26	45°40’23.10”N, 13°47’51.19”E	412
83	Bibione (VE)	Risaie IV Bacino	45°39’37.46”N, 13°01’55.70”E	1
84	**Aquileia (UD)**	**Not reported**	**Not reported**	**2**
85	**Marano Lagunare (UD), Grado (GO)**	**Laguna di Grado e Marano, Riserva Caneo**	**Not reported**	**0-1**
86	**Grado (GO)**	**Grado Pineta**	**Not reported**	**1**
87	**Duino Aurisina (TS)**	**Foci del Timavo**	**Not reported**	**2**
88	**Coastal municipalities (UD, GO, TS)**	**Coast and plains**	**Not reported**	**0-2**

Cx. martinii (Toma *et al.*, 2013), *Ae. dorsalis* (Stammer, 1932), *Cs. subochrea* (Dechigi *et al.*, 1967) and *An. sacharovi* (Sepulcri, 1963), were all reported from coastal and plain sites as well.

In the Carnic and Julian Pre-Alps and Alps (sites between 760-1613 m a.s.l.) seven species were recorded and three of them, the more microterm *Ae. communis*, *Ae. pullatus* and *Ae. punctor*, only in mountain areas (Table 3 and Table 4 in Supplementary material).

In the Karst plateau (sites between 125-412 m a.s.l.) a total of 10 species were sampled (Table 3).

In urban areas of all the region (sites between 0-900 m a.s.l.), a total of 12 species were found, but only four taxa, *Ae. koreicus, Ae. japonicus, An. maculipennis* s.l. and *Cx. pipiens,* were collected over 330 m a.s.l. (Table 3). The exotic *Ae. albopictus* and the native *Cx. pipiens* constantly occurred but the establishment of *Ae. albopictus* was restricted to plain and hilly areas up to an altitude of 350 m a.s.l., as no larvae and eggs were found in breeding sites of Pre-Alps and Alps. The presence of *Ae. koreicus* and *Ae. japonicus,* more resistant to cold temperatures than *Ae. albopictus* (Tanaka *et al*., 1979)*,* resulted still restricted to the hilly and mountain areas where they were firstly detected (Montarsi *et al*., 2015; Zamburlini & Cargnus, 2015; Seidel *et al*., 2016).

Ecosystems and habitats of pre-imaginal stages

Immature stages of 29 taxa (28 species plus the larvae of *An. maculipennis* s.l.) have been collected in a rich variety of stagnant water habitats included in the six major ecosystems identified on the regional territory (Table 2 and Table 4 in Supplementary material).

Table 2 – Habitats of pre-imaginal stages of Culicidae collected in Friuli Venezia Giulia (north-eastern Italy).

Geographic distribution	Ecosystem	Habitat of pre-imaginal stages
Northern Adriatic Sea coast	**1.** Coastal brackish marshes	**1a.** Temporary pools in halophytic marshes
		1b. Permanent brackish ponds and swamps
Friulian plains and Morainic hills 0–280 m a.s.l.	**2.** Wet woodlands and flooded shaded meadows	**2a.** Semi-permanent ponds and marshes
		2b. Permanent shaded ponds
		2c. Temporary freshwater pools
		2d. Tree-holes water-filled
	3. Crops	**3a.** Rice flooded parcels
		3b. Crop drains
Carnic and Julian Pre-Alps and Alps (mountains) 760–1620 m a.s.l.	**4.** Wet forests and marshes	**4a.** Pools in mixed deciduous woodlands
		4b. Flooded meadows and marshes. Snow melt pools
Karst plateau 120–420 m a.s.l.	**5.** Karst woods and marshes	**5a**. Shaded semi-permanent and permanent ponds and artificial containers
		5b. Tree-holes and rock-holes water-filled
Whole region 0–910 m a.s.l.	**6.** Urban areas	**6a.** Natural and artificial small containers of eutrophic water
		6b. Shaded containers filled with clear rainwater
		6c. Polluted water ditches

Northern Adriatic Sea coast
1. Coastal brackish marshes

1a. Temporary pools located within halophytic marshes, characterized mainly by *Salicornia* sp. and *Juncus* sp. (8 sites), were the main habitat of the constantly sampled *Ae. caspius* (8 sites) associated with the rare *Ae. detritus* (2 sites) (Table 4 in Supplementary material). Based on literature data, *Ae. dorsalis* shared this habitat with *Ae. caspius* (Stammer, 1932).

1b. Permanent brackish ponds and swamps (salinity below 2%) in the past were the main pre-imaginal habitat of *An. sacharovi* (De Faveri, 1939) (Table 4 in Supplementary material). This type of habitat has been greatly reduced by land reclamations and this species is currently absent (Zamburlini & Cargnus, 1998c).

Friulian plains and Morainic hills
2. Wet woodlands and flooded shaded meadows

2a. Semi-permanent ponds and marshes, placed along springs, river meanders or dismissed quarries, often characterized by the occurrence of *Populo-salicetum* and *Magnocariceton* vegetation; immature stages of a total of 9 species were collected (29 sites) (Table 4 in Supplementary material). *An. claviger* (27 sites), *Cs. morsitans* (21 sites), *Ae. annulipes* (21 sites), *Ae. cantans* (9 sites) and *Ae. cinereus* (9 sites), were the most frequent species, whereas *Ae. geminus*, *Ae. sticticus* and *Ae. vexans* were less common (4, 1 and 2 sites, respectively). Larvae of *An. hyrcanus* were collected in a semi-permanent pond at the edge of a grove; this record dates back to 1994 and, therefore, this species has to be considered as very rare so that its presence needs to be confirmed.

2b. In permanent shaded ponds, often covered by floating vegetation and by filamentous algae and surrounded by *Phragmites australis* and *Typha latifolia*, immature stages of seven mosquito species were found (13 sites) (Table 4 in Supplementary material). *An. maculipennis* s.l. (6 sites), *Cx. pipiens* and *Cx. territans* (4 sites, both) were the most frequent species, whereas *Cs. annulata* (2 sites) and *Cx. theileri* (1 site) were less frequent. *Cx. territans* was often found in pools rich in green algae situated along river streams. *U. unguiculata* was sampled in a freshwater pond within a *Phragmitetum* together with *An. maculipennis* s.l.. This record dates back to 1994 (Zamburlini, 1995) so the current presence of this species in FVG needs to be confirmed. Adults of *Cq. richiardii* were sampled near ponds (2 sites). Furthermore, *Cx. impudicus* and *Cx. martinii* (1 adult) (Toma *et al*., 2013), and *Cs. subochrea* (1 adult) (Dechigi *et al*., 1967) are reported from this type of habitat as well (Table 4 in Supplementary material).

2c. In temporary freshwater pools within *Querco-Carpinetum* woodlands situated in alluvial low plains, four mosquito species were collected in 10 sites (Table 4 in Supplementary material). Large numbers of larvae of *Ae. vexans* (10 sites) and *Ae. sticticus* (9 sites), more often in mixed populations, were found to exploit the springtime rainwater pools on thick leaf litter. Subsequent high densities of biting adults were observed in late springtime, causing strong annoyance to human activities in the woods and nearby areas. *Ae. annulipes* and *Ae. cinereus* were collected as well (1 and 2 sites respectively).

2d. In tree holes filled with rainwater, frequently in *Carpinus* sp. and *Fraxinus* sp. trees, larvae of *Ae. geniculatus* (all 12 sites) and *An. plumbeus* (8 sites) were collected (Table 4 in Supplementary material).

3. Crops

3a. In a single site with rice fields, five mosquito species were detected (Table 4 in Supplementary material). Intermittent cycles of flooding and drying up favoured the development, especially at the margins of the rice parcels, of *Ae. caspius* and of the less abundant *Ae. vexans*. Where the water persisted for a longer period, immatures of *An. maculipennis* s.l., *Cx. modestus* and *Cx. pipiens* were found well hidden within the superficial layer of filamentous algae.

3b. In field drains, where stagnant water is more persistent, *An. maculipennis* s.l. (2 sites) and *Cx. modestus* (1 site) were collected, whereas *Ae. caspius* developed in a temporary drain pools subjected to water level excursions (2 sites) (Table 4 in Supplementary material).

Table 3 – Checklist of 37 Culicidae species of Friuli Venezia Giulia (north-eastern Italy). Thirty-two species were recorded by the Authors of this paper from 1983 to 2017 (not in bold); five species were reported in literature from 1932 to 2017 (in bold). The asterisk (*) in the first column indicates a taxon not identified to species level. Two asterisks (**) in the second column indicate the 10 species recorded only by the Authors of this paper. Development stages are indicated as E = egg; L = larva; P = pupa; A = adult. Description of the types of habitat (1a- 6c) is reported in Table 2. – : no pre-imaginal stages collected.

N.	Species/taxon	Stage coll.	Year of collection	Habitat of pre-imaginal stages	References
1	*Anopheles* (*Anopheles*) *atroparvus* van Thiel, 1927	A	1994-1997	–	De Faveri, 1939; Sepulcri, 1963; Zamburlini & Cargnus, 1998b, 2009
2	*Anopheles* (*Anopheles*) *claviger* (Meigen, 1804)	LPA	1994-2015	2a, 6b	De Faveri, 1939; Sepulcri, 1963; Zamburlini, 1995; Zamburlini & Cargnus, 1998c, 2009; Mancini *et al.*, 2017; this paper
3	*Anopheles* (*Anopheles*) *hyrcanus* (Pallas, 1771) (**)	L	1994	2a	Zamburlini, 1995 (current presence to be confirmed)
4	*Anopheles* (*Anopheles*) *maculipennis* s.s. Meigen, 1818	A	1994-1997	–	De Faveri, 1939; Sepulcri, 1963; Zamburlini, 1995; Zamburlini & Cargnus, 1998b, 2009
(*)	*Anopheles* (*Anopheles*) *maculipenni*s s.l. Meigen	LP	1994-2015	2b, 3a, 3b, 5a, 6b	Stammer, 1932; Zamburlini & Cargnus, 1998b, Mancini *et al.*, 2017; this paper
5	*Anopheles* (*Anopheles*) *messeae* Falleroni, 1926	A	1994-1997	–	De Faveri, 1939; Sepulcri, 1963; Zamburlini, 1995; Zamburlini & Cargnus, 1998b, 2009
6	*Anopheles* (*Anopheles*) *plumbeus* Stephens, 1828	LPA	1994-2015	2d, 6a	Zamburlini, 1995; Zamburlini & Cargnus, 2009; Mancini *et al.*, 2017; this paper
7	***Anopheles sacharovi* Favr, 1903**	**ELPA**	**1920-1940s**	**1b**	De Faveri, 1939; Sepulcri, 1963 (to be considered extinct)
8	*Aedes* (*Stegomyia*) *albopictus* (Skuse, 1894)	ELPA	1995-2017	6a	Zamburlini, 1995; Zamburlini & Frilli, 2006; Zamburlini & Cargnus, 2009, 2015; Mancini *et al.*, 2017; this paper
9	*Aedes* (*Ochlerotatus*) *annulipes* (Meigen, 1830) (**)	LPA	1994-2017	2a, 2c	Zamburlini, 1995, 1996a; Zamburlini & Cargnus, 2009; this paper
10	*Aedes* (*Ochlerotatus*) *cantans* (Meigen, 1818)	LPA	1994-2017	2a, 4a, 4b, 5a	Zamburlini, 1995; Zamburlini & Cargnus, 2009; Mancini *et al.*, 2017; this paper
11	*Aedes* (*Ochlerotatus*) *caspius* (Pallas, 1771)	LPA	1983-2017	1a, 2a, 3a, 3b	Stammer, 1932; Dechigi *et al.*, 1967; Zamburlini, 1995; Zamburlini & Cargnus, 2009; Mancini *et al.*, 2017; this paper
12	*Aedes* (*Ochlerotatus*) *cataphylla* (Dyar, 1916) (**)	LPA	1996-2014	4b, 5a	Cargnus & Zamburlini, 1999; Zamburlini & Cargnus, 2009; this paper
13	*Aedes* (*Aedes*) *cinereus* Meigen, 1818	LPA	1994-2017	2a, 2c	Dechigi *et al.*, 1967; Zamburlini, 1995; Zamburlini & Cargnus, 1998a; this paper
14	*Aedes* (*Ochlerotatus*) *communis* (De Geer, 1776) (**)	LPA	1994-2014	4a, 4b	Cargnus & Zamburlini, 1999; Zamburlini & Cargnus, 2009; this paper
15	*Aedes* (*Ochlerotatus*) *detritus* (Haliday, 1833)	L	1983-2001	1a	Dechigi *et al.*, 1967; Zamburlini, 1995; Mancini *et al.*, 2017
16	***Aedes* (*Ochlerotatus*) *dorsalis* (Meigen, 1830)**	**L**	**1932**	**1a**	Stammer, 1932 (reported as doubtful; current presence to be confirmed)
17	*Aedes* (*Aedes*) *geminus* Peus, 1970 (**)	LPA	1994-2015	2a, 4a, 4b, 5a	Zamburlini & Cargnus, 1998a; 2009; this paper
18	*Aedes* (*Dahliana*) *geniculatus* (Olivier, 1791)	ELPA	1994-2017	2d, 5b, 6a	Zamburlini, 1995; Zamburlini & Cargnus, 2009, 2015; Mancini *et al.*, 2017; this paper
19	*Aedes* (*Hulecoeteomyia*) *japonicus* (Theobald, 1901)	LPA	2016	6a	Seidel *et al.*, 2016; this paper
20	*Aedes* (*Hulecoeteomyia*) *koreicus* (Edwards, 1917)	LPA	2015-2017	6a	Montarsi *et al.*, 2015 Zamburlini & Cargnus, 2015
21	*Aedes* (*Ochlerotatus*) *pullatus* (Coquillett, 1904) (**)	LPA	1994-2015	4a, 4b	Zamburlini, 1995; Cargnus & Zamburlini, 1999, 2009; this paper

(continued)

Continued Table 3

22	*Aedes* (*Ochlerotatus*) *punctor* (Kirby, 1837) (**)	LPA	1994-2015	4b	Zamburlini & Cargnus, 2009; this paper
23	*Aedes* (*Ochlerotatus*) *sticticus* (Meigen, 1838)	LPA	1994-2017	2a, 2c, 5a	Zamburlini, 1995, 1996b; Mancini *et al*., 2017; this paper
24	*Aedes* (*Aedimorphus*) *vexans* (Meigen, 1830)	LPA	1994-2017	2a, 2c, 3a, 5a	Stammer, 1932; Dechigi *et al*., 1967; Zamburlini, 1995; Zamburlini & Cargnus, 2009; Mancini *et al*., 2017; this paper
25	*Coquillettidia* (*Coquillettidia*) *richiardii* (Ficalbi, 1889)	A	1998-2014	–	Stammer, 1932; Zamburlini, 1995; Zamburlini & Cargnus, 2009; Toma *et al*., 2013; Mancini *et al*., 2017; this paper
26	*Culex* (*Maillotia*) *hortensis* Ficalbi, 1889 (**)	LPA	1994-2015	2b, 5a, 6b	Zamburlini, 1995; this paper
27	***Culex (Neoculex) impudicus* Ficalbi, 1890**	**LPA**	**2013**	**2b**	Toma *et al*., 2013
28	***Culex (Neoculex) martinii* Medschid, 1930**	**A**	**2013**	–	Toma *et al*., 2013 (presence to be confirmed)
29	*Culex* (*Barraudius*) *modestus* Ficalbi, 1889	LPA	1994-2013	3a, 3b	Stammer, 1932; Dechigi *et al*., 1967; Zamburlini, 1995; this paper
30	*Culex* (*Culex*) *pipiens* Linnaeus, 1758	LPA	1994-2017	2b, 3a, 5a, 6a, 6b, 6c	Stammer, 1932; Dechigi *et al*., 1967; Zamburlini, 1995; Zamburlini & Cargnus 2009; Mancini *et al*., 2017; this paper
31	*Culex* (*Neoculex*) *territans* Walker, 1856	LPA	1994-2011	2b	Stammer, 1932; Dechigi *et al*., 1967; Zamburlini, 1995; Zamburlini & Cargnus, 2009; Mancini *et al*., 2017; this paper
32	*Culex* (*Culex*) *theileri* Theobald, 1903	L	1994-2013	2b, 6b	Dechigi *et al*., 1967; Zamburlini, 1995; Toma *et al*., 2013
33	*Culiseta* (*Culiseta*) *annulata* (Schrank, 1776)	LPA	1994-2017	2b, 6c	Dechigi *et al*., 1967; Zamburlini, 1995; Mancini *et al*., 2017; this paper
34	*Culiseta* (*Allotheobaldia*) *longiareolata* (Macquart, 1838) (**)	LPA	1994-2015	5b, 6a	Zamburlini & Cargnus, 2015; this paper
35	*Culiseta* (*Culicella*) *morsitans* (Theobald, 1901)	LPA	1994-2017	2a	Dechigi *et al*., 1967; Zamburlini, 1995, 1996a; Zamburlini & Cargnus, 2009; this paper
36	***Culiseta (Culiseta) subochrea* (Edwards, 1921)**	**A**	**1967**	–	Dechigi *et al*., 1967 (current presence to be confirmed)
37	*Uranotaenia* (*Pseudoficalbia*) *unguiculata* Edwards, 1913 (**)	L	1994	2b	Zamburlini, 1995 (current presence to be confirmed)

Carnic and Julian Pre-Alps and Alps

4. Wet forests and marshes

4a. In semi-permanent rainwater pools within deciduous forest of sub-mountain and mountain areas (2 sites), immature stages of *Ae. pullatus* (2 sites), *Ae. cantans, Ae. communis* and *Ae. geminus* (1 site each) were collected. Additionally, some biting adults of *Ae. pullatus* were found (1 site) (Table 4 in Supplementary material).

4b. In flooded meadows and marshes of mountain areas, larvae of six species were detected (5 sites) (Table 4 in Supplementary material). Only *Ae. pullatus* was found in all sites, whereas *Ae. communis*, *Ae. geminus*, *Ae. punctor*, *Ae. cantans* and *Ae. cataphylla* were less frequent. *Ae. pullatus* was found in marshes flooded by snow melting water at higher altitudes (1570 m a.s.l.). Biting females of *Ae. geniculatus* were also captured (Table 4 in Supplementary material).

Karst plateau

5. Karst woods and marshes

5a. In shaded semi-permanent and permanent ponds and artificial containers, immatures stages of eight mosquito species were collected (7 sites) (Table 4 in Supplementary material). *An. maculipennis* s.l. (2 sites), *Cx. hortensis* (1 site) and *Cx. pipiens* (1 site) were detected in natural or artificial (cisterns and animal troughs) permanent water bodies, while *Ae. cantans* (2 sites), *Ae. sticticus* (3 sites), *Ae. vexans* (2 sites), *Ae. cataphylla* (1 site) and *Ae. geminus* (1 site) were found in temporary or semi-permanent water bodies subjected to level excursions at the margins of ponds, pools and swamps.

5b. In tree-holes and karst rock-holes filled with rainwater (3 sites), rich in decaying leaves and superficial microalgae, larvae of *Ae. geniculatus* and *Cs. longiareolata* (Macquart) (3 and 1 site, respectively) were collected (Table 4 in Supplementary material).

Whole region

6. Urban areas

6a. In natural and artificial small containers of eutrophic water (11 sites), larvae of eight species were collected (Table 4 in Supplementary material). *Ae. albopictus* (9 sites) was almost always detected, together with *Cx. pipiens* (8 sites) and with *Cs. longiareolata* (2 sites). The exotic *Ae.*

koreicus and *Ae. japonicus* (2 and 1 site, respectively) were also sampled. *An. plumbeus* (3 sites) and *Ae. geniculatus* (1 site) were collected in tree holes. Moreover, some adults of *Ae. albopictus* were sampled in one touristic site situated at 907 m a.s.l. (site N. 26) but no larvae were found in the surroundings.

6b. In shaded containers filled with clear rainwater (3 sites) larvae of *Cx. hortensis* (3 sites), *Cx. pipiens* (2 sites), *An. claviger*, *An. maculipennis* s.l. and *Cx. theileri* (1 site) were collected (Table 4 in Supplementary material).

6c. In large and permanent polluted water basins such as ditches rich in organic matter (3 sites), *Cx. pipiens* constantly occurred, sometimes found together with *Cs. annulata* (2 sites) (Table 4 in Supplementary material).

CONCLUSIONS

From a zoo-geographic point of view, all the native mosquito species recorded in FVG have a wide distribution in Europe (BECKER *et al*., 2010) and are already reported for other Italian regions (SEVERINI *et al*., 2009), except *Ae. geminus,* which is mainly distributed in central and western Europe and currently recorded only in FVG (ZAMBURLINI & CARGNUS, 1998a).

Many species recorded in FVG have a prevailing central-northern distribution in Europe. Among these, *Ae. annulipes*, *Ae. cantans* and *Ae. sticticus* are reported from Veneto (MANCINI *et al*., 2017), and the latter two species also from Piedmont (SABATINI *et al*., 1981) and Tuscany (BALDACCINI & GIANCHECCHI, 1989), respectively. *Ae. cataphylla, Ae. communis* and *Ae. pullatus* were found first in the Apennines at high altitudes (COLUZZI & COLUZZI, 1967). More recently, *Ae. cataphylla* and *Ae. pullatus* were found also in Piedmont (TOMA *et al*., 2013), and *Ae. communis* in Lazio (DE LIBERATO *et al*., 2015). On the contrary, *Cx. impudicus* and *Cx. theileri* have a prevailing Mediterranean gravitation and reach in FVG the northern limit of their European distribution.

After the spread of the Asian tiger mosquito, in the last years two new exotic species were introduced in FVG: *Ae. japonicus*, which is establish in the nearby Carinthia region (Austria) (SEIDEL *et al*., 2016), and *Ae. koreicus* which is spreading in the Italian regions adjacent to FVG (MONTARSI *et al*., 2015) and in north-eastern Slovenia (KALAN *et al*., 2017).

Further research, especially conducted in the mountain areas less investigated until now, will probably and hopefully enrich the mosquito inventory of FVG, in particular with species documented in the nearby Veneto region, such as in the far past *Ae. zammitii* (Theobald, 1903) [(sub *Ae. mariae* (Sergent & Sergent, 1903)] and *Cs. litorea* (Shute, 1928) (DECHIGI *et al*., 1967), and more recently *Ae. berlandi* (Seguy) (MANCINI *et al*., 2017), or detected in other Italian regions (SEVERINI *et al*., 2009), in the nearby Slovenia (SNOW & RAMSDALE, 1999), in Croatian Istria (MERDIĆ *et al*., 2008) and in Austria (ZITTRA *et al*., 2017).

Many of the mosquitoes present in FVG, both native or exotic species, have a great health concern as potential vectors of pathogens for humans and animals and they are object of an intensive surveillance (ZAMBURLINI & FRILLI, 2006; MANCINI *et al*., 2017). The study of the density and distribution of potential malaria vectors is still an important medical issue which needs to be updated, as the data here presented date back to the end of 1990s (ZAMBURLINI & CARGNUS, 1998b).

From a conservation perspective, the mosquito fauna represents in many natural ecosystems a precious resource of biodiversity, not always to be hasty eliminated but attentively controlled. Furthermore, the natural breeding sites of mosquitoes, and annexed fauna, need a particular protection since they have been greatly reduced in the last decades and are often at risk of disappearance, especially in the Friulian plains because of agriculture and urbanization expansion.

ACKNOWLEDGMENTS

We would like to thank dr. Roberto Romi and dr. Luciano Toma (Istituto Superiore di Sanità, Roma) for useful suggestions and critical review of a preliminary version of the manuscript.

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REDIA, 102, 2019: 23-26 http://dx.doi.org/10.19263/REDIA-102.19.03

PASQUALE TREMATERRA[a]

DESCRIPTION OF *COCHYLIMORPHA SCALERCIANA* SP. N. (LEPIDOPTERA, TORTRICIDAE) FROM CALABRIA (ITALY)

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Trematerra P. – Description of *Cochylimorpha scalerciana* sp.n. (Lepidoptera Tortricidae) from Calabria (Italy).

Cochylimorpha scalerciana sp. n. is described as new, the latter belongs to *straminea* group. As adult *C. scalerciana* resemble *C. tiraculana* from West-Alps, genetically it is near *C. straminea* and *C. alternana*.

Key Words: *Cochylimorpha scalerciana,* Lepidoptera Tortricidae, Calabria, Southern Italy, new species.

Over time, the Lepidoptera Tortricidae from the Calabria territory (Southern Italy) has received sporadic attention by both local and foreign entomologists. The only specific works refer to some catches taken on the southern slope of the Pollino Massif by Trematerra (1991) and in a chestnut area of the Sila Mountains by Trematerra *et al*. (1994). In a recent paper Trematerra *et al.* (2018) reported a tortricid list as updated and complete as possible for this region; up to a total of 181 taxa of the family Tortricidae, species belonging to all the tribes cited in the Italian fauna (Trematerra, 2003) have been found, with the exception of the members of the Sparganothini tribe.

During an expedition of 2015 by the entomologists dr Stefano Scalercio and dr Marco Infusino, few specimens of a new species belonging to genus *Cochylimorpha* Razowski, 1959 were collected. It is here described as new to science (Fig. I). The material was primarily identified morphologically, and supplemented by molecular data of the COI barcode region according to Hajibabaei *et al.* (2006) and Ratnasingham & Hebert (2007).

The Genus *Cochylimorpha* includes 83 palaearctic species (Razowski, 2009); 36 species have been recorded from Europe (Aarvik, 2013) and 14 species from Italy (Trematerra 2003).

Cochylimorpha scalerciana sp. n.

Material examined – Holotype 1 male, Calabria, Sila, Vivaio Sbanditi (Cosenza), 1351 m, 25.V.2015, leg. Scalercio and Infusino. Paratypes 2 males, Calabria, Sila, Vivaio Sbanditi (Cosenza), 1351 m, 18.V.2015, leg. Scalercio and Infusino; 1 male, Calabria, Sila, Montagna Grande (Cosenza), 1355 m, 18.V.2015, leg. Scalercio and Infusino; 1 male, Calabria, Colle Macchie, Pedace (Cosenza), 1450 m, 18.V.2015, leg. Scalercio and Infusino. Materials are deposited in the Trematerra Collection (University of Molise, Campobasso, Italy).

Description – Adult (Fig. II, 1). Wing span 18-20 mm (male). Head and palps whitish-cream; torax silvery grey sprinkled with yellowish scales; abdomen dark silvery grey. Ground colour of forewing silvery whitish cream, suffused and densely strigulated with yellowish-cream; cilia cream or pale whitish-cream. Hindwing greyish; cilia whitish-cream with brownish-grey basal line.

Male genitalia (Fig. II, 2-4) – Socii fused small; tegumen large and sclerotized; valva rather slender, convex caudally, well sclerotized above sacculus; sacculus simple, well slerotized without any angulation ventro-terminally; median part of transtilla broad, more or less concave, apically weakly spined; vinculum less developed; juxtae sub-trapezoidal; aedeagus elongated and robust, curved, sharp terminally; cornutus rather strong one fourth of aedeagus.

Female genitalia – Unknown.

Early stages – Unknown.

Biology - Moth collected on 18-25 May 2015 up to 1351 m a.s.l. (in Calabria, Southern Italy). Sampling localities are comprised between 1351 and 1450 metres of altitude, all included within the Sila National Park. At Vivaio Sbanditi and Colle Macchie the species was found in conifers woodlots, the former mainly composed by *Abies alba* Mill. with *Fagus sylvatica* L. and *Castanea sativa* Mill. as accessory species, and the latter in a pure stand of *Pinus nigra* ssp. *calabrica* (Loudon) Cesca & Peruzzi. The locality of Montagna Grande is covered by shrubs with *Sarothamnus scoparius* (L.) W.D.J. Koch and *Rubus* sp. as dominant elements.

Distribution – Southern Italy (in Calabria Region), on Sila Mountains.

Etymology – The new *Cochylimorpha* is dedicated to my colleague dr Stefano Scalercio (Rende, Cosenza, Italy) who contributed to collect the material studied.

DNA barcoding analyses – Two specimens were submitted to DNA barcoding (specimen ID: LEP-SS-830; LEP-SS-831). Voucher material is stored at the University of Molise, Department of Agricultural, Environmental and Food Sciences, Campobasso (Italy).

– Received 24 October 2018 Accepted 15 February 2019

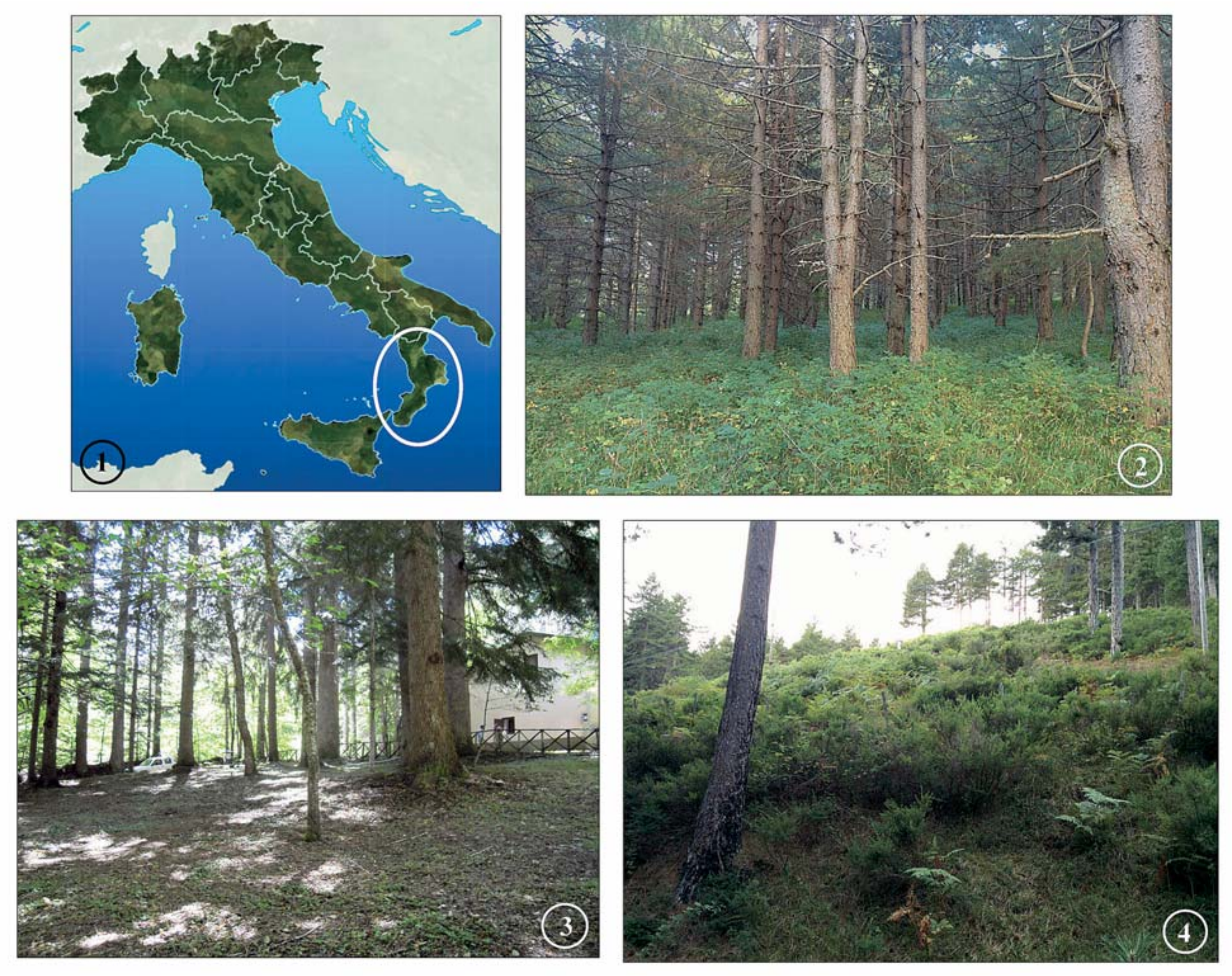

Fig. I – Calabrian territory (Southern Italy) (1), habitats frequented by *Cochylimorpha scalerciana* sp. n.: Colle Macchie, Pedace (Cosenza), 1450 m a.s.l. (2); Sila, Vivaio Sbanditi (Cosenza), 1351 m a.s.l. (3); Sila, Montagna Grande (Cosenza), 1355 m a.s.l. (4).

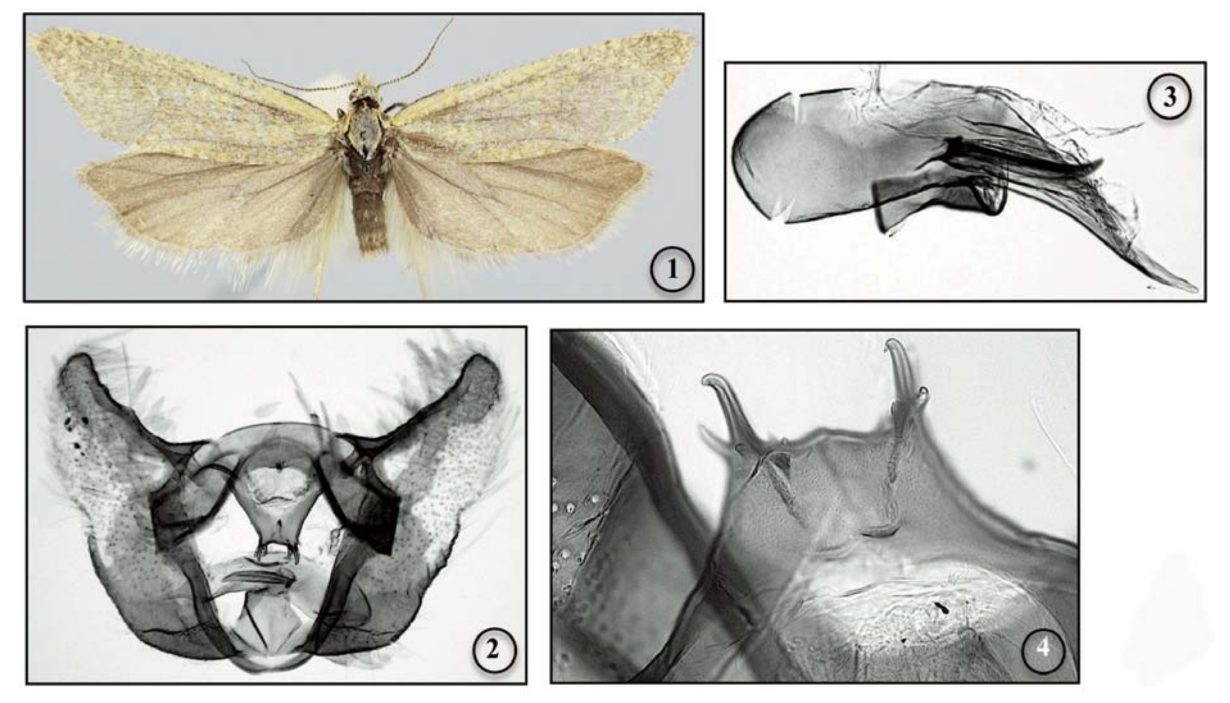

Fig. II – *Cochylimorpha scalerciana* sp. n.: adult male (1); male genitalia (2); aedeagus (3); central part of transtillae (4).

A small piece of a leg was transferred to a lysis plate under ca 0.3 ml 100% alcohol. Tissue samples were submitted to the standard procedures of the Canadian Centre for DNA Barcoding (CCDB) for sequencing the mitochondrial 5’ cytochrome oxidase gene, subunit 1 (COI), the standard marker for the identification of most animals. LepF1 and LepR1 were the primers used for PCR and sequencing (Hajibabaei *et al*., 2006). Sequences were blasted against the complete sequence database of BOLD data systems (Ratnasingham and Hebert, 2007) in order to investigate the closest matches using the BOLD Identification Engine (http://www.boldsystems.org/index.php/ IDS_OpenIdEngine). Sequences having no matches on BOLD were blasted against the complete sequence database of GenBank (Hajibabaei *et al*., 2006; Ratnasingham and Hebert, 2007). Genetic distances between sequences were measured using minimum pairwise distance and Neighbour joining tree was built using Kimura 2-parameter distance model for COI-5P marker.

A target 658-bp fragment of COI was aplified only from LEP-SS-831, as follow:
AACATTATACTTTATTTTTGGAATTTGAGCAGG
AATAGTAGGAACTTCTTTAAGTTTACTAATCCGT
GCTGAATTGGGAAATCCTGGATCTCTTATTGGA
GATGATCAAATTTATAATACTATTGTTACAGCACAT
GCATTTATTATAATTTTCTTTATAGTTATACCAAT
TATAATTGGAGGATTTGGAAATTGACTAGTACCTT
TAATATTAGGAGCGCCTGATATAGCTTTCCCCCG
TATAAATAATATAAGATTCTGGTTATTACCACCTTC
TATTATACTTCTAATCTCTAGAAGAATTGTA
GAAAATGGAGCAGGAACTGGATGAACGGTGTAC
CCCCCACTTTCATCTAATATTGCCCATAGTGGAA
GATCAGTAGACTTAGCAATCTTCTCTTTACATT
TAGCTGGAATTTCATCTATTTTAGGAGCTG
TAAATTTTATCACCACTATTATTAATATACGACC
TAATAATATAAGATTAGATCAAATACCTCTTTTTG
TATGAGCTGTAGGAATTACAGCTCTATTATTATTAT
TATCTCTTCCAGTTTTAGCGGGAGCTATTACTATAT
AACAGATCGTAATTTAAATACCTCATTTTTTGACC
CTGCTGGAGGGGGAGATCCTATTTTATATCAA
CATTTATTT

From BOLD it was not possible observe similarities. According to database of GenBank closer species, with 7% of genetic distances, are *Cochylimorpha straminea* (Haworth, 1811) Identifiers: TLMF Lep 08445 [sampleid], Depository: inatura, Dornbirn Collected in: Austria, Vorarlberg; GenBank accession number KP253321.1 and *Cochylimorpha alternana* (Stephens, 1834), Identifiers: MM22625 [sampleid], Depository: University of Helsinki Collected in: Finland, Nylandia; GenBank accession number KX049789. Barcode of *C. tiraculana* is unknown.

Comments

The new species *C. scalerciana* belongs to *straminea* group. As adult *C. scalerciana* resemble *C. tiraculana* (Bassi & Scaramozzino, 1989) from Western Alps. *C. scalerciana* differs clearly from *C. straminea* (Fig. IV) and *C. alternana* (Fig. V) both in the external morphology of wings and in the male genitalia (Razowski, 1970 and 2009).

The adult of *C. scalerciana* is closely related with *C. tiraculana* (Fig. III, 1-4); the latter is externally distinct by dense strigulation of forewing that are silvery cream suffused and densely strigulated with ochreous cream, in *C. scalerciana* wings are silvery whitish-cream suffused and densely strigulated with yellowish-cream; male genitalia differ from those of *C. tiraculana* mainly in shape of valvae and in aedeagus. In *tiraculana* valva is broad and convex caudally with sacculus simple (Fig. III, 2-4), in *scalerciana* valva is rather slender, convex caudally and well sclerotized above sacculus (Fig. II, 2-4). Aedeagus is similar to *C. sparsana* (Staudinger, 1879).

C. tiraculana type localities are high alpine meadows in

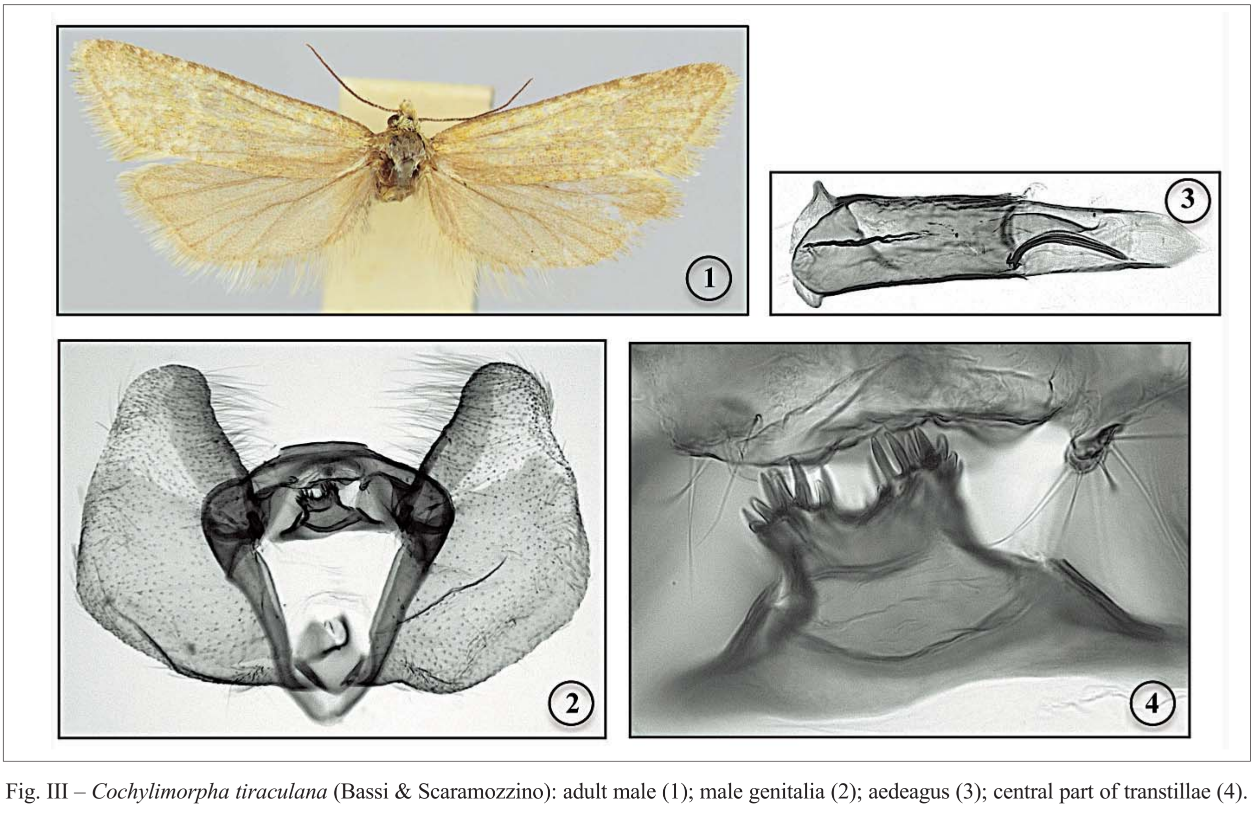

Fig. III – *Cochylimorpha tiraculana* (Bassi & Scaramozzino): adult male (1); male genitalia (2); aedeagus (3); central part of transtillae (4).

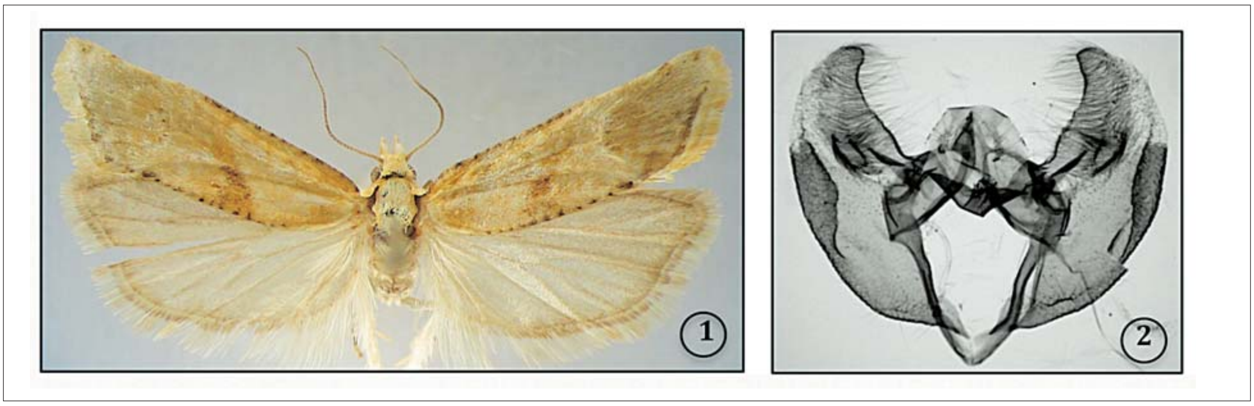

Fig. IV – *Cochylimorpha straminea* (Haworth, 1811): adult male (1); male genitalia (2).

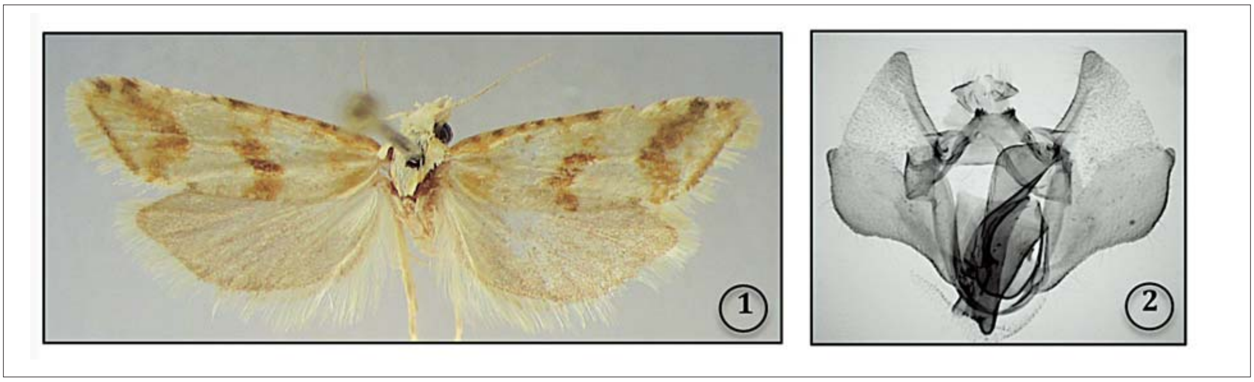

Fig. V – *Cochylimorpha alternana* (Stephens, 1834): adults male; male genitalia (2).

the border massifs between Italy and France (Italian valleys of Susa and Viù and French Hautes Alpes), and it is also reported for Alps of Switzerland (AARVIK, 2013), while *C. scalerciana* is only present in the Sila mountains (Calabria, Italy) in the Southern Apennines.

ACKNOWLEDGEMENTS

I am grateful to dr Stefano Scalercio and dr Marco Infusino (Consiglio per la Ricerca in agricoltura e l'analisi dell'Economia Agraria, Rende, Cosenza, Italy) for giving the material in study, information, comments and helpful suggestions. The investigation was funded as part of the "Monitoring Project for Nocturnal Lepidoptera through the use of Rothamsted light traps", by the Sila National Park at the Research Center for Forestry and Wood in Rende, and of the Project "ALForLab" (PON03PE_00024_1) co-funded by the National Operational Programme for Research and Competitiveness (PON R&C) 2007-2013, through the European Regional Development Fund and National Resource [Revolving Fund - Cohesion Action Plan, MIUR].

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REDIA, 102, 2019: 27-34 http://dx.doi.org/10.19263/REDIA-102.19.04

ARNALDO BORDONI [a] - JOHN T. NUNN [b]

DESCRIPTION OF THE MATURE LARVA OF *WAITATIA MAORIANA* BORDONI, 2005 (COLEOPTERA STAPHYLINIDAE STAPHYLININAE XANTHOLININI) FROM NEW ZEALAND (*)

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(*) 279 th Contribution to the knowledge of the Staphylinidae.
Corresponding Author: Arnaldo Bordoni; arnaldo.bordoni@fastwebnet.it

Bordoni A., Nunn J. – Description of the mature larva of *Waitatia maoriana* Bordoni, 2005 (Coleoptera Staphylinidae Staphylininae Xantholinini) from New Zealand.

The larva of *Waitatia maoriana* Bordoni, 2005 from New Zealand is described and figured for the first time.

KEY WORDS: Rove-beetles, staphylinid, larval morphology, immature, preimaginal, stages, external structure.

INTRODUCTION

The genus *Waitatia* was described (BORDONI, 2005) for two species from New Zealand (*W. bellicosa* Bordoni, 2005 and *W. maoriana* Bordoni, 2005) with distribution in both the North and the South Islands (Fig. I, 1-2). *Waitatia* is a very distinctive genus in the following characters: massive aspect and length of the body (10-14 mm long), robust cylindrical head, with a wide neck, maxillary and labial palpi, labrum only emarginated in the middle, tarsi anteriorly dilated, upper epipleural line of the pronotum not joining with the lower line, and genitalia of male and female. In its original description *Waitatia* has been compared with *Zeteotomus* Jaquelin du Val, 1857 but, in fact, it is morphologically unique among known Xantholinini.

Published descriptions of larvae of Xantholinini by REY (1887) and PAULIAN (1941) are not detailed and some of doubtful identity. Later, POTOTZKAYA (1967) and KASULE (1970) contributed more detailed descriptions and TOPP (1978) proposed a key for the determination of Staphylinidae laevae.

In particular PAULIAN (1941) schematically described *Leptacinus batychrus* (Gyllenhal, 1827), *Xanthophius serpentarius* Motschulsky, 1859 from North India (sub *Leptacinus;* wrong citation because the species is known only from Ceylon), *Metolinus leucocnemis* (Kraatz, 1859) (wrong citation because this larva is listed as collected in Bengal but *M. leucocnemis* occurs only in Ceylon), *Nudobius lentus* (Gravenhorst, 1806) from Europe and *N. cephalus* (Say, 1834) (sub *cephalotes*) from Arizona, *N. cavicola* (Cameron, 1938) (sub *Xantholinus*), *N. jeanneli* (Bernhauer, 1939) (sub *Xantholinus*) from Africa, *Thyreocephalus anachoreta* (Erichson, 1839) from North India (probably *T. jocheni* Bordoni, 2002, because the type of *T. anachoreta* was not found and the specimens related to this name was attributed to five species [BORDONI, 2002]), *Xantholinus elegans* (Olivier, 1795) (sub *meridionalis*) from France.

In the seventies of the last century POTOTZKAYA (1967) described larvae of the following species: *Leptacinus batychrus (*Gyllenhal, 1827), *Xantholinus maykopensis* Coiffait, 1966, *X. tricolor* (Fabricius, 1787), *X. laevigatus* Jacobson, 1849, *X. longiventris* Heer, 1839, *Gyrohypnus ochripennis* (Eppelsheim, 1892), *G. angustatus* Stephens, 1833, *G. punctulatus* (Paykul, 1789), and *Nudobius lentus* (Gravenhorst, 1806).

KASULE (1970) studied larvae of the following species: *Xantholinus glabratus* (Gravenhorst, 1802), *X. linearis* (Olivier, 1795) (sub Gravenhorst!), *Leptacinus formicetorum* Märkel, 1841 (not described and figured), *Nudobius lentus* (Gravenhorst, 1806).

The only recent descriptions are related to *Phacophallus pallidipennis* (Motschulsky, 1858) from Philippines (BORDONI, 2002) and some larvae from New Zealand [*Whangareiella fulvipes* (Broun, 1880), *Neoxantholinus brouni* (Sharp, 1876), *N. pseudorufulus* Bordoni, 2005, *Pseudocorynus mediocris* (Broun, 1880), *P. nelsonianus* Bordoni, 2005, *P. cultus* (Broun, 1880), and *Linosomus socius* (Fauvel, 1877)] (BORDONI, 2005). Recently PIETRYKOWSKA-TUDRUJ & STANIEC (2006) published the morphology of the developmental stages of *Hypnogyra angularis* (Ganglbauer, 1895) (see all species details in Table 1).

MATERIAL AND METHODS

The examined larva was obtained *ex societate imaginis* (2 females in the authors collections) by splitting the bole of a dead *Pseudopanax* tree from Leith Saddle, near Dunedin, Otago (J. Nunn 19.III.2015). The larvae was preserved in a solution of glycerine and alcohol. For microscopic, permanent slides the larvae were rinsed in distilled water and cleared in KOH 10%. The drawings of parts of the body were made using light microscopy (Optika B-290) and the habitus illustrations were prepared from photograph taken with Canon EOS 700 camera.

BIONOMICS

The *Pseudopanax* tree bole was riddled with the tunnels of wood-boring cryptorhynchine weevils and contained adults

– Received 10 October 2018 Accepted 6 March 2019

Fig. I – Habitus of *Waitatia maoriana* Bordoni, 2005 (1) (total length: 10.5 mm) and *Waitatia bellicosa* Bordoni, 2005 (2) (total length: 14 mm).

and larvae of *Psepholax* and *Mesoreda* species (cfr Broun, 1893), plus adults and possibly larvae of *Mecistostylus douei* Lacordaire, 1866. It seems likely that the larvae of *Waitatia* prey on the larvae of these wood-boring weevils. Adults of *W. maoriana* have been found by J. N. close the same locality under bark of a dead *Pseudopanax* tree and freely on a low forest vegetation. It is worthwhile to record that a series of adults of *Pseudoxantholinus sharpi* (Broun, 1880) were found by J. N. in a tree stump on Tinakori Hill (Wellington), which was also affected by beetles that made tunnels of larger diameter in the wood. These observations allow assume that species of both of these genera live in the tunnels of dead plants hunting for other larvae.

Pseudopanax is a small genus of 12-20 species of evergreen plants, the majority of which are endemic to New Zealand, although they also occur in Tasmania (Australia) and some plants from South America have at times been included in this genus. *Pseudopanax arboreus* (common name “five-finger”) is a very common small tree in New Zealand native forests.

Table 1 – Larval descriptions of species of Xantholinini.

Species	References	Notes
Phacophallus parumpunctatus (Gyllenhal, 1827)	Rey (1887)	Uncertain identification
Leptacinus batychrus (Gyllenhal, 1827)	Paulian (1941)	Schematic description, uncertain identification
Xanthophius serpentarius Motschulsky, 1859 (sub *Leptacinus*)	Paulian (1941)	Schematic description, uncertain identification, incorrect locality
Metolinus leucocnemis (Kraatz, 1859)	Paulian (1941)	Schematic description, uncertain identification, incorrect locality
Nudobius lentus (Gravenhorst, 1806)	Paulian (1941)	Schematic description, from Europe
Nudobius cephalus (Say, 1834) (sub *cephalotes*)	Paulian (1941)	Schematic description, from Arizona, U.S.A.
Nudobius (Xantholinus) cavicola (Cameron, 1938) (sub *Xantholinus*)	Paulian (1941)	Schematic description, from Africa
Nudobius (Xantholinus) jeanneli (Bernhauer, 1939) (sub *Xantholinus*)	Paulian (1941)	Schematic description, from Africa
Thyreocephalus anachoreta (Erichson, 1839)	Paulian (1941), Bordoni (2002)	From North India, but probably Thyreocephalus jocheni Bordoni, 2002
Xantholinus elegans (Olivier, 1795) (sub *meridionalis*)	Paulian (1941)	Schematic description, from France
Leptacinus batychrus (Gyllenhal, 1827)	Pototzkaya (1967)	Description lacks many details
Xantholinus maykopensis Coiffait, 1966	Pototzkaya (1967)	Description lacks many details
Xantholinus tricolor (Fabricius, 1787)	Pototzkaya (1967)	Description lacks many details
Xantholinus laevigatus Jacobson, 1849	Pototzkaya (1967)	Description lacks many details
Xantholinus longiventris Heer, 1839	Pototzkaya (1967)	Description lacks many details
Gyrohypnus ochripennis (Eppelsheim, 1892)	Pototzkaya (1967)	Description lacks many details
Gyrohypnus angustatus Stephens, 1833	Pototzkaya (1967)	Description lacks many details
Gyrohypnus punctulatus (Paykul, 1789)	Pototzkaya (1967)	Description lacks many details
Nudobius lentus (Gravenhorst, 1806)	Pototzkaya (1967), Kasule (1970)	Descriptions lack many details From UK (almost alone head)
Xantholinus glabratus (Gravenhorst, 1802) (now *Megalinus*)	Kasule (1970)	Uncertain identification (almost alone head)
Xantholinus linearis (Olivier, 1795) (sub Gravenhorst)	Kasule (1970)	Misidentification (almost alone head)
Leptacinus formicetorum Märkel, 1841	Kasule (1970)	Cited, but not described or figured
Phacophallus pallidipennis (Motschulsky, 1858)	Bordoni (2002)	From Philippines
Pachycorynus minimus (Bernhauer, 1920) *Australinus cyaneipennis* (McLeay, 1873) *Thyreocephalus margaretae* Bordoni 2005 *Grevillia subtropicalis* Bordoni, 2005	Bordoni (2005) Bordoni (2005) Bordoni (2005) Bordoni (2005)	From Australia, schematic description From Australia, schematic description From Australia, schematic description From Australia, schematic description
Whangareiella fulvipes (Broun, 1880)	Bordoni (2005)	From New Zealand
Neoxantholinus brouni (Sharp, 1876)	Bordoni (2005)	From New Zealand
Neoxantholinus pseudorufulus Bordoni, 2005	Bordoni (2005)	From New Zealand
Pseudocorynus mediocris (Broun, 1880)	Bordoni (2005)	From New Zealand
Pseudocorynus nelsonianus Bordoni, 2005	Bordoni (2005)	From New Zealand
Pseudocorynus cultus (Broun, 1880)	Bordoni (2005)	From New Zealand
Linosomus socius (Fauvel, 1877)	Bordoni (2005)	From New Zealand
Hypnogyra angularis (Ganglbauer, 1895)	Pietrykowska-Tudruj & Staniec (2006)	From Poland
Neoxantholinus koghianus Bordoni & Staniec, 2018	Bordoni & Staniec, 2018	From New Caledonia

DESCRIPTION

Mature larva. Body length (from anterior margin of nasale to the end of pygopod): 12 mm; head length: 1.48 mm; head width, oculi level: 1.11 mm; pronotum length: 1 mm; pronotum width, anterior angles level: 1.11 mm. Body cylindrical (Fig. II, 1-2). Head and pronotum sclerotized (Fig. III, 1-4), brown, abdominal sclerites feebly yellowish; legs light yellow, mandibles dark brown; setae light brown. Body elongated, prothorax as wide as head; pro- meso- and metathorax gradually wider; abdomen with widened segments IV-V and the others slightly narrowed. Setae of head and thorax, most of setae of abdominal sternite I, longest setae of abdominal tergite II-IV, some microsetae on other tergites and urogonphi are simple. Remaining macro and micro setae of abdominal segments I-X are rod-shaped and frayed.

Head (Fig. II, 2) longer than wider with sides almost rectilinear, slightly narrower forward. Surface almost shiny and without micro-sculpture. Epicranial suture very evident, bifurcate long before the anterior glands. Chetotaxy of dorsal side of head with 20 setae and 6 microsetae; posterior part with 4 microsetae on the two ends. Ventral surface of head with the sculpture as in Fig. III, 2, with only 10 setae and 2 tentorial pits (tp). Apotome (Fig. III, 2) with 4 short setae. Nasale (Fig. III, 1) with 6 setae, 2 gland pits (gp) and 2 olfactory organs (og). Anterior margin of nasale with 9 teeth divided by a median tooth in 2 distinct cluster of 4 teeth. The paramedian teeth about twice as long as median tooth; the teeth of the external cluster less developed, apart

Fig. II – Larva of *Waitatia maoriana* Bordoni (1), with particular of head and pronotum (2).

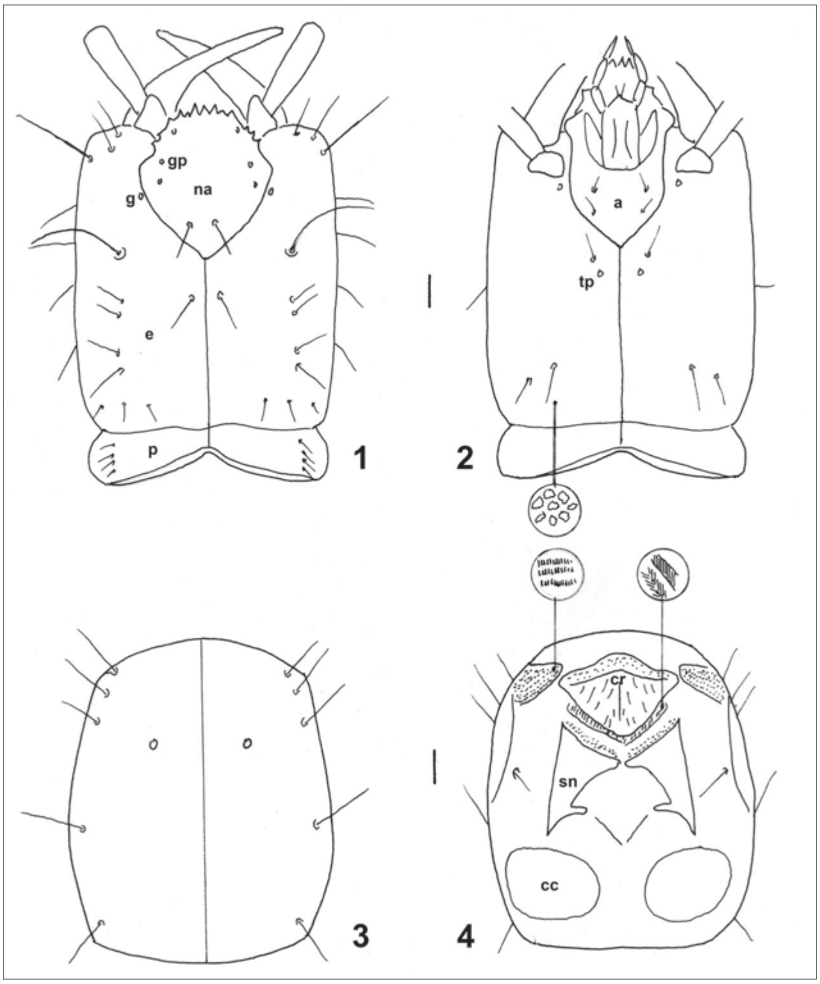


Fig. III – *Waitatia maoriana*, larva: head in dorsal view (1) (na- nasale, e- epicranial part, gp- glandular pit, g- gland, p- posterior part, head in ventral view (2) (a- apotome, tp- tentorial pit), pronotum in dorsal view (3), prothorax in ventral view (4) (cr- cervicosterum, sn- sternite, cc- coxal cavity).

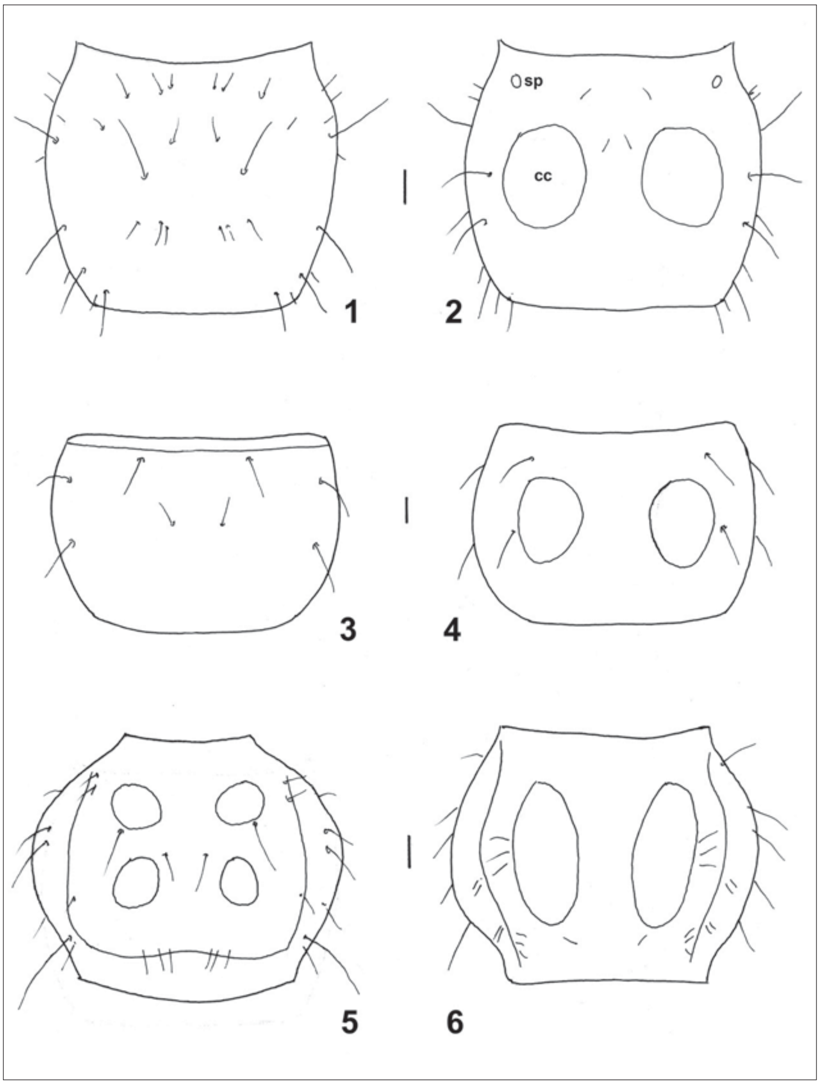


Fig. IV – *Waitatia maoriana*, larva: mesothorax in dorsal and ventral view (1-2) (sp- spiracle, cc- coxal cavity), metathorax in dorsal and ventral view (3-4), VII abdominal segment in dorsal and ventral view (5-6).

from the penultimate that is prominent. Epipharynx in the shape of half moon.

Antennae broken, with only the first three segments visible (Fig. V, 5). Mandible symmetrical (Fig. V, 4) slender, 0.55 mm long, with 2 setae on outer margin. Maxilla (Fig. V, 6) partially damaged and partly rebuilt, 0.37 mm long, with cardo (cd) shorter than stipes (st), about 1.3 times as long as wide; stipes gradually narrowed to apical portion, about 2.2 times as long as wide, with 5 setae. Mala short, with 2 apical setae; palpifer (pf) very short; maxillary palp 4-segmented gradually shorter, with few setae. Labium (Fig. V, 7) slightly dilated forwards; 0.18 mm long; prementum (pmn) with two rounded lines and 2 anterior setae. Ligula conical (lg), with 2 short setae. Labial palp 3-segmented, the second article longer than the first, the last short.

Forelegs on ventral view (Fig. VI, 1): coxa (cx) 0.59 mm long, with 6 setae, 4 microsetae and few pores; trochanter (tr) 0.33 mm long, with only 4 setae and 2 microsetae; femur (fe) 0.51 mm long, with 8 spine-shaped setae of different length and 4 microsetae; tibia (tb) 0.38 mm long, with 13 spine-shaped setae of different length and 3 microsetae and with 3 micro cuticular process at the apex; tarsungulus (tu) 0.26 mm long, with 3 short spine-shaped setae. (Fig. VI, 1-2)

Median and posterior legs in ventral view (Fig. VI, 3): coxa 0.59 mm long, with only 4 setae; trochanter 0.27 mm long, with 3 setae and 1 spine; femur 0.48 mm long, with 4 short and 1 more long spine-shaped setae; tibia 0.36 mm long, with short spine-shaped setae; anterior margin of the apex with 10 micro cuticular processes (Fig. VI, 4); tarsungulus very short, 0.11 mm long, with 3 setae.

Prothorax in dorsal view subrectangular (Fig. III, 4), 1 mm long and 1.11 mm wide, with 10 setae and 2 pores; the same in ventral view with only 2 setae near the sternites (Fig. III, 4); cervicosternum (cr) sub-triangular, largely rounded posteriad, with median incision; surface with 16 microsetae; prosternal area with 2 triangular sternites (sn); coxal cavity (cc) sub-rounded. The anterior edge surface of prothorax and surface between cervicosternum and prosternal area covered respectively by very short, microprocess and extremely fine and very dense, very tiny spinulae.

Pro- meso- and metanotum of thorax with mid-longitudinal ecdysial line.

Mesothorax in dorsal view (Fig. IV, 1) 0.81 mm long and 1.22 mm wide, with 14 setae and 20 microsetae; the same in ventral view (Fig. IV, 2) with coxal cavities (cc), 10 setae and 2 spiracles (sp).

Metathorax (Fig. IV, 3-4) in dorsal view 0.92 mm long and 1.48 mm wide, with 8 setae; in ventral view it presents 4 setae and coxal cavities.

Abdominal segments I-VIII each with tergite, sternite, a pair of paratergites and a pair of parasternites laterally; tergites and sternites I-VIII divided by membranous area

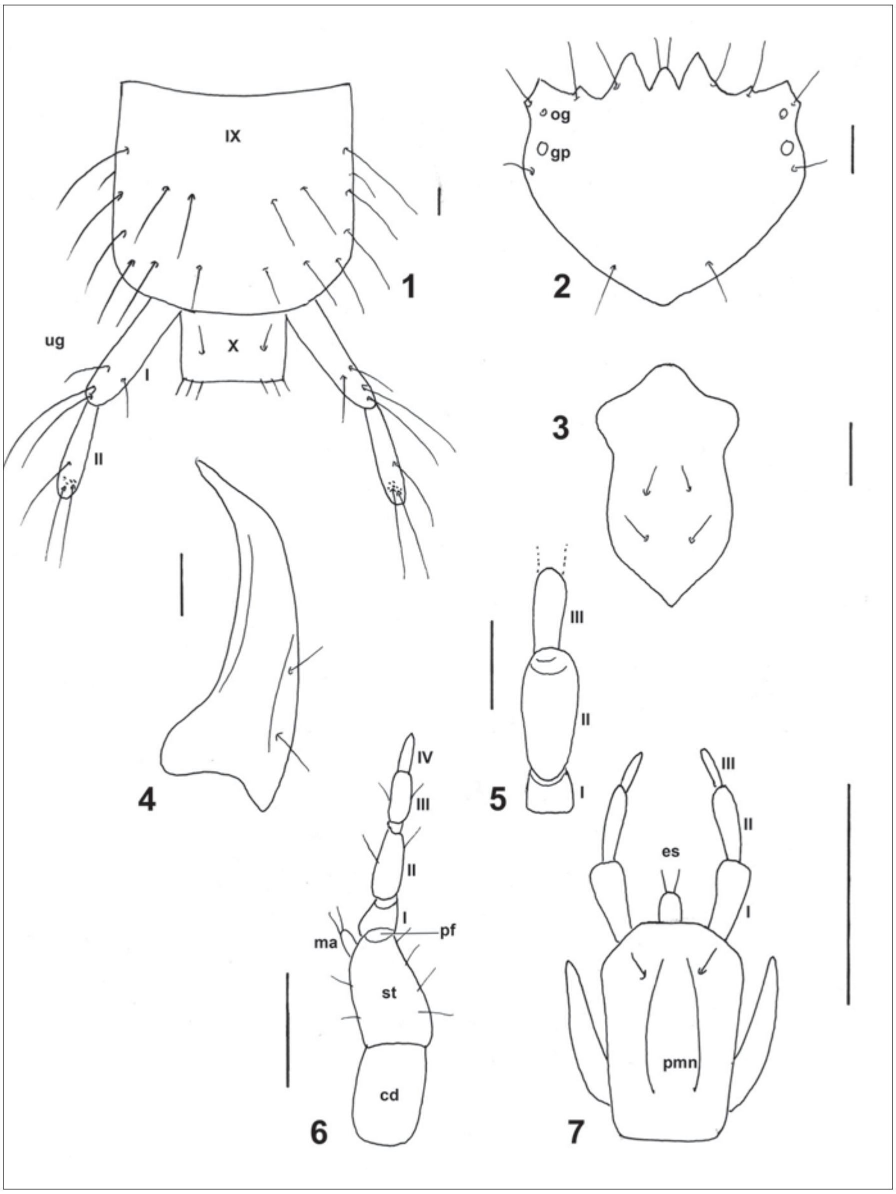
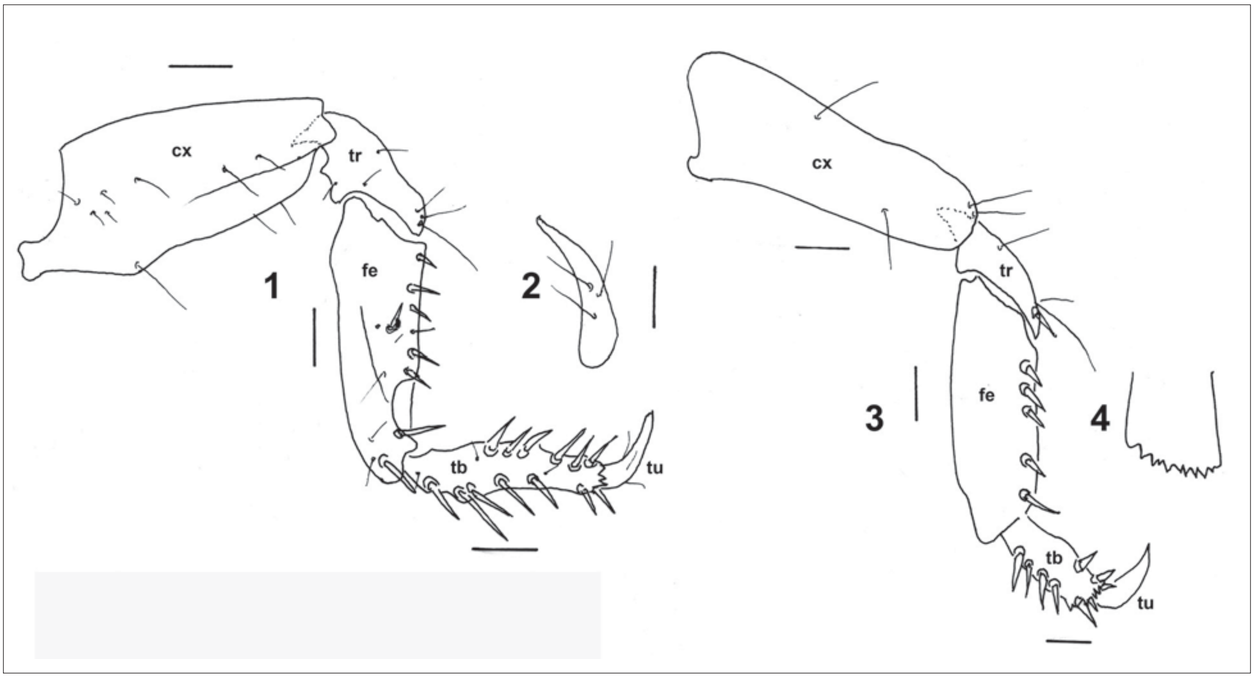


Fig. V – *Waitatia maoriana*, larva: IX-X segments (1) (ug- urogomphi, I-II- first and second segments), nasale in dorsal view (2) (og- olfactory organ, gp- glandular pit), apotome (3), right mandible (4), antenna (5), right maxilla (6) (cd- cardo, st- stpes, pf- palpifer, ma- mala), labium in ventral view (7) (pmn- prementum, lg- ligula).

Fig. VI – *Waitatia maoriana*, larva: anterior leg in ventral view (1) (cx- coxa, tr- trochanter, fe- femur. tb- tibia, tu- tarsungulus (2), median and posterior legs in ventral view (2) (cx- coxa, tr- trochanter, fe- femur. tb- tibia, tu- tarsungulus) micro cuticular process (4).



mid-longitudinally, each with a pair of elliptical spiracles between tergites and sternites. Abdominal segment VII 0.81 mm long and 0.80 mm wide, with 28 setae in dorsal view (Fig. IV, 5-6) and with 6 setae and 16 microsetae in ventral view.

Segment IX in dorsal side (Fig. V, 1) 0.66 mm long and 0.74 mm wide, with 18 setae; X segment (pygopod) 0.55 mm long and 0.37 mm wide, with 8 setae and some frayed, shorter setae on the surface. Tergite IX with pair of two-segmented, slender urogomphi; segment I 0.33 mm long, with 2 long setae and 2 shorter setae; segment II 0.18 mm long, with 1 lateral seta and 2 apical, long setae.

TAXONOMIC REMARKS

To make a comparison with the larvae of *Waitatia maoriana* and related species of the tribe Xantholinini is problematic, not only because of the peculiarity of the New Zealand species, but also due to the difficulty of identifying correctly the species of the larvae described in the past (c. g. Paulian, 1941, Kasule, 1970). Kasule (1970) nominally described *Xantholinus glabratus* and *X. linearis,* but *X. glabratus* belongs to the genus *Megalinus* Mulsant & Rey, 1877. Another fact that rises serious doubts about the species identities of the larvae described by Kasule (1970) is that he claimed the specimens of *X. linearis* that he were collected from Uganda and Tanzania, but the genus *Xantholinus* does not occurs in Africa South of the Sahara.

It should also be mentioned that the larval descriptions of Pototzkaya (1967) are rather cursory and lacking in many details.

In view of the fact that our knowledge of the Xantholinini of New Zealand is incomplete and that the known species, even their larvae, have few features in common with those from the Palaearctic which larvae have been studied, we refrain from further considerations. We only mention that the chaetotaxy of the body of *Waitatia maoriana* larvae appears less extensive than that of larvae of Palaearctic Xantholinini.

ACKNOWLEDGEMENTS

We wish to thank B. Staniec (Department of Zoology, Maria-Curie Sklodowska University, Lublin, Poland) for the help and useful suggestions, S. Cuoco (Livorno, Italy) for the photo and F. Cianferoni (Museo di Storia naturale, sezione di Zoologia, Firenze, Italy) for technical support.

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REDIA, 102, 2019: 35-40 http://dx.doi.org/10.19263/REDIA-102.19.05

JUAN M. NIETO NAFRÍA[a] - PAUL A. BROWN[b] - SARA I. LÓPEZ CIRUELOS[c] - JAIME ORTEGO[d]

A NEW SPECIES OF *APHIS* (HEMIPTERA APHIDIDAE) LIVING ON *TROPAEOLUM* SPECIES (TROPAEOLACEAE) FROM CHILE AND ARGENTINA

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Nieto Nafría J.M., Brown P.A., López Ciruelos S.I., Ortego J. – A new species of *Aphis* (Hemiptera Aphididae) living on *Tropaeolum* species (Tropaeolaceae) from Chile and Argentina.

A species of *Aphis* collected in a low-altitude Chilean locality in the region of Coquimbo and in two high-altitude Argentinian locations in the Mendoza province on plants of the genus *Tropaeolum* (Brassicales: Tropaeolaceae) is described from apterous and alate viviparous females and is named as *Aphis tropaeoli* Nieto Nafría and Brown, **sp. n.**. Similarities of and differences between 71 species known in South America belonging to five genera of the subtribe Aphidina (Aphidinae, Aphidini) are presented in a partial identification key; the new species is most similar to *Aphis berberidorum* Ortego & Mier Durante. The new species must be able to live on several species of *Tropaeolum* and may be widespread in a large area of South America. It is recommended the monitoring plants of *Tropaeolum* species used in gardening anywhere in the World.

KEY WORDS: Aphids, Aphidina, new species, South America, Tropaeolaceae, identification key.

INTRODUCTION

In August 1974, Roberto Carrillo found two apterous female aphids on an unspecified species of *Tropaeolum* in Chile. These he gave to Dick Hille Ris Lambers, who mounted them on slides and which he considered to be a new species and labelled them as manuscript “Aphis tropaeoli n. sp.”.

He did not describe the species and both slides were transferred with the rest of his collection to the British Museum of Natural History (currently Natural History Museum, London) in 1984 after the death of that eminent aphidologist. BLACKMAN and EASTOP (2006, 2018) maintained that these specimens could not belong to any species known and it was not one of the 14 species of *Aphis* described in South America between both 2006 and 2018.

In the aphid collection of the University of Leon (Leon, Spain) there are several apterous and alate viviparous females belonging to three aphid samples collected on *Tropaeolum incisum* Speg. (Sparre) in Argentina between 1996 and 2000 by J. Ortego, who was alone or in company with M.P. Mier Durante and J.M. Nieto Nafría.

A comparative study of all of these specimens together and with specimens of other South American *Aphis* species, has allowed us to establish that they are of a single new species.

The new species exhibits larger and smaller apterous viviparae (“big” and “small” forms, respectively), a bimodal phenomenon also seen in other Argentinian *Aphis* species, e. g.: *Aphis cuyana* López Ciruelos & Ortego, 2017, *A. eucollinae* López Ciruelos & Ortego, 1999, *A. mendocina* Mier Durante & Ortego, 2006 and *A. melosae* Mier Durante & Ortego, 1999, as well in some species in other parts of the World. The “big form” is present during most of the parthenogenetic phase of the species and the “small form” is the aestivating form, a form to resist the adverse conditions of drought and higher temperatures and can more easily survive on stressed plants. “Big” and “small” apterae of the mentioned South American species differ from each other by body length and volume, on different thoracic-abdominal sclerotisation which is extensive in “big” apterae and is not extensive or may be absent in “small” apterae, and sometimes by other qualitative or quantitative features.

MATERIAL AND METHODS

Aphids collected were preserved in 70% ethanol and cleared and mounted using the method described in NIETO NAFRÍA and MIER DURANTE (1998). The mounting medium in Hille Ris Lambers’s slides is Canada balsam. Measurements of specimens were taken as detailed by NIETO NAFRÍA and MIER DURANTE (*op. cit.*).

Papers describing all native South American species of Aphidina have been consulted, and particularly the description of *Aphis berberidorum* and the comparative table of features of *Andinaphis*, *Aphis* and *Protaphis* species (ORTEGO and MIER DURANTE, 1997; LÓPEZ CIRUELOS *et al.*, 2017). In addition, we have studied specimens of several South American native and introduced species of Aphidina that are preserved in the collection of the University of

– Received 17 October 2018 Accepted 15 March 2019

Leon, only three South American species of Aphidina are not represented in this collection.

The distribution of host plant species have been taken from Belov (2018), Instituto de Botánica Darwinion (2018) and The Catalogue of Life Partnership (2018).

Microphotographs were taken in the Service of Microscopy of the University of Leon using an Olympus set: BX61 microscope, DP70 digital camera and DP Manager Software version 2.1.1.163.

RESULTS AND DISCUSSION

Aphis* (*Aphis*) *tropaeoli Nieto Nafría & Brown, sp. n.
(Fig. I; Table 1)

Types

Holotype, apterous viviparous female (measured specimen number 1, mounted separately, "big form"): CHILE, Region de Coquimbo (IV), province Elqui, La Serena [probably and approximately 29º 55' S 71º 14' W, 90 m.a.s.l.], on *Tropaeolum* sp., 23rd August 1974, Carrillo *leg.*, Natural History Museum London collection (BMNH(E) 1984-340).

Paratypes: 50 apterous viviparous females [apt] and 29 alate viviparous females [al]; Natural History Museum London and *Universidad de León* collections. CHILE: same data as the holotype (1 big apt). ARGENTINA, Mendoza province, Malargüe, Refugio del Club Andino (35º 23' S, 69º 54' W, 2160 m.a.s.l.), on *Tropaeolum incisum*, 7th December 1996 (8 big apt, 3 small apt, 3 al) and 6th January 1997 (23 big apt, 8 small apt, 26 al), Ortego *leg.*; ARGENTINA, Mendoza province, Malargüe, Provincial Route 222 Valle Hermoso viewpoint, near Las Leñas ski resort (35º 08' S, 70º 11' W, 2370 m.a.s.l.), on *Tropaeolum incisum*, 5th February 2000 (1 big apt, 6 small apt), Nieto Nafría, Mier Durante & Ortego *leg.*

Description

Apterous viviparous females, big form (Figs. I, 1 and 2). From 34 specimens. When alive bright brown and slightly waxy white powdered. 1.95-2.40 mm long with cauda. Metric and meristic features in Table 1. Head, including clypeus and mandibular and maxillar lames and rostrum brown. Frons sinuate. Antennal segments I and II dark-brown and at least as pigmented as cephalic dorsum and antennal segment VI, with spines and small transversal striae; antennal flagellum progressively pigmented from the

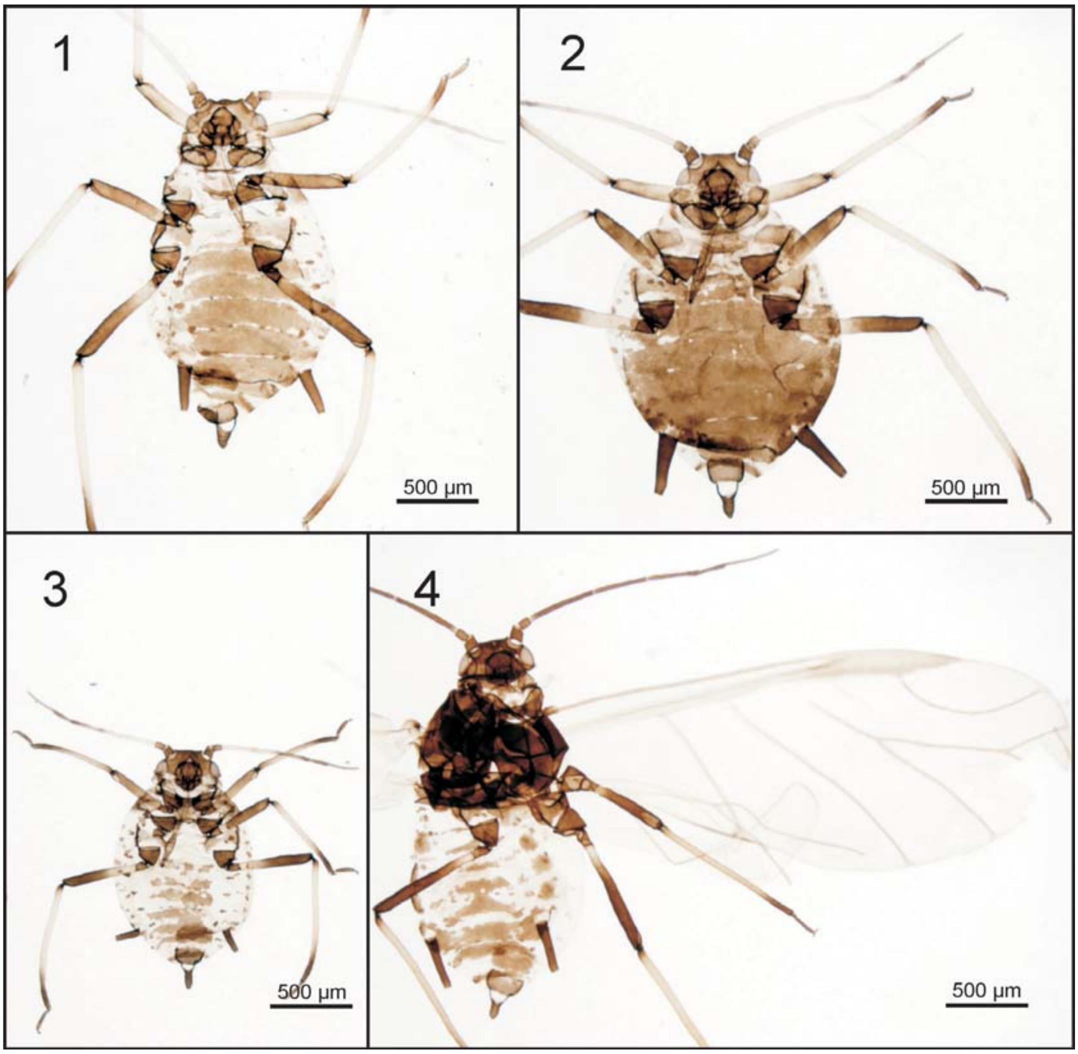

Fig. I – *Aphis* (*A.*) *tropaeoli* Nieto Nafría & Brown, **sp. n.** - 1. Apterous viviparous female big form (with dorsoabdominal transversal bands), holotype; 2. Apterous viviparous female big form (with dorsoabdominal wide plate); 3. Apterous viviparous female small form; 4, Alate viviparous female.

Table 1 – Metric and meristic features of apterous (big and small forms) and alate viviparous females of *Aphis tropaeoli* Nieto Nafría & Brown, **sp. n.** Note: trochanter, femur, tibia and tarsus of the hind legs. Abbreviations: Abd, abdominal; Ant, antennal segment; *D*, subarticular diameter of Ant. III; segm, segment or segments.

	apterous viviparous females			alate viviparous females
	small	big	in all	
Body / hind tibia [times]	1.63–1.97	1.58–2.09	1.58–2.09	*1.733–1.96*
Antenna [mm]	0.98–1.31	1.34–1.79	0.98–1.79	*1.47–1.77*
Antenna / Body [times]	0.66–0.85	0.67–0.84	0.66–0.85	*0.70–0.77*
Antennal segm. III [mm]	0.23–0.35	0.36–0.50	0.23–0.50	*0.39–0.46*
Antennal segm. IV [mm]	0.15–0.25	0.24–0.35	0.15–0.35	*0.25–0.38*
Antennal segm. V [mm]	0.17–0.22	0.21–0.29	0.17–0.29	*0.24–0.32*
Antennal segm. VI base [mm]	0.09–0.12	0.12–0.14	0.09–0.14	*0.12–0.16*
Antennal segm. VI processus terminalis [mm]	0.21–0.28	0.26–0.35	0.21–0.35	*0.31–0.34*
Antennal segm. III / Antennal segm. processus terminalis [times]	1.05–1.50	1.30–1.68	1.05–1.68	*1.25–1.38*
Antennal segm. VI: processus terminalis / base [times]	1.92–2.45	1.89–2.62	1.89–2.62	*2.09–2.71*
Femur [mm]	0.45–0.55	0.63–0.83	0.45–0.83	*0.60–0.73*
Tibia [mm]	0.75–0.95	1.05–1.43	0.75–1.43	*1.05–1.33*
Ultimate rostral segm. [mm]	0.12–0.14	0.13–0.15	0.12–0.15	*0.13–0.14*
Ultimate rostral segm. / its basal width [times]	2.00–2.80(3.13)	1.81–2.90	1.81–2.90(3.13)	*2.17–3.11*
Ultimate rostral segm. / Ant. VI base [times]	1.13–1.25(1.50)	(0.96)1.07–1.26	(0.96)1.07–1.26(1.50)	*(0.93)1.07–1.13*
Ultimate rostral segm. / tarsus, 2nd segm. [times]	0.81–0.93	0.78–0.91	0.78–0.93	*0.74–0.81*
Tarsus, 2nd segm. [mm]	0.14–0.16	0.15–0.19	0.14–0.19	*0.16–0.19*
Siphunculus [mm]	0.16–0.28	0.28–0.43	0.16–0.43	*0.24–0.33*
Siphunculus / its middle width [times]	3.00–5.25	3.65–5.67	3.00–5.67	*3.79–5.91*
Siphunculus / cauda [times]	0.84–1.56	1.22–1.71	0.84–1.71	*1.23–2.00*
Cauda [mm]	0.16–0.20	0.22–0.27	0.16–0.27	*0.16–0.21*
Cauda / its basal width [times]	1.17–1.73	1.31–2.08	1.17–2.08	*1.00–1.56*
Setae on …				
… vertex [µm]	27–40	27–48	27–48	*30–40*
… vertex [times *D*]	1.1–2.0	1.0–1.9	1.0–2.0	*1.3–1.8*
… Antennal segm. III [number]	6–13	8–14	6–14	*8–10*
… Antennal segm. III [µm]	12–25	20–30	12–30	*20–25*
… Antennal segm.. III [times *D*]	0.6–1.3	0.7–1.2	0.6–1.3	*0.9–1.1*
… trochanter, posterior [µm]	37–53	40–73	37–73	*40–50*
... trochanter, posterior / trochantero-femoral suture [times]	0.8–1.2	0.6–1.2	0.6–1.2	*0.8–1.0*
… femur, dorsal [µm]	25–30	30–40	25–40	*28–33*
… femur, dorsal [times *D*]	1.1–1.7	1.1–1.5	1.1–1.7	*1.2–1.4*
… femur, ventral [µm]	27–48	40–50	27–50	*35–50*
… femur, ventral [times *D*]	1.2–2.1	1.3–2.2	1.2–2.2	*1.6–2.2*
… tibia, at middle, dorsal [µm]	25–38	27–43	25–43	*28–35*
… tibia, at middle, dorsal / tibial diameter at middle [times]	0.3–0.4	0.3–0.5	0.3–0.5	*0.3–0.4*
… Abdominal segms. 2 to 4, marginal [µm]	15–28	25–35	15–35	*28–30*
… Abdominal segms. 2 to 4, marginal [times *D*]	0.6–1.4	0.9–1.4	0.6–1.4	*1.2–1.3*
… Abdominal segm. 8 [number]	2–3	2–4	2–4	*2–2*
… Abdominal segm. 8 [µm]	25–40	35–58	25–58	*38–63*
… Abdominal segm. 8 [times *D*]	1.0–2.1	1.2–2.3	1.0–2.3	*1.7–2.8*
… genital plate, discal [number]	2(3)	2(4)	2(4)	*2–5*
… genital plate, marginal [number]	9–12	10–14	9–14	*8–12*
… cauda [number]	5–8	5–12	5–12	*7–10(12)*

distal half of segment V and imbricate from the proximal part of segment III. Rostrum reaches nearly to the hind leg coxae with darkened ultimate rostral segment, which carries 2 long accessory setae. Coxae, trochanters, tarsi, most femora (progressively) and apex of tibiae brown like head; proximal portion of femora and most of tibiae pale. First segment of tarsi with 3 or occasionally 2 setae. Thoracic segments with variable dorsal sclerotisation, from very small and disperse sclerites to discontinuous transversal bands, always brown. Dorsal abdominal sclerotisation is also variable, abdominal segments 1 to 6 with brown spinopleural bands and brown and spinuled marginal sclerites, although the latter are absent on segment 1; each band and closely associated sclerites, can be fused in a transverse band more frequently on segment 4; in most sclerotized specimens spinopleural or complete transverse bands can merge with each other, forming a more-or-less wide and compact patch. Abdominal segments 7 and 8 have individual transverse and spinulate bands. Intersegmental and spiracular sclerites on thorax and abdomen are darker than segmental sclerotisation. Prothorax and abdominal segments 1 and 7 with marginal papillae, pronounced but less voluminous than the triommatidium. Intermediate abdominal segments without marginal tubercles. Siphunculi more or less tapered on proximal half and sub-cylindrical on distal half, with small flange and conspicuously longer than cauda, homogeneously pigmented and at least as dark as the abdominal dorsum and with scales. Genital and anal plates dark-brown. Cauda long-triangular, sometimes with a very slight proximal constriction or tenuously enlarged at the beginning of its distal half. Setae in general slender and pointed.

Apterous viviparous females, small form (Fig. I, 3). From 17 specimens. When alive matt-brown with greyish powder. 1.42–1.75 mm long with cauda. Metric and meristic features in Table 1. When mounted yellowish brown in general, with head including clypeous and laminae brown, antennae and legs pigmented as with “big form” except femora, which are uniform pale-brown. Most sclerotized specimens with a near complete transversal band on prothorax, wide spinal and marginal sclerites on each segment from mesothorax to abdominal segment 6 and isolated transverse bands on segments 7 and 8; in the less pigmented specimens sclerites only occur on prothorax and reduced or fractured isolated transverse bands on abdominal segments 7 and 8. Frons delicately sinuous or almost flat. Rostrum reaching hind coxae. Siphunculi tapering. Other qualitative features as with “big forms”.

Alate viviparous females (Fig. I, 4). From 29 specimens. When alive matt-dark-brown to matt-black covered with greyish powder. 1.92-2.43 mm long with cauda. Metric and meristic features in Table 1. Head, including clypeus and mandibular and maxillary lames and rostrum, antennae (except a very small proximal part of segment III, which is pale), most part of legs (see below), dorsal-abdominal sclerites (see below), siphunculi, genital and anal plates and cauda brown to very dark-brown. Frons flat. Antennal segments IV–VI and ventral face of antennal segment III strongly imbricate. Secondary sensoria on antennal segment III circular, with slight flange, somewhat different in size, more or less aligned along the ventral side of almost the entire segment; 9–17 on each antenna (21-32 on both). Coxae, trochanters, dorsal face of most of the length of front femora, most part of intermediate and hind femora, apex of tibiae and tarsi pigmented. Abdominal segments 1–6 usually with a spinal band with irregular edges and marginal round sclerites (pre- and post-siphuncular sclerites on segments 5 and 6), on segment 1 sometimes reduced to a small setiferous sclerite or are absent; segments 2-6 frequently with pleural sclerites; sometimes segments 5 and 6 with transverse bands; in some poorly sclerotized specimens sclerites reduced in size and pleural on all segments and spinal anterior ones can be absent. Abdominal segments 7 and 8 with individual transverse bands. Siphunculi very imbricated. Cauda narrow and with more-or-less marked proximal constriction, losing the triangular aspect usual in apterae. Other qualitative feature as in “big large” apterous viviparous females.

BIOLOGY

Aphis tropaeoli sp. n. has been collected on *Tropaeolum incisum* in the Argentinian province of Mendoza at above 2,000 meters altitude, and on an unidentified species of *Tropaeolum* in the Chilean Region of Coquimbo, a very low altitude. *Tropaeolum incisum* is present in several Argentinean provinces, including Mendoza, and in some Chilean regions, including Coquimbo in localities between 1,000 and 3,000 m.a.s.l. The host plant for specimens collected in Chile could belong to *T. azureum* Bert. ex Colla, *T. hookerianum* Bérneoud, *T. majus* L. or *T. tricolor* Sweet, which are known in this Region at low altitude. Thus it can be assumed that *Aphis tropaeoli* sp. n. might live on several *Tropaeolum* species.

Sexual forms are not known so we are uncertain of their life cycle, but as the populations survive at high altitude, the species may be holocyclic with as yet undiscovered sexual forms.

DISTRIBUTION

It is possible that the range of *Aphis tropaeoli* sp. n. corresponds with that of *Tropaeolum* species, and might be found in other Chilean regions and Argentinian provinces in addition to Coquimbo and Mendoza, respectively, as well as in other parts of the Andes mountain range. It must also be considered that it could affect cultivated plants of several species of the genus (i.e. *T. majus*, *T. peregrinum* L., *T. tuberosum* Ruiz & Pav.) in their natural distribution and in other parts of the World where they have been introduced.

ETYMOLOGY

The specific epithet *tropaeoli* is the name of the genus plant hosts of the new species in genitive.

TAXONOMIC DISCUSSION

Typical features of the genus *Aphis* are present in the studied specimens. Along with *Aphis*, there are 21 genera included in Aphidina (Aphidinae Aphidini); *Aphis* includes 588 species and the other 21 genera include 140 species (FAVRET, 2018) worldwide. In South America there are 70 species of Aphidina currently known (LÓPEZ CIRUELOS *et al.*, 2017; LÓPEZ CIRUELOS *et al.*, 2018; GONZÁLEZ RODRÍGUEZ *et al.*, 2018), which are distributed as follows: 63 species in *Aphis* (including two species belonging to subgenus *Toxoptera*, which was considered in the past as an independent genus), 1 in *Andinaphis*, 2 in *Brachyunguis* (one of each subgenera *Brachyunguis* and *Xerophilaphis*), 2 in *Pehuenchaphis* and 2 in *Protaphis*. Of these, 44 are South American native species consisting of 39 *Aphis* species and all the *Andinaphis*, *Brachyunguis* and *Pehuenchaphis* species. Most of the introduced species are widely distributed and are polyphagous or oligophagous species.

Aphis tropaeoli sp. n. is the 71st species recorded in South America and the 45th South American native species belonging to subtribe Aphidina. To distinguish it from the other species currently known in South America, including the new species, the following identification key for apterous viviparous females can be used. This key does not pretend to identify all Aphidina species in South America as to include them here would be inappropriate. The number of characters used has been reduced to a minimum, and those that are more easily discernible have been chosen. Dorso-abdominal sclerotisation has not been used because of the extreme variability of this character in many species. Seven species are accessible by two, or exceptionally three, places within the keys, for example *A. fabae* and *A. martinezi* by two and *A. rumicis* by three.

A Siphunculi absent or placed on abdominal segment 6 .... *Pehuenchaphis* species [Table 2, group **1**]

– Siphunculi placed on abdominal segment 5, as is typical in Aphidinae .... **B**

B Clypeous enormous .... *Brachyunguis* (*X.*) *blanchardi*

– Clypeous not enlarged .... **C**

C Stridulatory apparatus (a pattern of ridges on ventro-lateral areas of abdominal sternites 5 and 6 and a row of short peg-like hairs on hind tibiae) present .... *Toxoptera* species [Table 2, group **2**]

– .... Stridulatory apparatus absent **D**

D Marginal tubercles absent on abdominal segments 1, 8 or both two .... *Andinaphis paradoxa* and three *Aphis* species [Table 2, group **3**]

– Marginal tubercles on both abdominal segments 1 and 8 present [sometimes one of them absent in *A.* (*A.*) *patagonica*] .... **E**

E Genital plate without posterior setae .... *Aphis* (*A.*) *paravanoi*

– Genital plate with posterior setae .... **F**

F Antennal segment III with secondary sensoria .... *Protaphis* species and four *Aphis* species [Table 2, group **4**]

– Antennal segment III without secondary sensoria (they can be present in alatiform specimens of several species, and unusually in *Aphis fabae*) .... **G**

G Marginal tubercles present in several intermediate abdominal segments .... sixteen *Aphis* species [Table 2, group **5**]

– Marginal tubercles absent in intermediate abdominal segments; some may be sporadic in some species .... **H**

H Siphunculi pale, sometimes darkened to apex .... eight *Aphis* species [Table 2, group **6**]

– Siphunculi dark or black, sometimes with a small proximal paler portion .... **I**

I Ultimate rostral segment longer than second segment of hind tarsi .... *Brachyunguis* (*B.*) *bahamondesi* and twenty six *Aphis* species [Table 2, group **7**]

– Ultimate rostral segment as long as second segment of hind tarsi at most .... **J**

J Abdominal segment 8 with three setae at least (sometimes 2 in

Table 2 – Groups of species mentioned in the identification key. In order to distinguish the species included in each group between them, features of each species provided by López Ciruelos *et al.* (2017) or the keys by Blackman and Eastop (2018) for apterous viviparous females of species associated with different plant genera, should be used.

Group 1
P. agilissima Mier Durante, Nieto Nafría & Ortego, 2003
P. americana (Mier Durante & Ortego, 1998)

Group 2
A. (Toxoptera) aurantii Boyer de Fonscolombe 1841
A. (Toxoptera) citricidus (Kirkaldy, 1907)

Group 3
A. matilei Nieto Nafría, Ortego & Mier Durante, 2000
A. maulensis Mier Durante & García-Tejero, 2016
A. vurilocensis Nieto Nafría, Brown & López-Ciruelos, 2016
An. paradoxa (Mier Durante, Ortego & Nieto Nafría, 1997)

Group 4
A. acaenaevora Mier Durante & Ortego, 1998
A. hederae Kaltenbach, 1843
A. intrusa Ortego, 1998
A. melosae Mier Durante & Ortego, 1999 *in part*
Pr. middletonii (Thomas, 1879)
Pr. terricola (Rondani, 1847)

Group 5
A. carrilloi Ortego, Mier Durante & Nieto Nafría, 2013
A. coridifoliae Mier Durante & Ortego, 1999
A. fabae Scopoli, 1763 *in part*
A. malalhuina Mier Durante, Nieto Nafría & Ortego, 1999
A. mulini Hille Ris Lambers, 1974
A. mulinicola Hille Ris Lambers, 1974
A. papillosa Mier Durante, Nieto Nafría & Ortego, 2003
A. pomi De Geer, 1773
A. pseudopulchella Blanchard, 1944
A. rumicis De Geer, 1773 *in part*
A. sambuci Linnaeus, 1758
A. schinivora Ortego, Nieto Nafría & Mier Durante, 2007
A. senecionicoides Blanchard, 1944
A. solanella Theobald, 1914 *in part*
A. tehuelchis Nieto Nafría & López Ciruelos, 2016
A. zapalina Mier Durante & Ortego, 2016

Group 6
A. eucollinae López Ciruelos & Ortego, 2016
A. farinosa Gmelin, 1790
A. lippiae Ortego & Nieto Nafría, 2018
A. nasturtii Kaltenbach, 1843
A. schinifoliae Blanchard, 1939
A. patagonica Blanchard, 1944 *in part*

continue in the next column

Group 6 (continuation)
A. renjifoanae Ortego & Nieto Nafría, 2016
A. ruborum (Börner, 1931)

Group 7
A. acuminata Nieto Nafría & von Dohlen, 2016
A. affinis Del Guercio, 1911
A. alstroemeriae Essig, 1953
A. amaranthi Holman, 1974
A. asclepiadis Fitch, 1851
A. biobiensis Nieto Nafría & Mier Durante, 2016
A. conflicta Nieto Nafría, Ortego & Mier Durante, 2008
A. coreopsidis (Thomas, 1878)
A. craccivora Koch, 1854 *in part*
A. cytisorum Hartig, 1841 *in part*
A. forbesi Weed, 1889
A. gossypii Glover, 1877
A. illinoisensis Shimer, 1866
A. intybi Koch, 1854
A. junelliae González Rodríguez & Nieto Nafría, 2018
A. luzuriagae López Ciruelos, Brown & Nieto Nafría, 2018
A. marthae Essig, 1953 *in part*
A. martinezi Nieto Nafría, Ortego & Mier Durante, 1999 *in part*
A. melosae Mier Durante & Ortego, 1999 *in part*
A. mendocina Mier Durante, Ortego & Nieto Nafría, 2006
A. mulguraeae Nieto Nafría & Mier Durante, 2018
A. nerii Boyer de Fonscolombe, 1841
A. roberti Nieto Nafría, Ortego & Mier Durante, 1999
A. rumicis De Geer, 1773 *in part*
A. sedi Kaltenbach, 1843
A. spiraecola Patch, 1914
B. bahamondesi Remaudière & Halbert, 1996

Group 8
A. cuyana López Ciruelos & Ortego, 2017
A. danielae Remaudière, 1994
A. fabae Scopoli, 1763 *in part*
A. rumicis De Geer, 1773 *in part*
A. solanella Theobald, 1914 *in part*

Group 9
A. cinerea Nieto Nafría & Ortego, 2002
A. craccivora Koch, 1854 *in part*
A. cytisorum Hartig, 1841 *in part*
A. gaultheriae López Ciruelos, Brown & Nieto Nafría, 2018
A. patagonica Blanchard, 1944 *in part*

apterous viviparous “small” females of *A. cuyana* ....five species of subgenus *Aphis* [Table 2, group **8**]

– ....Abdominal segment 8 with only 2 setae **K**

K Cauda finger-shaped, sub-proximal constriction marked ....five species of subgenus *Aphis* [Table 2, group **9**]

– Cauda more-or-less triangular, sub-proximal constriction absent or very small ....**L**

L Intersegmental sclerites (muscular sclerites) not darker than other sclerites (segmental and spiracular) ....*Aphis* (*A.*) *marthae*, in part

– Intersegmental sclerites (muscular sclerites) darker than other sclerites, very distinguishable from them, even if they are included into segmental ones ....**M**

M Siphunculus 1.6–3.0 times its width at middle, sometimes curved out. In antennal segment VI, processus terminalis 1.2-1.9 times base ....*Aphis* (*A.*) *martinezi*

– Siphunculus 2.7-5.7 times width at middle (3.0-5.7 times in *A. tropaeoli*, 2.7-5.1 but usually more than 2.9 times in *A. berberidorum*) and never curved out ....**N**

N Longest seta on vertex 7-18 µm. Abdominal segments 2 and 3 usually without complete transversal bands or patch even in well sclerotized specimens; abdominal segments 4-6 more widely sclerotized, with transversal bands or a wide plate (in this species there are not two forms, big and small) .... *Aphis* (*A.*) *berberidorum*

– Longest seta on vertex 27-48 µm. Abdominal segments 2 and 3 with similar pattern of sclerotization to abdominal segments 4-6 in “big” form (which are well sclerotized specimens) ....*Aphis* (*A.*) *tropaeoli* **sp. n.**

Apterous viviparous females of *Aphis berberidorum* and *A. tropaeoli* sp.n. present different quantitative characters although with some overlap. Alate viviparous females of both species separate very well from each other, because *A. tropaeoli* have abdominal segmental cross bands while *A. berberidorum* only have marginal sclerites and scarce and scattered spinal and pleural sclerites.

ACKNOWLEDGMENTS

The field work in Argentina was partially financed by the Regional Government of Castile and Leon, Spain (research projects LE 45/02 and LE 034A05).

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GRAZIANO BASSI [a]

NOTES ON SOME AFROTROPICAL CRAMBINAE (LEPIDOPTERA PYRALOIDEA), CHIEFLY COLLECTED IN THE IVINDO NATIONAL PARK IN GABON, WITH DESCRIPTIONS OF NEW SPECIES

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Bassi G. – Notes on some Afrotropical Crambinae (Lepidoptera Pyraloidea), chiefly collected in the Ivindo National Park in Gabon, with descriptions of new species.

The results of multi-year studies on undetermined specimens integrated with those of a research expedition in the Ivindo forest in Gabon are given. Three new species are described and illustrated: *Chrysocatharylla larseni* sp. n., *Calamotropha hausmanni* sp. n. and *Calamotropha paradiakonoffi* sp. n.; the new synonymy *Pseudocatharylla ruwenzorella* Błeszyński = *Pseudocatharylla tisiphone* Bassi syn. n. is established; seven species are reported as new to Gabon.

Key Words: Cameroon, Crambidae, distribution, Gabon, Kenya, new synonymy, Uganda.

INTRODUCTION

Crambinae are found the world over and comprise 2047 described species, of which 436 are represented in the Afrotropical region (Nuss *et al.,* August 2018). The Gabonese fauna is among the more poorly known, with only two species cited for the country: *Calamotropha subdiodonta* Błeszyński and *Culladia troglodytella* (Snellen) (De Prins & De Prins, August 2018).

This work is based on the specimens collected during 10 days of field work at the Ipassa Research Station (Ivindo National Park, Ogooué-Ivindo Province) in Gabon, integrated with the data recorded from the material studied in the last decades.

The present material is rich enough to permit the description of three new species, establish a new synonymy and to extend the documented geographic distribution of some previously described species.

MATERIAL AND METHODS

Genitalia preparations were made following Robinson (1976). The terminology of the genitalia follows Klots (1970) and Kristensen (2003). Genitalia photographs were taken with a Canon S120 digital camera. The habitus photos were made with a Nikon D300 digital camera. The images were enhanced with Adobe Photoshop Elements. DNA sequencing was performed at the Canadian Centre for DNA Barcoding following standard high-throughput protocols (Ivanova *et al.,* 2006; deWaard *et al.,* 2008). The length of the labial palpus is compared to the maximum diameter of the composite eye in side view. The distribution data are taken, if not otherwise stated, from the material personally studied and from Afromoths database (De Prins & De Prins, accessed August 2018). The endings of specific epithets have been changed, where applicable, in accordance with the International Code of Zoological Nomenclature, chap. 7, art. 34.2.

Abbreviations used:
BC ZSM = Bar Code Zoologische Staatssammlung München.
BMNH = Natural History Museum, London, UK (formerly British Museum (Natural History).
HNHM = Hungarian Natural History Museum, Budapest.
MHNG = Muséum d'histoire naturelle, Genève.
RMCA = Royal Museum of Central Africa, Tervuren.
CGB = Bassi personal collection, Avigliana (Torino).
GS...BM = Genitalia slide.... British Museum.
GS...GB = Genitalia slide.... G. Bassi.
ZSM = Zoologische Staatssammlung München.

TAXONOMY

Chrysocatharylla larseni sp. n.

Holotype male: Gabon, Ivindo-Ogooué Prov[ince], Ipassa-Makokou Res[earch] St[ation], 500m, 14-24.III.2015, 00°31'N 12°48'E, lux, G. Bassi legit, GS 5882 GB, BC ZSM 92316, 49010 CGB.

Paratype male: Nigeria, Benin City, Sapoba Forest Reserve, 10-13.III.1972, E. W. Classey [legit], BM 1972-190, GS 2373 GB, BMNH.

Etymology – The species is dedicated to Dr Knud Larsen, well-known Danish specialist of Tortricidae, to thank him for his amicable donation of the holotype specimen, caught in one of his light-traps.

Diagnosis – *C. larseni* sp. n. is well distinguished from others congeners in the wing coloration (Fig. I, 1), especially for the two well-defined fasciae in the forewings. The male genitalia (Fig. II, 5) are most similar to those of *C. oenescentella* (Hampson) (see Bassi, 1999: figs 3, 4) in the structure of the phallus and cornutus, but the valva without processes is diagnostic. The cup-shaped scent organ (Fig. II, 6) distinguish *C. larseni* from any other known Afrotropical Crambinae.

– Received 18 October 2018 Accepted 15 March 2019

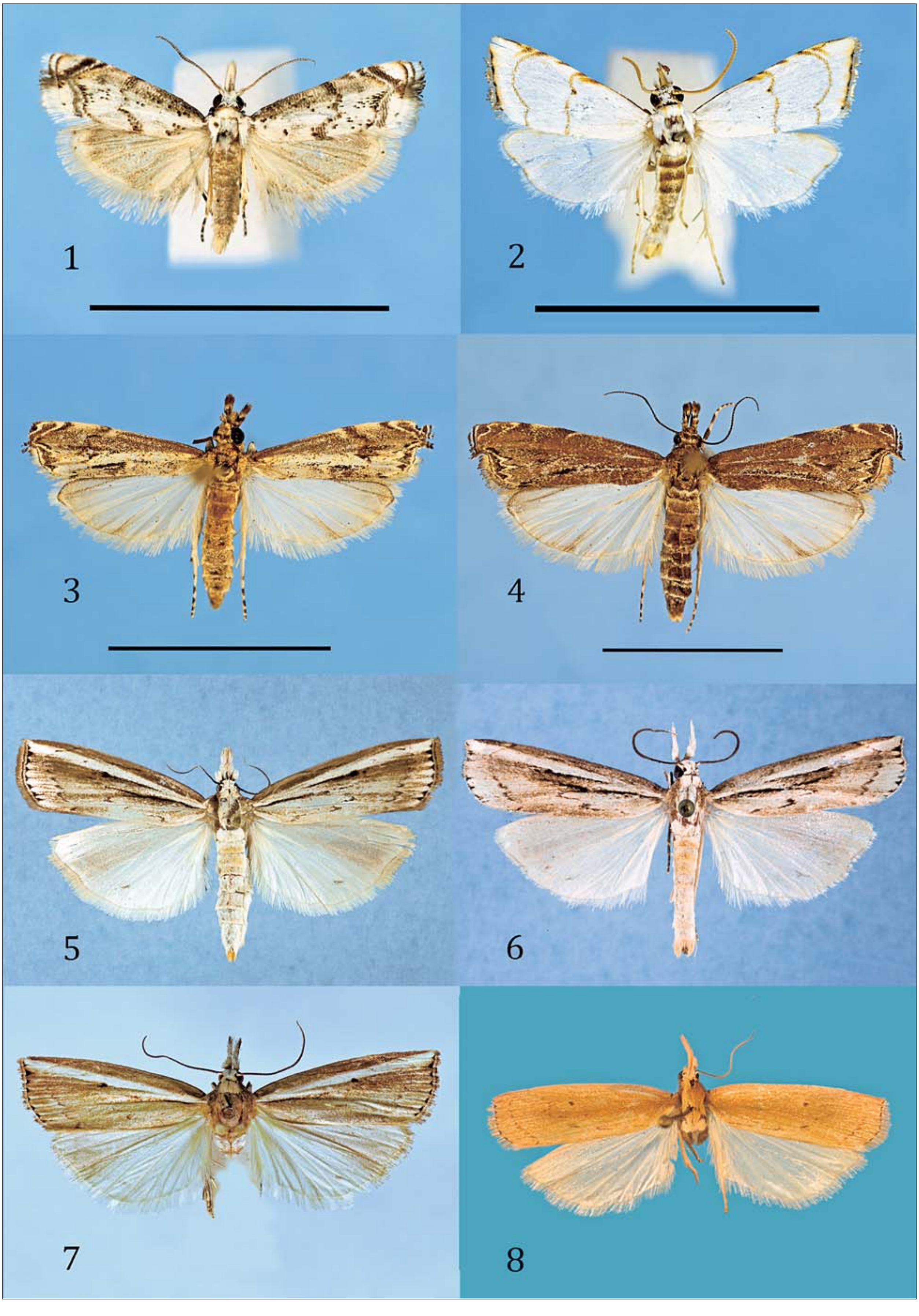

Fig. I – Crambinae spp. habiti. Scale bars = 10 mm. (1) *Chrysocatharylla larseni* sp. n., holotype (2) *Pseudocatharylla argenticilia* (Hampson), male, Gabon (3) *Mesolia microdontalis* (Hampson), male, Gabon (4) *Mesolia microdontalis* (Hampson), female, Gabon (5) *Calamotropha hausmanni* sp. n., female paratype, wingspan 36 mm (6) *Calamotropha hausmanni* sp. n., holotype (7) *Calamotropha athena* Błeszyński, female, Cameroon, wingspan 27 mm (8) *Calamotropha paradiakonoffi* sp. n., holotype.

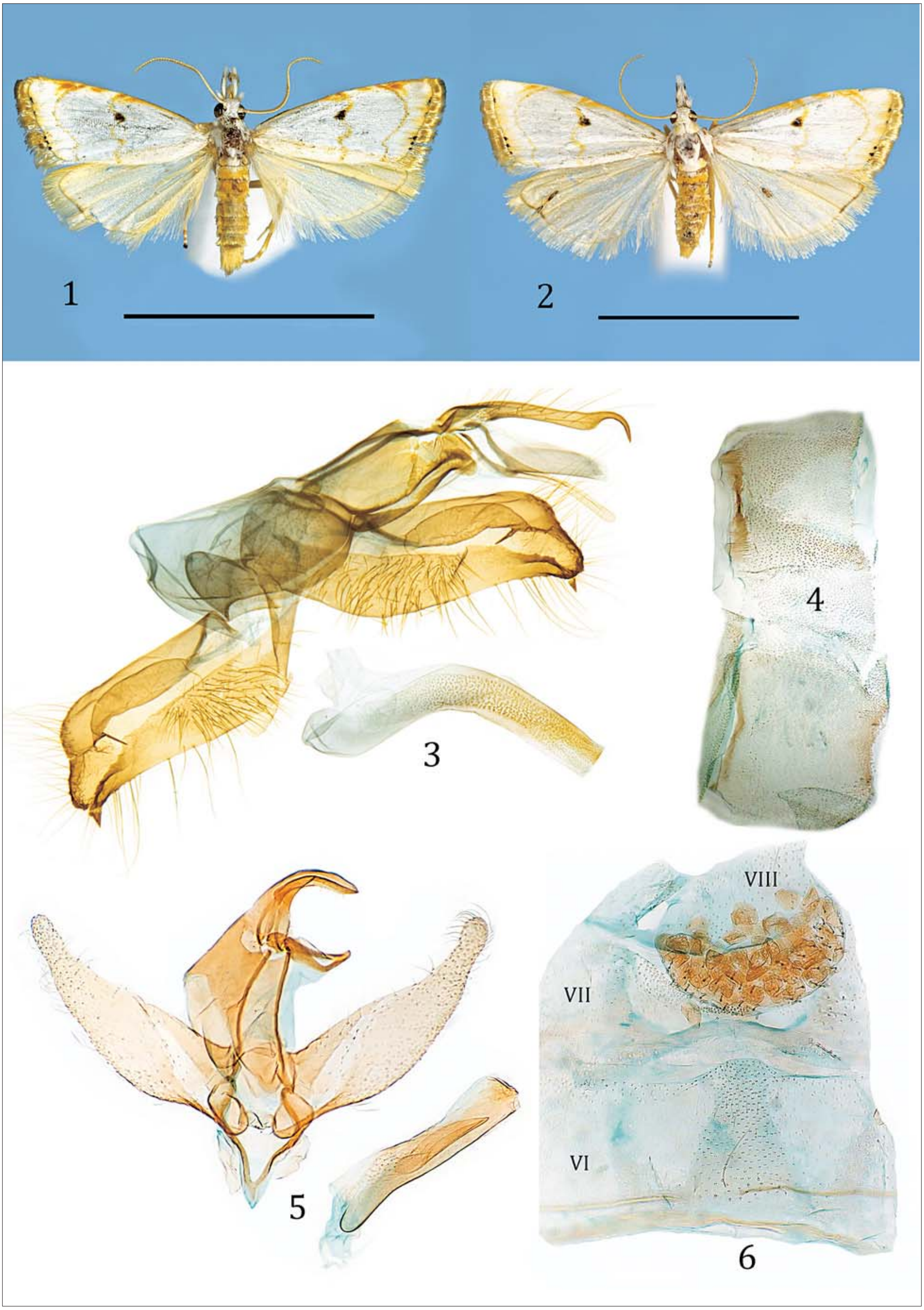

Fig. II – Crambinae spp. habiti and male genitalia. Scale bars = 10 mm., genitalia not to scale. (1) *Pseudocatharylla ruwenzorella* Błeszyński, male, Gabon (2) *Pseudocatharylla ruwenzorella* Błeszyński, female, Gabon (3) *Calamotropha hausmanni* sp. n., paratype, male genitalia (4) *Calamotropha hausmanni* sp. n., same paratype, eighth abdominal segment sclerotizations (5) *Chrysocatharylla larseni* sp. n., holotype, male genitalia (6) *Chrysocatharylla larseni* sp. n., holotype, abdominal structure, segments specified.

COI barcode sequence of the holotype (658 bp):
AACTTTATATTTTATCTTTGGAATTTGAGCAGGAA
TAGTAGGTACATCATTAAGTCTTCTAATTCGAGCTG
AATTAGGAAACCCTGGCTCACTAATTGGGGATGAT
CAAATTTACAATACTATTGTAACAGCTCATGCATTTA
TTATAATTTTTTTCATAGTAATACCAATTATAATTGGA
GGATTTGGAAATTGATTAGTACCTTTAATATTAGGA
GCCCCCGATATAGCATTCCCCCGAATAAATAATATAA
GATTTTGACTATTGCCCCCCCTCTTTAACCTTATTAAT
TTCCAGCAGAATTGTTGAAAATGGGGCAGGTACAG
GATGAACAGTGTATCCCCCCCCTTTCATCTAATATCG
CTCACGGAGGCAGTTCTGTTGATCTAGCTATTTTTT
CATTACATTTAGCTGGTATTTCTTCTATTTTAGGAGC
TATTAATTTTATTACCACTATTATTAACATACGAATTA
ATAGATTATCATTTGATCAAATACCTTTATTTGTATGA
TCAGTGGGAATTACTGCCTTACTCCTTCTTCTCTCAT
TACCTGTATTGGCAGGAGCTATTACTATATTATTAAC
AGATCGAAACTTAAATACATCTTTTTTTGACCCTGCT
GGAGGGGGAGATCCTATTCTTTACCAACATTTATTT.

Description (Fig. I, 1) – Wingspan: holotype 12 mm, paratype 11 mm. Labial palpi 3.5 × longer than widest diameter of eye, white on inner side, brown on lower outer side, white on upper outer side. Maxillary palpi white. Antennae thickened basally, slightly serrate distally, grey with silvery grey costa. Frons rounded, produced, white sprinkled with grey. Ocelli absent. Chaetosemata minute. Head white. Patagia basally grey, then silvery white. Tegulae white suffused with yellow, with scattered silvery grey scales. Thorax white suffused with yellow, sprinkled with few silvery grey scales. Forewing ground colour white with yellow apical suffusion and sprinkled with black scales; costa grey; antemedial fascia grey, bowed in medially; postmedial fascia silvery white, angled at R5-M1, bordered grey brown from angle to costa and silvery grey from angle to dorsum; terminal line brown grey around termen, then white; two small terminal dots medially and subdorsally; fringes silvery white, with short scales bordered grey brown at termen, and a medial silvery grey dot; underside strongly suffused with black. Hindwings grey suffused with yellow; fringes concolorous; underside yellowish white with costa suffused with black. Forelegs black; mid- and hindlegs yellowish white with tarsi annulated black and white; first pair of spurs longer than second pair. Abdomen grey with yellow suffusion on first segments; anal tuft white with light yellow suffusion; eight sternite modified into cup-shaped (Fig. II, 6) structure spiny on inner side and apparently full of a liquid that solidified during the boiling process; sixth and seventh abdominal sternites modified to support the eighth.

Male genitalia (Fig. II, 5): Uncus and gnathos of equal lengths, both curved and apically pointed; tegumen subtriangular; juxta shield-shaped, lightly sclerotized; vinculum triangular; valva without processes, slendering toward rounded cucullus; phallus as long as valva, with small phallobase; vesica with one large and elongated cornutus and some scobinations.

Female unknown.

Remarks – The general aspect of the moths and some characteristics of the genitalia, i.e. the simple valva and the phallus features, justify the placement of *larseni* in *Chrysocatharylla* Bassi, even if the presence of a peculiar abdominal structure (probably a scent organ) could inspire its placement in a new genus. The holotype is barcoded, but the lack of a female and the paucity of available barcoded specimens of *Chrysocatharylla* and allied genera for a relevant DNA comparison suggest to defer an eventual generic description.

Pseudocatharylla argenticilia (Hampson, 1919)
Crambus argenticilia Hampson, 1919a: 439.
Type locality: Sierra Leone.

Material examined – a male and 2 females, Gabon, Ivindo-Ogooué Prov[ince], Ipassa-Makokou Res[earch] St[ation], 500m, 14-24.III.2015, 00°31'N 12°48'E, lux, G. Bassi legit, CG 5759 and 5765 GB, CGB.

Remarks – The species (Fig. I, 2) is known from Cameroon, Central African Republic, Gabon (new record), Ghana, Guinea, Nigeria, and Sierra Leone.

Pseudocatharylla ruwenzorella Błeszyński, 1964
Type locality: Uganda.
Pseudocatharylla tisiphone Bassi, 1999:167, 168.
Type locality: Cameroon. syn. n.

Material examined – 3 males, 2 females, Gabon, Ivindo-Ogooué Prov[ince], Ipassa-Makokou Res[earch] St[ation], 500m, 14-24.III.2015, 00°31'N 12°48'E, lux, G. Bassi legit, BC ZSM 92299 and 92300, GS 5718 and 5722, CGB.

Remarks – Species known from the Republic of the Congo (new record: CGB), Democratic Republic of the Congo (new record: MHNG), Cameroon (new record: CGB), Gabon (new record), Uganda. The examination of the Gabonese specimens of both sexes, both through genitalia and DNA analysis, allows their correct match and to establish the synonymy of *P. ruwenzorella* with the holotype of *P. tisiphone* Bassi. Females (Fig. II, 2) are paler and slightly larger than males (Fig. II, 1). COI barcode sequence of specimen ZSM 92299 (658 bp):
AACTTTATATTTTATTTTTGGTATTTGAGCTGGAATA
GTAGGAACATCATTAAGATTATTAATTCGAGCTGAA
TTGGGAAACCCTGGATCTTTAATTGGAGATGATCAA
ATTTATAATACTATTGTTACAGCTCATGCATTTATTAT
AATTTTTTTTATAGTTATACCCATTATAATTGGAGGAT
TTGGAAACTGATTAGTTCCCCTAATACTAGGAGCTC
CGGATATGGCATTCCCGCGAATAAATAATATAAGATT
TTGATTATTACCTCCATCTTTAACCTTACTAATTTCA
AGAAGAATTGTAGAAAATGGAGCTGGAACAGGTT
GAACAGTTTACCCCCCCCCTTTCATCAAATATTGCTC
ATGGGGGAAGATCAGTTGACCTTGCAATTTTTTCCT
TACATTTAGCTGGTATTTCATCTATTTTAGGAGCAAT
TAATTTTATCACAACAATTATTAATATACGAATTAAT
GAATTAACTTTTGATCAAATACCCCTATTTGTCTGAT
CCGTGGGTATTACAGCTTTATTACTTCTTCTTTCCCT
ACCTGTATTAGCTGGAGCTATCACCATATTATTAACA
GATCGAAACTTAAATACTTCTTTTTTTGACCCTGCT
GGAGGGGGGGATCCAATTTTATACCAACATTTATTT.

Culladia achroella (Mabille, 1900)
Crambidion achroellum Mabille, 1900: 748.
Type locality: Madagascar.

Material examined – 4 females, Gabon, Ivindo-Ogooué Prov[ince], Ipassa-Makokou Res[earch] St[ation], 500m, 14-24.III.2015, 00°31'N 12°48'E, lux, G. Bassi legit, CGB.

Remarks – Species known from Botswana, Democratic

Republic of the Congo, Egypt, Ethiopia, Gabon (new record), Gambia, Kenya, Madagascar, Mauritius, Mozambique, Namibia, Nigeria, Réunion, Sao Tomé and Principe (new record: CGB), Saudi Arabia, Seychelles, South Africa, Sudan, Somalia, Tanzania, Yemen, Zimbabwe.

Culladia inconspicuella (Snellen, 1872)

Crambus inconspicuellus Snellen, 1872: 102, 103.
Type locality: Lower Guinea.

Material examined – a male, Gabon, Ivindo-Ogooué Prov[ince], Ipassa-Makokou Res[earch] St[ation], 500m, 14-24.III.2015, 00°31'N 12°48'E, lux, G. Bassi legit, GS 5716 GB, CGB.

Remarks – Species known from Cameroon (new record: CGB), Democratic Republic of the Congo, Ethiopia (Błeszyński, 1970), Gabon (new record), Ivory Coast (Błeszyński, 1970), Malawi (new record: CGB), Kenya (Błeszyński, 1970), Mautitius, Réunion, Seychelles, Sierra Leone (Błeszyński, 1970), South Africa.

Culladia troglodytella (Snellen, 1872)

Crambus troglodytellus Snellen, 1872: 103, 104.
Type locality: Lower Guinea.

Material examined – 2 males, Gabon, Ivindo-Ogooué Prov[ince], Ipassa-Makokou Res[earch] St[ation], 500m, 14-24.III.2015, 00°31'N 12°48'E, lux, G. Bassi legit, BC ZSM 92338, GS 5723 GB, CGB.

Remarks – Species known from Cameroon (new record: CGB), Gabon, Ghana (new record: HNHM) Ivory Coast, Mauritius, Nigeria, Réunion. Data from Mauritius and Réunion seem doubtful and probably refer to *C. achroella* (Mabille) or *C. inconspicuella* (Snellen).

Mesolia microdontalis (Hampson, 1919)

Prionopteryx [sic] *microdontalis* Hampson, 1919b: 66, 67.

Material examined – A male and a female, Gabon, Ivindo-Ogooué Prov[ince], Ipassa-Makokou Res[earch] St[ation], 500m, 14-24.III.2015, 00°31'N 12°48'E, lux, G. Bassi legit.

Remarks – Described from 3 males and 6 females from Nigeria, "Gold Coast" and Sierra Leone. Even if in the BMNH there is a male labelled "Type, Gold Coast, G.C. Dudgeon, 1907-332, *Prionapteryx microdontalis* Type male Hmpsn, GS 7530 BM", the original description does not mention the word "type". *M. microdontalis*, like *M. meyi* Bassi (2013: figs. 3, 4) shows sexual dimorphism, with males (Fig. I, 3) paler and smaller than females (Fig. I, 4). Species known from Cameroon (new record: CGB, MHNG), Gabon (new record), The Gambia (new record: ZSM), Ghana, Nigeria, Sierra Leone.

Calamotropha athena Błeszyński, 1961

Type locality: Nigeria.

Material examined – A female, Gabon, Ogooué-Ivindo Province, Ipassa-Makokou Res[earch] St[ation], 500m, 14-24.III.2015, 0031N 12°48'E, lux, G. Bassi legit, GS 5770 GB, BC ZSM 92318, CGB.

Redescription of the female genitalia (Fig. III, 2) – Papillae anales elongated, moderately up-curved dorsally and slightly down curved ventrally. Apophyses posteriores 1.9X longer than apophyses anteriores, narrow and bulged basally. Apophyses anteriores sub-triangular, slightly curved inwards apically. Abdominal segment VIII large, strongly developed, sclerotized, bulged dorsally and also slightly bulged ventrally, joined to sterigma by sclerotized bridge. Ostium bursae sub-oval, with sterigma tubular, slightly bent outward, strongly produced for almost all of length of abdominal segment VIII, moderately enlarging apically. Ductus bursae twice as long as sterigma, moderately sclerotized basally, then wrinkled and distally scobinate. Ductus seminalis branching off at anterior one-third of ductus bursae. Corpus bursae suboval, shorter than ductus bursae, completely scobinate.

Remarks – Described from a male holotype from Old Calabar [Nigeria], and two female paratypes from Sierra Leone and Masindi [Uganda], this species seems broadly distributed in Central and Western Africa. I know it from the Democratic Republic of the Congo (new record: RMCA), Cameroon (new record: CGB), Liberia (new record: Coll. Poltavsky, Rostov-on-Don, Russia). A redescription of the female genitalia was warranted because of the unclear illustration in the original description and for a better comparison with the new species described below.

Calamotropha hausmanni sp. n.

Holotype male: Kenya, 1600-1700 m, Nandi Prov[ince], Kakamega-Regenwald [Kakamega Forest National Reserve], Umg[ebung] [surroundings] Rondo, 5-10.V.1997, Eitschberger, Bauer & Traub leg[unt], ex coll. EMEM, ZSM.

Paratypes: a male and two females, same data as holotype, GS 5035 and 5042 GB, ZSM and CGB.

Etymology – The species is dedicated to Dr Axel Hausmann, Head of the Lepidoptera Section in the ZSM, to thank him for his more than twenty-year friendship and for his great help during my visits to his Museum.

Diagnosis – In wing pattern *C. hausmanni* (Figs I, 5 and 6) resembles *C. athena* Błeszyński (Fig. I, 7). However, *C. hausmanni* is larger and with more contrasted colours. The male genitalia of *C. hausmanni* (Fig. II, 3) are easily distinguishable by the strongly hooked uncus and two teeth on the valva . The female genitalia of *C. hausmanni* (Figs III, 1 and 3) can be distinguished from those of *C. athena* (Fig. III, 2) by the almost straight eighth abdominal tergite, the shorter apophyses posteriores and the sterigma almost straight as opposed to the arched and apically enlarged sterigma in *C. athena*. The other Afrotropical congeners with a strongly produced sterigma are *C. diakonoffi* Błeszyński (Figs III, 5 and 7) and *C. paradiakonoffi* sp. n. described below (Figs III, 4 and 6): both have longer apophyses anteriores and the sterigma is enlarging towards apex and wrinkled.

Description (Figs I, 5 and 6) – Wingspan: holotype 27 mm, male paratype 21 mm, female paratypes 32 and 36 mm. Labial palpi 4 × longer than widest diameter of eye, brown with upper and inner sides white. Maxillary palpi subtriangular, white with brown base. Antenna: in male thickened basally, slightly serrate distally, brown with silvery brown costa; in female thickened, brown to dark

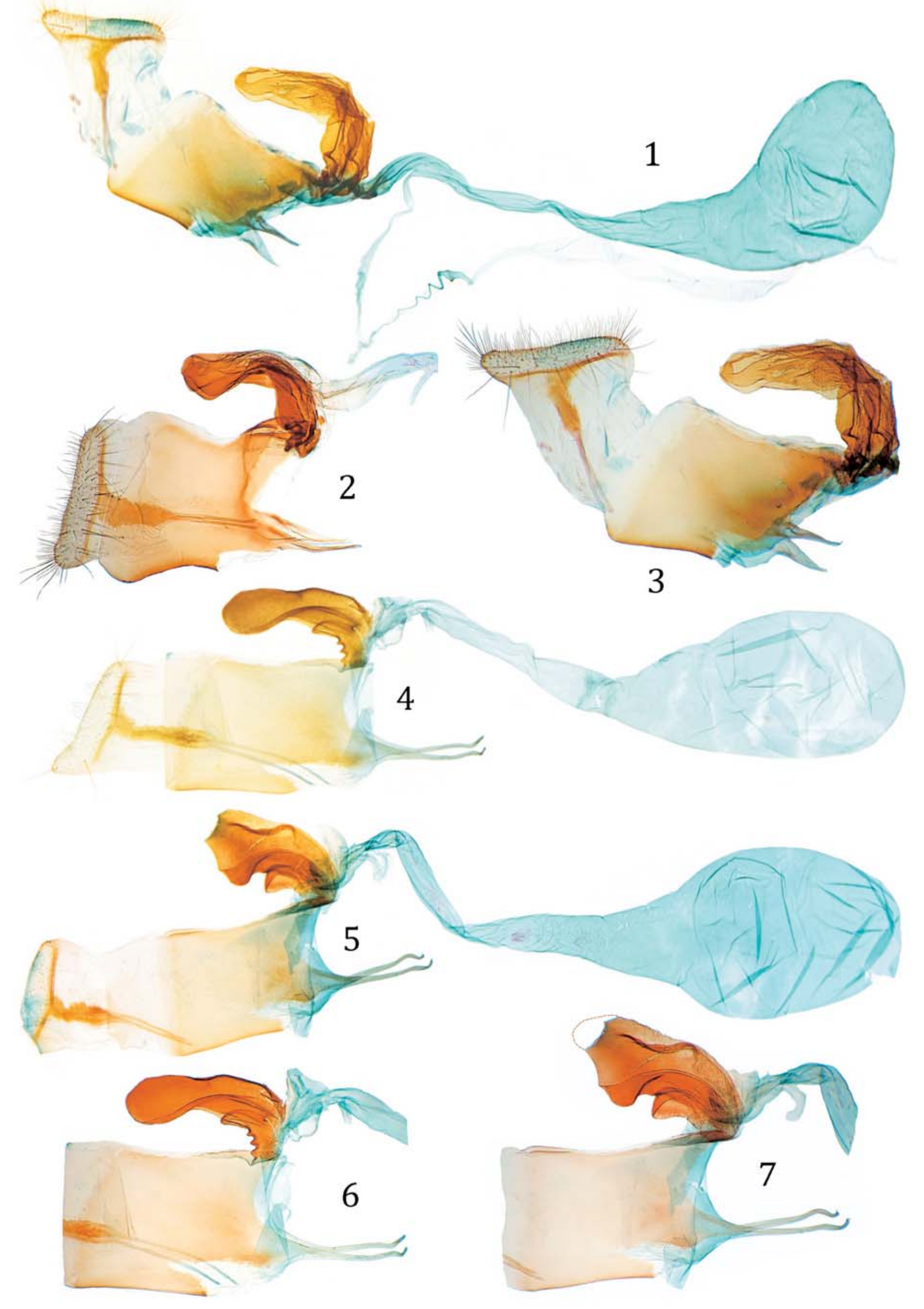

Fig. III – Crambinae spp. female genitalia. Not to scale. (1) *Calamotropha hausmanni* sp. n., paratype (2) *Calamotropha athena* Błeszyński, lateral view of distal part and sterigma – antrum complex, Cameroon (3) *Calamotropha hausmanni* sp. n., paratype, lateral view of distal part and sterigma – antrum complex (4) *Calamotropha paradiakonoffi* sp. n., holotype (5) *Calamotropha diakonoffi* Błeszyński, South Africa, Kwa-Zulu Natal (6) *Calamotropha paradiakonoffi* sp. n., lateral view of eighth abdominal segment and sterigma – antrum complex (7) *Calamotropha diakonoffi* Błeszyński, lateral view of eighth abdominal segment and sterigma – antrum complex.

brown. Frons rounded, slightly produced, white. Ocelli and chaetosemata strongly reduced. Head white. Patagia white in middle, brown laterally. Tegulae brown, distally creamy brown. Thorax white with thin medial line brown. Forewing ground colour white and brown with well-developed medial dot; medial fascia ill-defined, wrinkled, running along dorsum up to the cell, without reaching costa; postmedial fascia strongly dentate, thin and widely outcurved at R5-M1; with eight subtriangular terminal dots; medial stripe white, ending at termen, basally bordered brown up to cell; apex moderately pointed; termen sinuous; fringes brown, with medial line darker; underside brown with distal costa, apex and termen golden yellow. Hindwings glossy white lightly suffused with yellow and brown; fringes ivory yellow dorsally, white ventrally; underside white with dorsal half strongly suffused with brown. Legs brown with well-developed tibial spurs. Abdomen with tergites white suffused with yellow; sternites yellow with two brown stripes laterally; anal tuft ivory yellow, darker in female. Sclerotizations of eight abdominal segment in male as in Fig. II, 4.

Male genitalia (Fig. II, 3): Uncus slightly longer and more sclerotized than gnathos, with long setae, apically strongly hooked, with pointed tip. Gnathos slender, slightly curved, with rounded tip. Tegumen subrectangular, more strongly sclerotized ventrally, with large membranous window at junction of uncus with gnathos. Vinculum stout, moderately bifid dorsally. Juxta membranous. Pseudosaccus subconical, large, 0.6 as long as vinculum. Valva subrectangular, 1.5 longer than vinculum, slightly curved distally; costa with rounded tip bearing long setae; sacculus longer than costa, tapering in a strongly sclerotized apical tooth; ventral fold sclerotized apically and covered with strong medium sized setae in basal half; costal fold bulged medially; distal end of bulge with slender and pointed tooth. Phallus large, gently curved, with several rough scobinations.

Female genitalia (Figs III, 1 and 3): Papillae anales elongated, slightly up-curved dorsally and slightly down curved ventrally. Apophyses posteriores 2.15 longer than apophyses anteriores, bulged basally, then narrowing. Apophyses anteriores sub-triangular. Abdominal segment VIII strongly developed, slightly oblique in side view, joined to sterigma by strongly sclerotized and spiny bridge. Ostium bursae sub-oval, spiny, with sterigma tubular, strongly produced to 0.75 length of abdominal segment VIII. Ductus bursae two times longer than corpus bursae, moderately sclerotized basally, then wrinkled and distally scobinate. Ductus seminalis branching off anteriorly at one-third of ductus bursae. Corpus bursae suboval and completely scobinate.

Calamotropha paradiakonoffi sp. n.

Holotype female: [Uganda] Kampala, 8.VI.[19]25, B:[?, unknown collector], Coll. Audéoud, GS 2592 GB, MHNG.

Etymology – The name of the new species refers to its similarity to *C. diakonoffi* Błeszyński in female genitalia.

Diagnosis – A large *Calamotropha*, but smaller and paler in ground colour than *C. diakonoffi*, with postmedial fascia clearly defined and curved, and termen arched, as opposed to the postmedial fascia ill-defined and only slightly arched distally and to straight and oblique termen in *C. diakonoffi*. The female genitalia of *C. paradiakonoffi* sp. n. (Figs III, 4 and 6) are similar to those of *C. diakonoffi* (Figs III, 5 and 7), but the bridge between the eighth abdominal segment and the sterigma is longer and strongly dentate, and the sterigma is longer and less enlarged with the first inner fold smaller and closer to the bridge.

Description (Fig. I, 8) – Wingspan 30 mm. Labial palpi 4 × longer than widest diameter of eye, brown with inner side creamy brown. Maxillary palpi well developed, brown with white tip. Antenna simple, brown with silvery brown costa. Frons rounded, slightly produced, creamy brown. Ocelli absent. Chaetosemata strongly reduced. Head creamy brown, with brown tuft around chaetosemata. Patagia brown, paler in middle. Tegulae brown. Thorax light brown. Forewing ground colour tawny with subdorsal dot dark brown; medial fascia very narrow, crossing straight almost all of dorsal area, then in vertical section strongly serrate; postmedial fascia serrate, gently outcurved at R5-M1; terminal line absent; with eight dark brown terminal dots; apex slightly acuminate; termen barely concave near apex and clearly convex in middle; fringes creamy brown; underside glossy light brown with interveins brown. Hindwings glossy white with concolorous fringes; underside glossy white suffused with yellow. Legs brown with well-developed first pairs of tibial spurs and minute second pairs of spurs.

Male unknown.

Female genitalia (Figs III, 4 and 6): Papillae anales elongated, moderately up-curved dorsally and slightly down curved ventrally. Apophyses posteriores slightly more than twice as long as apophyses anteriores, enlarged basally, then strongly narrowing. Apophyses anteriores subtriangular at base, slender. Abdominal segment VIII strongly developed, tubular, joined to sterigma by a strongly sclerotized and tridentate bridge. Ostium bursae sub-oval; sterigma strongly produced for 0.8 length of eighth abdominal segment, slightly bent inwards, moderately enlarging apically, folded in inner side basally and subapically. Ductus bursae three times as long as sterigma, moderately sclerotized basally, then wrinkled and distally scobinate. Ductus seminalis branching off anteriorly at one-third of ductus bursae. Corpus bursae suboval, shorter than ductus bursae, completely scobinate.

ACKNOWLEDGMENTS

I thank Dr A. Durante, the Museo di Storia naturale del Salento, the CENAREST (Centre National de la Recherche Scientifique et Technologique), the IRET (Institut de Recherche en Ecologie Tropicale), and the ANPN (Agence Nationale des Parcs Nationaux du Gabon) for permitting and organizing the research expedition to Ipassa in the Ivindo National Park. A particular word of thanks to the staff of BMNH, HNHM, MRAC and Dr A. Poltavsky (Rostov on Don) for the loan of material, to Dr A. Hausmann for the loan of material and for his help in DNA barcoding, and to Dr B. Landry (MHNG), for the loan of material, for reviewing the text, and for his valuable suggestions. I also owe a great deal of gratitude to Pier Luigi Scaramozzino, Dept. of Agriculture, Food & Environment, University of Pisa, for his crucial help with the photographic work performed for this article. Finally I thank the reviewers of the manuscript for their valuable suggestions.

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REDIA, 102, 2019: 49-54 http://dx.doi.org/10.19263/REDIA-102.19.07

PASQUALE TREMATERRA[a]

DESCRIPTION OF *CYDIA ALDOCATANIAE* SP. N. AND REPORT OF *CYDIA RYMARCZKI* VARENNE & NEL, 2013 FROM MALTA (LEPIDOPTERA TORTRICIDAE)

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Trematerra P. – Description of *Cydia aldocataniae* sp. n. and report of *Cydia rymarczki* Varenne & Nel, 2013 from Malta (Lepidoptera Tortricidae).

Cydia aldocataniae sp. n. is described as new. Adults and genitalia are very similar to *C. fagiglandana* (Z.) but DNA barcodes are different. Moreover, the presence of *Cydia rymarczyki* Varenne & Nel, 2013 is reported for the first time in the Maltese fauna.

Key Words: *Cydia aldocataniae,* new species, *Cydia rymarczki*, Lepidoptera Tortricidae, Malta.

INTRODUCTION

In the past the Tortricidae from the Maltese Islands have received only sporadic attention by both local and foreign lepidopterists (T.B. Fletcher 1904-05; 1905), Alfredo Caruana Gatto (1905). More recently Valletta (1973) listed 33 species of tortricids out of a list of 413 species. Sammut (1984) and Razowski (1996) mentioned 41 and 50 species respectively. Sammut (2000) in a catalogue of over 600 species brought the number of known tortricids from Malta to 59. In the latest checklist of lepidoptera, the electronic version of the Fauna Europaea, the number of Tortricidae reported from Malta is 51 (Aarvik, 2013). The species listed by Trematerra and Sammut (2007) ammout to 68. Seguna in 2008 added *Cydia blackmoreana* (Walsingham) from Naxxar.

In the present paper a new species collected by Aldo Catania from Buskett Garden in 2017 is described and the presence of *Cydia rymarczki* Varenne & Nel, 2013 is cited for the first time. With these two new records the genus *Cydia* in Malta is represented by 12 species.

Cydia aldocataniae sp. n.

Material Examined – Holotype, 1♂, Malta, Buskett, 35°51’21.13’’N – 14°23’49.66’’E, Alt. 190 mt, 28.VI.2017, Leg. A. Catania (Sample ID: LEP-SS-00826) (in collection Pasquale Trematerra, Campobasso, Italy).

Paratypes, 1♀, Malta, Buskett, 35°51’21.13’’N – 14°23’49.66’’E, Alt. 190 mt, 15.VI.2017, Leg. A. Catania (in collection P. Trematerra, Italy); 1 ♀, Malta, Buskett, 35°51’21.13’’N – 14°23’49.66’’E, Alt. 190 mt, 2.VI.2017, Leg. A. Catania (in collection Aldo Catania, Malta).

Additional material (Paratypes) from Malta, Buskett, 35°51’21.13’’N – 14°23’49.66’’E, Alt. 190 mt, Leg. A. Catania: 1♀, 25.V.2017; 1♂ and 1♀, 30.V.2017; 1♂, 2.VI.2017; 1♂, 10.VI.2017; 1♂, 11.VI.2017; 1♂, 20.VI.2017; 1♂, 25.VI.2017; 1♀, 28.VI.2017; 2♀, 30.VI.2017; 4♂, 3.VII.2018 (in collection Aldo Catania, Malta). 1♀, 30.V.2017 (in collection Paul Sammut, Malta). 1♀, 2.VI.2017 (in collection Antony Seguna, Malta).

Adult (Fig. I) – Wingspan 14-16 mm. Head brownish sprinkled with cream scales, palps light brown-cream; antennae brownish-cream. Thorax brownish, abdomen cream colour with brownish stripes. Forewing grey-brownish, sprinkled whitish-cream with creamish strigulae; costal strigulae delicate, whitish or white-cream, dividings dark brown; post-apical strigula white-cream; speculum olive grey, with refractive lines and blackish inner spots; outer lines of speculum silvery. Dorsal patch white-creamish, diffused distally and extending towards middle of wing, marked by brownish lines. Basal blotch brown-creamish, slightly convex medially; subtornal blotch dark brown. Cilia brownish grey; basal line creamish. Hindwing brownish with cilia brownish-cream, darker at base, basal line cream. Males with creamish-white blotch in basal and anal areas with similarly coloured cilia.

Male Genitalia (Fig. II, 1-2) – Tegumen developed with enlarged base; small prominence at top of tegumen proximally and scales on lateral surfaces; valvae large, elongate bean shaped with costa convex in the median part; cucullus elongate, convex caudally covered with group of spines; ventral incision quite pronounced; basal cavity rather rounded; neck of valva broad; caudal angle of sacculus little pronounced; aedeagus very slender long and curved, basally enlarged and at the terminal part pointed.

Female Genitalia (Fig. II, 3-4) – Sub-genital sternite trapezoidal, cut laterally; postostial part of sterigma small, slightly expanding posteriorly; ostium bursae wide, sterigma funnel shaped; colliculum membranous; cingulum rather elongated; ductus seminalis sub-median; bursa copulatrix sculptured, developed and broad; signum developed.

Distribution – Known only from the type locality: Buskett Garden, Rabatt (Malta).

– Received 19 November 2018 Accepted 18 March 2019

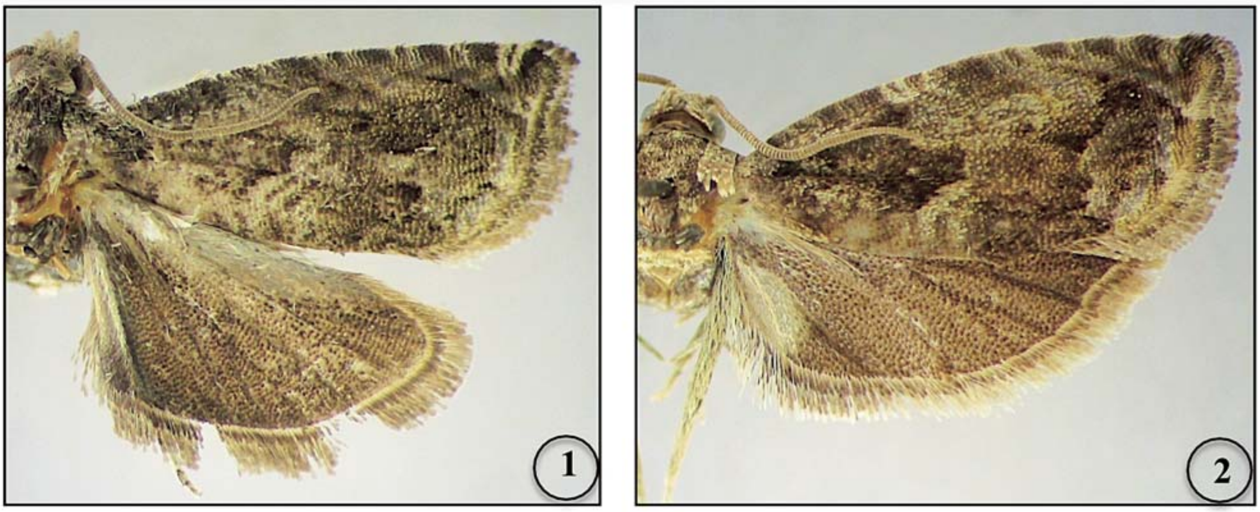

Fig. I – *Cydia aldocataniae* sp.n.: adult male (1); *Cydia fagiglandana* adult male (2).

Biology – Probably univoltine. The specimens of *C. aldocataniae* sp. n. came out from acorns of *Quercus ilex* L. collected at Buskett Garden (Malta), in October/November, and adults emerged from end of May to the first week of July (Fig. III, 1-4).

Diagnosis – Externally *Cydia aldocataniae* sp. n. looks like *Cydia fagiglandana* (Z.) but differs by wing-patterns (Danilevski & Kuznetsov, 1968; Razowski, 2003) (Fig. I, 1-2). From the latter species, in male genitalia it differs by the conformation of tegumen and of valvae, the caudal angle of the sacculus, by cucullus being larger and by aedeagus slender, longer and pointed (Fig. II, 1-2; Fig. IV, 1-2). The female genitalia differ by subgenital sternite being trapezoidal, cut laterally, ostium bursae wider, cingulum being elongated, bursa copulatrix smaller and sculptured (Fig. II, 3- 4; Fig. IV, 3-4).

Barcoding Analyses – A small piece of a leg was transferred to a lysis plate under ca. 0.3 ml 100% alcohol. Tissue samples were submitted to the standard procedures of the Canadian Centre for DNA Barcoding (CCDB) for sequencing the mitochondrial 5’ cytochrome oxidase gene, subunit 1 (COI), the standard marker for the identification of most animals. LepF1 and LepR1 were the primers used for PCR and sequencing (Hajibabaei *et al.*, 2006). Sequences were blasted against the complete sequence database of BOLD data systems (Ratnasingham and Hebert, 2007) in order to investigate the closest matches using the BOLD Identification Engine (http://www.boldsystems.org/index.php/ IDS_OpenIdEngine). Sequences having no matches on BOLD were blasted against the complete sequence database of Gen Bank (Hajibabaei *et al.*, 2006; Ratnasingham and Hebert, 2007). Genetic distances between sequences were measured using minimum pairwise distance and Neighbour joining tree was built using Kimura 2-parameter distance model for COI-5P marker. The distance of the *C. aldocataniae* (ID: BCLEP376-18) from the nearest neighbour (NN) is equal to 2,99%. The NN is *C. fagiglandana* (BIN: BOLD AAC5023).

A target 658-bp fragment of COI was amplified only from LEP-SS-831, as follow:

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AACATTATATTTTATTTTTGGTATTTGAGCTGGCATA
GTAGGAACTTCCCTAAGTTTATTAATTCGTGCAGA
ATTAGGAAATCCTGGATCTCTAATTGGAGATGATCA
AATTTATAATACTATTGTAACTGCTCATGCTTTTATTA
TAATTTTCTTTATAGTAATACCTATTATAATCGGTGGA
TTTGGTAACTGATTAGTACCATTAATACTAGGAGCC
CAGATATGGCATTTCCTCGAATAAATAATATAAGATT
TTGATTATTACCTCCCTCTATTATACTTTTAATTTCAA
GTAGAATTGTAGAAAATGGAGCAGGTACAGGATGA
ACAGTATACCCCCCTCTGTCATCTAATATTGCCCATA
GTGGAAGATCTGTAGACTTAGCTATTTTCTCCCTTAT
TTAGCTGGAATTTCTTCTATTTTAGGAGCTGTTAATT
TTATTACAACTATTATTAATATACGACCTAATAATATA
TCATTAGACCAAATACCCCTATTTGTATGAGCTGTG
GTATTACCGCTCTTCTCTTACTTCTATCATTACCAGT
ATTAGCAGGTGCCATTACTATACTTCTCACAGACCG
AAATCTCAATACTTCATTTTTTGATCCTGCTGGTGG
AGGTGATCCAATTCTCTACCAACATCTATTT
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Etymology – The new *Cydia* is dedicated to my colleague Mr. Aldo Catania (Żebbuġ, Malta) who has contributed to collections of the material studied.

Cydia rymarczyki Varenne & Nel, 2013

Material Examined – 1 ♀, Malta, Buskett, Bred ex-larva on *Quercus ilex* 30.V.2014, leg. A. Catania. 1 ♂ Malta, Buskett, Bred ex-larva on *Quercus ilex* Alt. 190mt., 1.VI.2014, leg. A. Catania. 1 ♀, Malta, Buskett, 35°51’21.13”N – 14°23’49.66”E, Alt. 190mt., 30.V.2017, Leg. A. Catania.

Cydia rymarczyki Varenne & Nel, which is closely related to *Cydia amplana* (Hübner), and *Cydia molybdana* (Constant), was described from southern France (Varenne & Nel, 2013). *C. rymarczyki* presents a habitus rather similar to that of *C. amplana* but more contrasted and browner, especially in the males (Fig. V, 1). The male genitalia are closely related to those of *C. molybdana* and the female genitalia are related to those of *C. amplana* (Varenne & Nel, 2013) (Fig. V, 2-5).

Biology – Probably bivoltine. According to Varenne & Nel (2013), in France *C. rymarczyki* coexists with *C.*

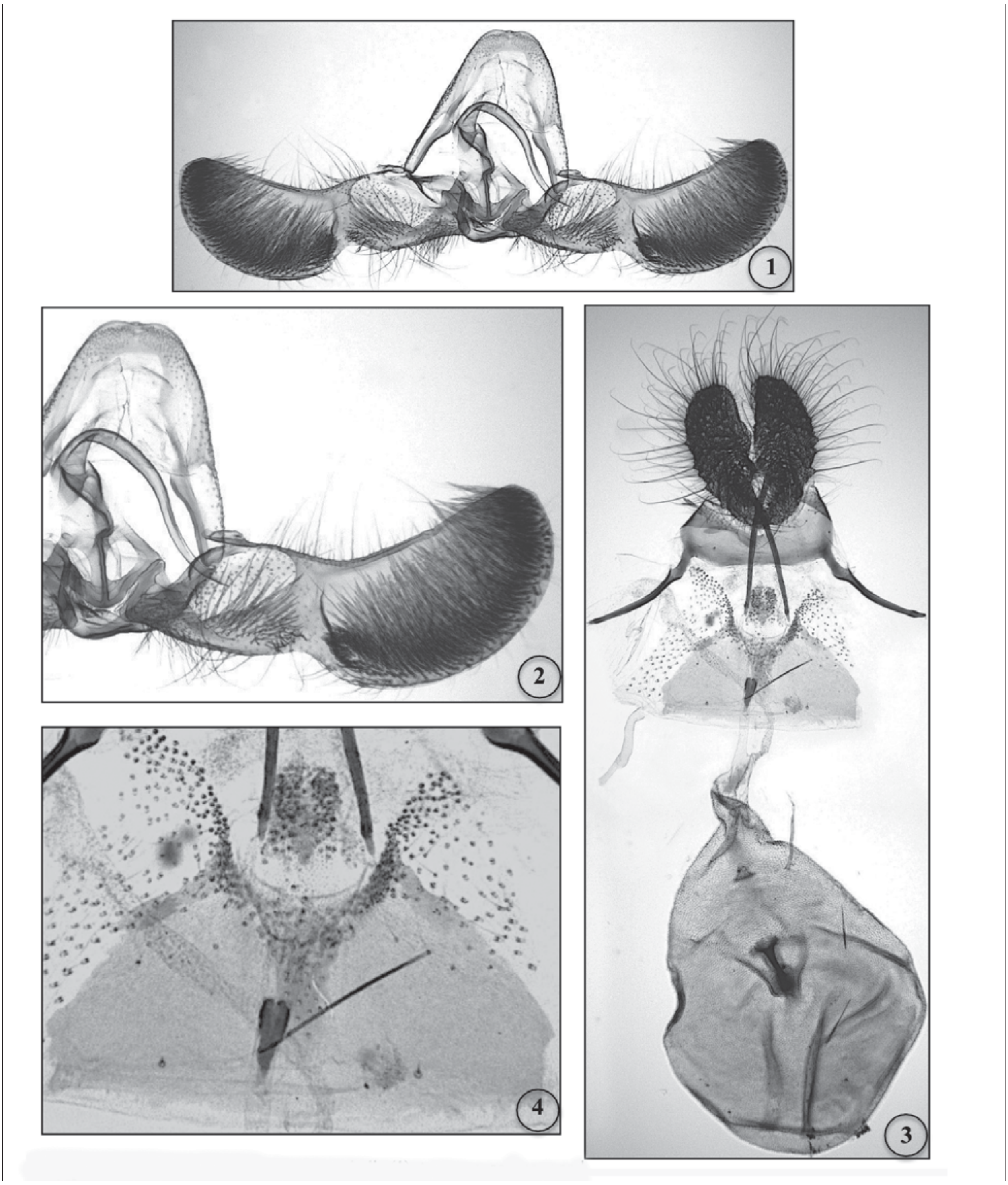

Fig. II – *Cydia aldocataniae* sp. n.: male genitalia (1); tegumen, aedeagus and valva (2); Female genitalia (3); antrum and colliculum (4).

amplana and *C. molybdana*. As with these two species, hatching occurs mostly in September. From 2013 to 2017, in Malta adults of C. *rymarczyki* were obtained from late May to early June from larvae in acorns of *Quercus ilex*.

Distribution – France (coastal plain of Alpes-Maritimes) and Malta (Buskett), also in Greece (Crete) published as *Cydia amplana* (Huemer in litt.). Before this new distribution record, *C. rymarczyki.* was known only from the Var and the Alpes-Maritimes, on the coastal plains (altitude around 100 m), from le Destet, Mouriès, 95 m, Bouches-du-Rhone; le Brûlat, Grand Vallat, Sainte-Anne-du-Castellet, 115 m; Tamarin, Biot, 100 m, Alpes-Maritimes.

ACKNOWLEDGEMENTS

I thank Mr. Aldo Catania and Mr. Paul Sammut (Malta), Dr. Marco Colacci and Lorenzo Goglia (Italy) for information, comments and helpful suggestions. I am grateful to Dr. Paul Hebert and his team (Canadian Centre for DNA Barcoding Guelph, Canada) for DNA sequencing work.

Fig. III – Plant of *Quercus ilex* in Buskett Garden (Malta) (1); acorns of *Quercus ilex* (2); larva of *Cydia aldocataniae* in an acorn of *Quercus ilex* (3); cocoon of *C. aldocataniae* (4).

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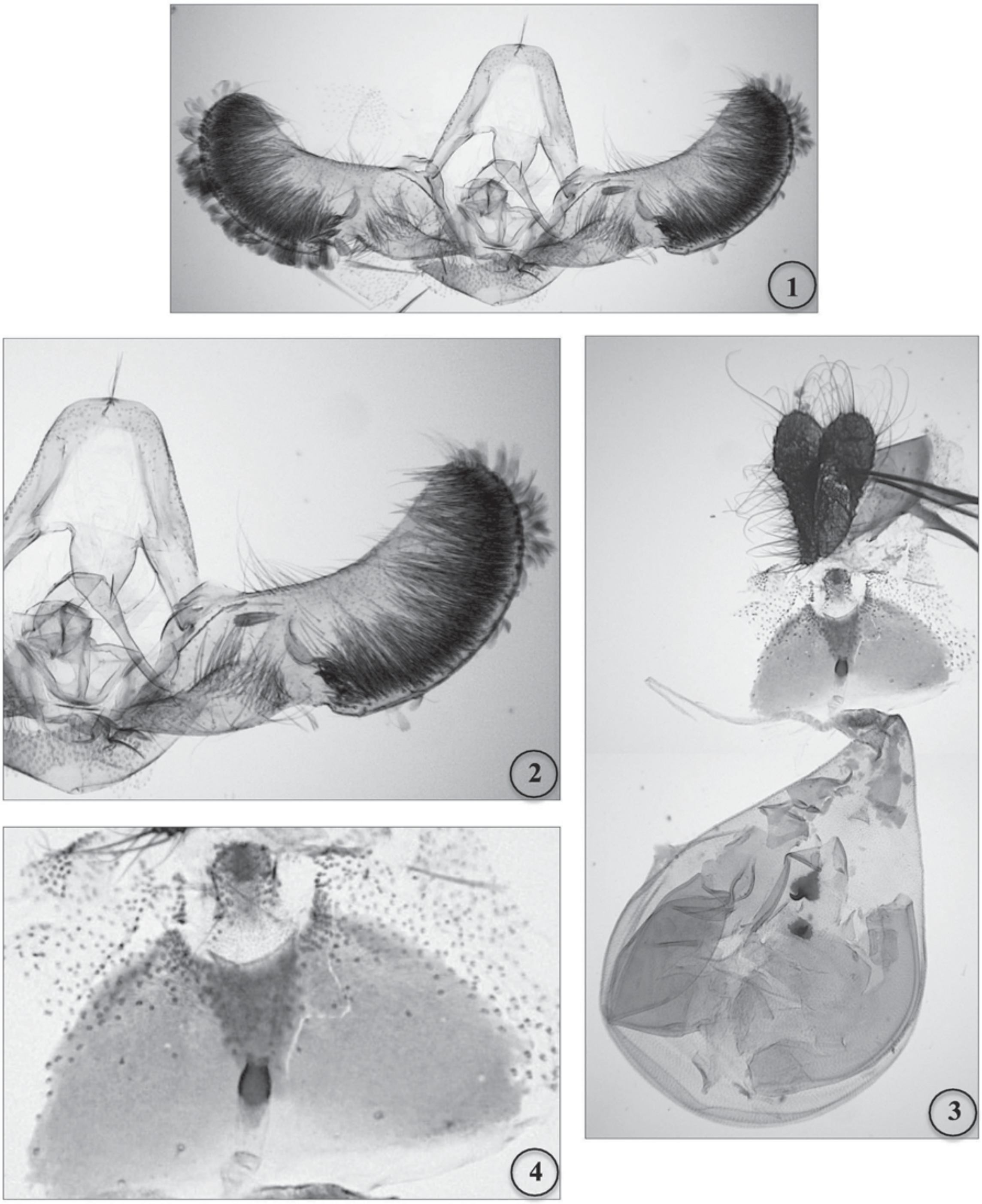

Fig. IV – *Cydia fagiglandana* (Zeller, 1841): male genitalia (1); tegumen, aedeagus and valva (2); Female genitalia (3); antrum and colliculum (4).

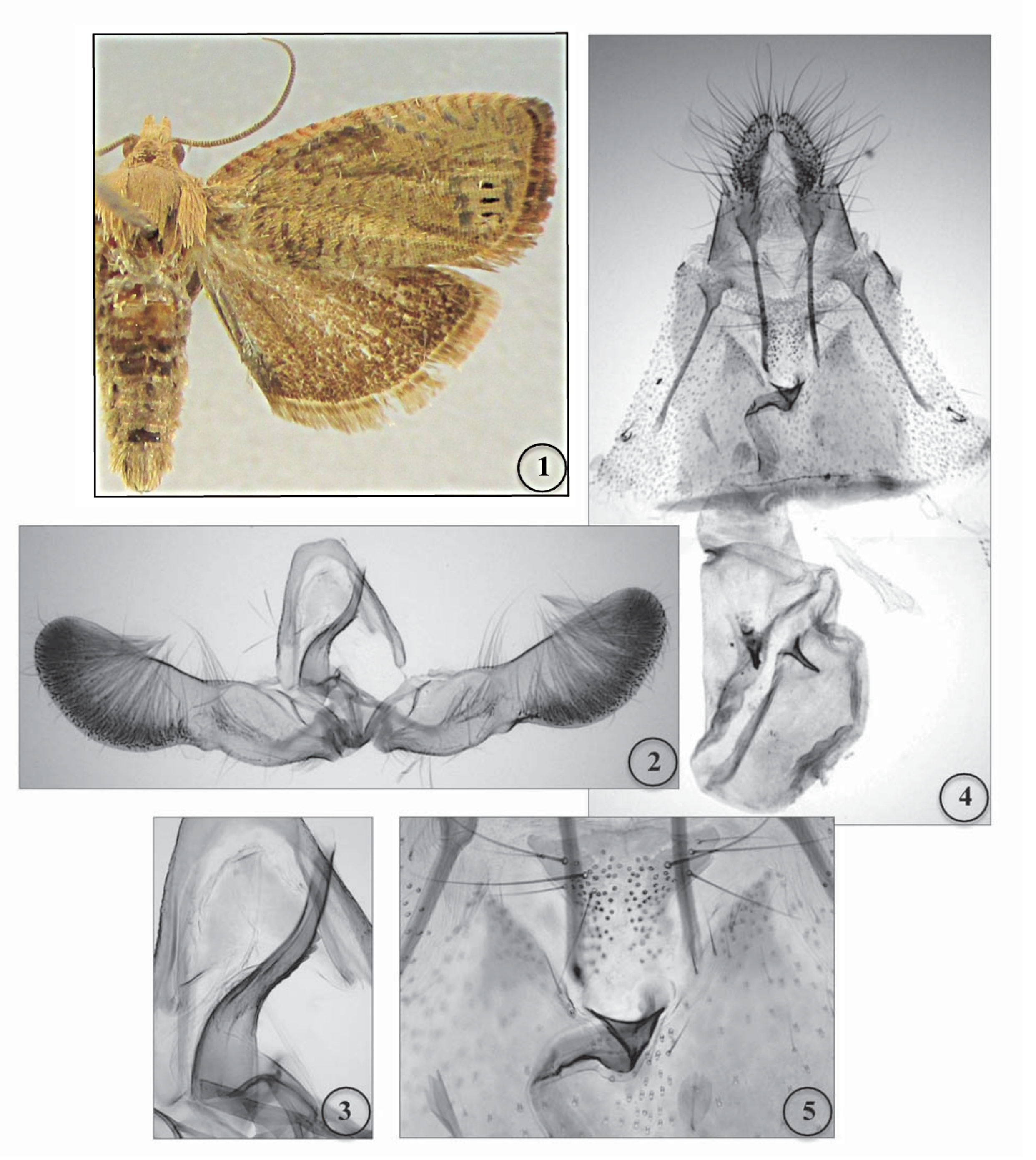

Fig. V – Cydia *rymarczki* Varenne & Nel, 2013: adult male (1); male genitalia (2); aedeagus (3); female genitalia (4); sterigma and antrum (5).

REDIA, 102, 2019: 55-60 http://dx.doi.org/10.19263/REDIA-102.19.08

PARISA ABDOLI [a] – ALIREZA POURHAJI [b]

DESCRIPTION OF A NEW SPECIES OF THE GENUS *PHOLETESOR* MASON, 1981 AND HOST ASSOCIATION FROM IRAN (BRACONIDAE MICROGASTRINAE)

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Abdoli P., Pourhaji A. – Description of a new species of the genus *Pholetesor* Mason, 1981 and host association from Iran (Braconidae Microgastrinae).

A new species of Microgastrine parasitoid wasps, *Pholetesor pseudocircumscriptus* Abdoli sp. nov., is described, and its host association, *Phyllonorycter corylifoliella* (Hübner, 1796) (Lepidoptera: Gracillariidae) is identified from Iran. The new species is fully illustrated and diagnosed. An identification key and updated checklist for the Iranian species of the genus *Pholetesor* along with their distribution, host associations and references are provided.

Key Words: Taxonomy, Microgastrinae, *Pholetesor*, new species, *Phyllonorycter corylifoliella*.

INTRODUCTION

The Braconidae is the second largest family of Hymenoptera, and the Microgastrinae Förster, 1862 is a large and diverse subfamily in the Braconidae with over 2,710 described species worldwide (Yu *et al.,* 2016). This subfamily is one of the most important groups of parasitoids in terms of both species richness and economic importance (Rodriguez *et al.*, 2013).

The genus *Pholetesor* Mason, 1981 is a relatively small group, consisting of 49 described species worldwide, including 12 in the West Palearctic region, 25 in the East Palearctic and 3 species in Iran (Liu *et al.,* 2016; Yu *et al.*, 2016).

The known species of *Pholetesor* (at the time considered to belong to *Apanteles*) were placed by Nixon (1965) in two species-groups (*circumscriptus* and *bucculatricis* groups). Later, Nixon (1973), followed by Papp (1983) and Tobias (1986) revised the European species (*circumscriptus* group of Nixon, 1973), whereas the Nearctic ones were revised by Whitfield (2006). Kotenko (2007) and Akhtar (2010) described four new species from the Russian Far East and one species from the Oriental region, respectively. Recently Liu *et al.* (2016) revised Chinese species of this genus and added eleven new species.

Previously, only three species of the genus *Pholetesor, P. circumscriptus* (Nees, 1834), *P. bicolor* (Nees, 1834) and *P. viminetorum* (Wesmael, 1837) were recorded from Iran (Yu *et al.*, 2016). *Apanteles pedias* Nixon, 1973 was synonymized with *Microgaster bicolor* Nees, 1834 by Papp (1983) and raised (as *Pholetesor bicolor*) from synonymy with *Pholetesor circumscriptus* (Nees) (e.g., see Shaw, 2012).

The species of *Pholetesor* are solitary koinobiont endoparasitoid that often parasitize leaf mining Lepidoptera, such as Gracillariidae, Elachistidae and Bucculatricidae (Papp, 1988; Whitfield, 2006; Shaw, 2012). The family Gracillariidae is one of the largest families of plant-mining Lepidoptera with 2000 species currently recognized (Fochezato *et al.*, 2018). *Phyllonorycter corylifoliella* with other related species such as *P. blancardella* and *P. turanica*, has become an important pest of rosaceous trees in many apple-producing areas in Iran (Radjabi, 1986). *P. corylifoliella* (Hübner) was first reported from Iran in 1970 and gradually spread to across north, northwest and central regions (Radjabi, 1986; Modarres Aval, 1997). Amiri *et al.* (2008) studied the species richness of the parasitoid complex of *P. corylifoliella* in Iran and found *Pholetesor bicolor* (Nees, 1834) as an important parasitoid of *corylifoliella*.

The objective of this study is to improve our knowledge of the genus *Pholetesor* in Iran.

MATERIAL AND METHODS

The specimens of the present study were collected from an apple orchard in East Azarbaijan province, northwest of Iran (Fig. I). Apple leaves with mines on the upper surface were kept in cylindrical plastic boxes (Diameter: 18 and High: 13 cm) at 25±2°C in greenhouse conditions, until the adults of miner moths or parasitoid wasps emerged. The specimens were identified using the keys of Nixon (1973), Tobias (1986), Kotenko (2007) and Liu *et al.*, (2016). Morphological terminology follows Wharton *et al.* (1997) for wing venation, and Karlsson & Ronquist (2012) for other characters. The abbreviations T1, T2, and T3 refer to metasomal mediotergites 1, 2 and 3; F2,8,15 refer to antennal flagellomeres 2, 8 and 15; POL means posterior ocellar line (minimum distance between the posterior ocelli), and OOL means ocular ocellar line (minimum distance between posterior ocellus and eye).

The species was photographed with an Olympus™ SZX9 stereomicroscope equipped with BMZ-04-DZTM digital imaging system (Behin Pajouhesh Co., Iran). The specimens are deposited in the insect collection of the

– Received 24 May 2018 Accepted 21 May 2019

Department of Entomology, Tarbiat Modares University (TMUC), Tehran and Plant Protection Department and East-Azarbaijan Agricultural and Natural Resources Research & Education Center, Agricultural Research, Tabriz (EANREC) Iran and Canadian National Collection of Insects, Agriculture and Agri-food Canada (CNC).

RESULTS

Pholetesor Mason, 1981

Pholetesor Mason, 1981: 37. Type species.
Apanteles ornigis Weed 1887: 6.

Diagnosis of *Pholetesor*: Members of this genus are diagnosed by having hypopygium short, usually pointed apically and without median striae although usually folded along the mid-line; ovipositor sheaths hairy throughout; ovipositor sheaths not longer than metatibia; T1 usually parallel-sided or narrowed towards apex and longer than wide but occasionally (*bucculatricis* group in the New World) wider at apex and only a little longer than wide; T2 wider than long, the sides either diverging posteriorly or parallel; T3 often smooth but sometimes basally or completely rugose; propodeum typically with two fields of irregular aciculations diverging forwards from the polished apical boss, the remainder of the surface variously sculptured; a more or less well-defined areola and costulae present in members of the *bucculatricis* group, but the carinae often obscured in dense rugosities; anterior margin of metanotum with a pair of sublateral setiferous projections; anteromesoscutum usually with weak and ill-defined punctures and strong microsculpture giving metallic reflections; some species of the *bucculatricis* group with coarse, separately defined punctures; vannal lobe of hind wing convex and hairy along the margin (Mason, 1981).

In the current study, one new species of the genus *Pholetesor* was collected in the Northwest of Iran. An identification key and updated checklist for the Iranian species of genus *Pholetesor* along with their distribution, host associations and references are provided (Table 1).

Pholetesor pseudocircumscriptus Abdoli sp. nov.

(Figs. II, 1-5; III, 1, 2)

Type material: Holotype: female, (TMUC), East Azarbaijan province, Maragheh (37°22′25″ N, 46°24′12″ E, 1726 m. a. s. l.), 06.vii.2017. Paratypes: Same data as holotype, 4 females, 5 males (TMUC), 3 females, 5 males (EANREC), 2 females, 1 male (CNC). Leg. Alireza Pourhaji

Diagnosis: Anteromesoscutum densely punctate; posterior half of T1 shallowly rugulose; T1 strongly narrowed at apex; posterior width of T1 distinctly shorter than median length of T2; T2 subtriangle, strongly narrowed basally and T2 greatest width 2.00× its length medially; T1 posteriorly and T2 basally more narrowed; T1–2 and basal half of T3 yellow (Fig. II, 4); in forewing, vein r shorter than vein 2RS (Fig. III, 1).

Description Female: Body length: 1.45 mm. Head: Antenna almost as long as body, its segments finely setose; F2,8,15 L/W ratio 2.70, 2.50, and 1.65; mouthparts not elongated; gena, lower face, vertex and clypeus punctate; lower face width/height ratio 1.20; basal width of mandible 2.40 × malar space (Figs. II, 1,2); length ratio of OOL/diameter of ocellus/POL: 1.06/0.04/0.80 (Fig. II, 3).

Mesosoma: Anteromesoscutum length/width ratio 0.70 and densely punctate (Fig. II, 3); notauli not defined; scutoscutellar sulcus narrow with scarcely visible crenulation; mesoscutellar disc with fine and sparse punctures to

Table 1 – Distribution, association hosts and references for the genus *Pholetesor* in Iran.

Species	**Sampling locality**	**references**	**hosts**
P. circumscriptus	Markazi	(Radjabi, 1986), reported as *Apanteles circumscriptus*	*Phyllonorycter corylifoliella*
	Alborz	(Shojai, 1989), reported as *Apanteles lautellus*	*Phyllonorycter platani* (Staudinger)
	no locality cited	(Behdad, 1991)	*Phyllonorycter blancardella* (Fabricius)
	Isfahan	(Ghahari *et al.*, 2010), reported as *Apanteles circumscriptus*	*Phyllonorycter blancardella*
	West Azarbaijan	(Samin *et al.*, 2014)	
	Fars	(Samin *et al.*, 2015), reported as *Apanteles circumscriptus*	
	East Azarbaijan	(Ghahari & van Achterberge, 2016)	
P. pseudocircumscriptus **sp.nov**.	West Azarbaijan	New species (present study)	*Phyllonorycter corylifoliella*
P. bicolor	Markazi	(Radjabi, 1986), reported as *Apanteles bicolor*	*Phyllonorycter corylifoliella*
		(Modarres Awal, 1997) as *Apanteles bicolor*	
	Ilam	(Ghahari *et al.*, 2011)	
P. viminetorum	Golestan	(Telenga, 1955)	

Fig. I – Map of Iran, with the province from which the new species was collected coloured yellow.

smooth; side of mesoscutellar disc with wider crenulate depression, and posterior area of mesoscutellum disc polished; metanotum almost smooth on anterior parts and with coarse wrinkles posteriorly; propodeum almost smooth; pronotum and propleuron smooth; prepectal carina absent; mesopleuron almost smooth on antero- and ventrolateral parts, weakly punctate; metapleuron smooth.

Wings: Fore wing: length: 2.15 mm; pterostigma length/width ratio 2.70, vein R1 slightly longer than pterostigma; vein R1 5.00 × as long as distance of vein R1 to vein 3RSb; vein r shorter than vein 2RS; 3RSa indistinct; areolet open; vein 1-CUb slightly longer than vein 1-CUa (Fig. III, 1). Hind wing: vein M+CU/1-M ratio 1.25; vein cu-a slightly curved, almost straight; vannal lobe of hind wing evenly convex and with almost even fringe of setae (Fig. III, 2).

Legs: Metacoxa punctate; metafemur length/width ratio 4.15; length ratio of metafemur/metatibial/metabasitarsus/ sum of other metatarsal segments: 0.55/0.70/0.32/0.50; metatibial spurs unequal; length of inner metatibial spurs slightly shorter than metabasitarsus half (Fig. II, 5).

Metasoma: T1 gradually narrowing posteriorly and its posterior half shallowly ruguslose; T1 length 1.80 × basal width; posterior width of T1 distinctly shorter than median length of T2 ;T2 with satin-like sheen and covered with minute points, T2 subtriangular and posterior width 2.00× its length medially; T3 longer than T2 (T3 1.60× as long as T2); ovipositor sheath slightly curved setose with uniform width and slightly narrowed apically; ovipositor sheath slightly shorter than metatibia half; ovipositor sheath length 0.33 mm; hypopygium short, usually pointed apically and without median striate with a weak fold along the mid-line.

Coloration: Body black; labrum, mandible, palpi, scape, tegula, legs, T1-2 and basal half of T3 yellow; pterostigma and wing venation light brown or brownish yellow (Fig. II, 5).

MALE: Antenna distinctly longer than body; scape brown; legs and tegula darker, reddish yellow to light brown; T1–3 black or dark brown; other characters similar to female.

NOTES: The newly described species runs in the keys by NIXON (1973), TOBIAS (1986) and KOTENKO (2007) to *P. circumscriptus* (Nees, 1834). The differences are: in *P. pseudocircumscriptus* T1 is weakly sculptured posteriorly, T1 length 1.80× basal width, T1 posteriorly and T2 basally more narrowed; T1-2 and basal half of T3 yellow; posterior width of T2 2.00× its medial length; body length 1.40-1.50 mm, whereas in *P. circumscriptus* T1 is smooth posteriorly, T1 1.50× as long as posterior width, T1 posteriorly and T2 basally in compared with *pseudocircumscriptus* rather less narrowed posteriorly; T1-3 black or blackish, less frequently yellow; posterior width of T2 1.50× (less than 2.00×) its medial length; body larger, about 1.80-2.00 mm.

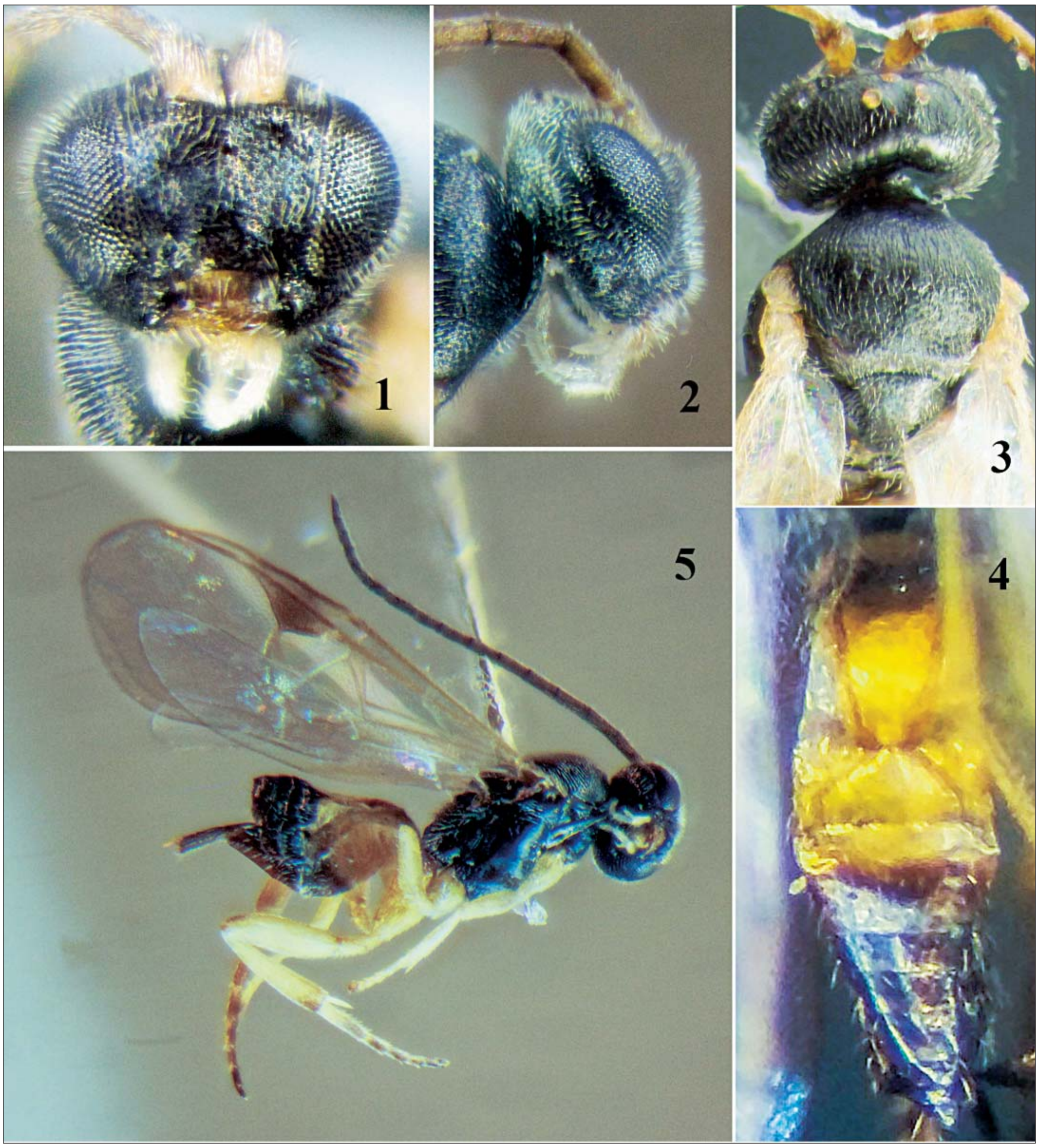

Fig. II – *Pholetesor pseudocircumscriptus* Abdoli **sp. nov.** (Holotype). Female: 1. Head, facial view; 2. Head, lateral view; 3. Head and mesosoma, dorsal view; 4. Metasoma, dorsal view; 5. General habitus, lateral view.

The new species is very similar to *P. bicolor* (Nees, 1834) [=*P. pedias* (Nixon, 1973), = *P. schillei* (Niezabitowski, 1910)]. The differences are: in *P. pseudocircumscriptus* sp. nov. the fore wing vein R1 is slightly longer than pterostigma and vein R1 5.00 × as long as distance of vein R1 to vein 3RSb, whereas in *P. bicolor* the vein R1 is short, not longer than pterostigma, and not more than 1.50–2.00× longer than the distance from it to apex of wing.

Also, *Pholetesor psudocircumscriptus* sp. nov. is very similar to *Pholetesor laetus* (Marshall, 1885), it differs from *P. laetus* as follows: in *P. psudocircumscriptus* sp. nov. anteromesoscutum densely punctate; posterior width of T1 distinctly shorter than median length of T2; T2 subtriangle; in forewing, vein r shorter than vein 2RS; F15 L/W ratio 1.65; body length 1.45 mm. (whereas in *laetus* anteromesoscutum without or with fine punctuation; T2 subrectangular; posterior width of T1 about equal to median length of T2; in forewing, vein r as long as vein 2RS); F15 L/W ratio 1.8-2.00; body larger, about 2.50 mm.

ETYMOLOGY: The species name “*pseudocircumscriptus*” is a Latin adjective referring to its resemblance to the species “*circumscriptus*”.

BIOLOGY: The type series was reared from larvae of *Phyllonorycter corylifoliella* (Hübner, 1796) [Lepidoptera: Gracillariidae] on its host plants (*Malus* sp. [family: Rosaceae]).

KEY TO SPECIES OF *PHOLETESOR* FROM IRAN (FEMALE SPECIMENS)

The following identification key is adapted for Iranian species based on original descriptions and NIXON (1973), PAPP (1983), TOBIAS (1986) keys with inclusion of the one new described species.

1 Vein R1 short, not longer than pterostigma and not more than 1.50-2.00 × longer than the distance from it to apex of wing; T1-2(-3) frequently yellow to vivid yellow, otherwise black or blackish ....***P. bicolor*** (Nees, 1834)

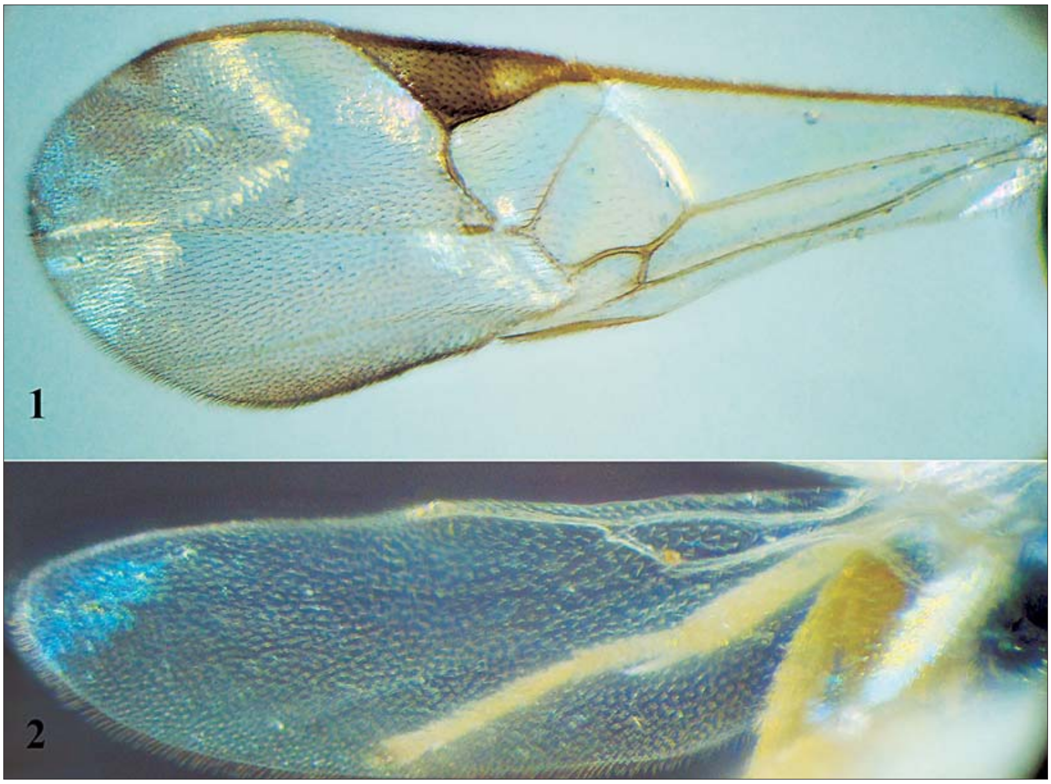

Fig. III – *Pholetesor pseudocircumscriptus* Abdoli **sp. nov.** (Holotype). Wings, female: 1. Fore wing; 2. Hind wing.

Vein R1 long, longer than pterostigma and not less than 3.00–4.00 × longer than the distance from to the apex of the wing (Fig. III, 1); T1–3 yellow or black 2

2(1) Ovipositor valves strongly expanded apically and sharply truncate; T1 only weakly or not at all narrowed posteriorly; anterior margin of postscutellum between the forwards-pointing projection and mid-point of postscutellum not concave and phragma of scutellum completely hidden, projection thus hardly emphasized, though its presence indicated by two minute, suberect hairs .... ***P. viminetorum*** (Wesmael, 1837)

- Ovipositor valve much less expanded apically (Fig. II, 5); T1 distinctly narrowed posteriorly and T1 posterior width not more than one third of basal width (Fig. II, 3); anterior margin of postscutellum between the forwards-pointing projection and mid-point of postscutellum concave and phragma of scutellum strongly revealed 3

3(2) T1 smooth posteriorly, T1 length 1.50× basal width and T1 posteriorly and T2 basally in compared with *pseudocircumscriptus* rather less narrowed posteriorly; posterior width of T2 1.50× (less than 2.00×) its medial length; T1–3 black or blackish, less frequently orange or yellow; body larger, about 1.80-2.00 mm .... ***P. circumscriptus*** (Nees, 1834)

- T1 shallowly rugulose posteriorly, T1 length 1.80× basal width and T1 posteriorly and T2 basally more narrowed; posterior width of T2 2.00× its medial length; T1–2 and basal half of T3 yellow (Fig. II, 4); body length 1.40-1.50 mm .... ***P. pseudocircumscriptus*** Abdoli **sp. nov.**

DISCUSSION

These samples were collected from the East Azerbaijan province in the Northwest of Iran (Table 1). The sampled regions in this study belong to the Irano-Anatolian hotspot that forms a natural barrier between the Mediterranean basin and the dry plateaus of western Asia (Kiani *et al.*, 2017), which is expected to harbour the highest biodiversity in Iran.

This study has added one new species of *Pholetesor*, increasing the total number to 50 species worldwide and 14 species in the West Palearctic. The number of Iranian *Pholetesor* species has risen from three to four, which compares to adjacent countries as follows: 12 species in Russia and one species in Turkmenistan (Yu *et al.*, 2016). Among several families that have been reported as *Pholetesor* hosts, over 50 percent of hosts belong to family Gracillaridae, an indication that perhaps this family is the most important host for this genus.

As regards, DNA barcoding data along with traditional taxonomy has improved the accuracy of identification, we are doing to get COI sequence of new species and all sequence data will available in BOLD and GenBank.

Finally, more research on *Pholetesor* is needed to identify biological characteristics for their use in pest control, or to protect and conserve them.

ACKNOWLEDGMENTS

This work is supported by East-Azarbaijan Agricultural and Natural Resources Research & Education Center, Tabriz. Thanks to Department of Entomology, Tarbiat Modares University for providing facilities. The authors wish to thank Dr. Helen Alipanah for confirming the identification of the Lepidoptera species which is host of parasitoid. We cordially thank Dr. Donatella Goggioli as Redia Managing Editor and Dr. Mark Shaw and Dr. Gavin Broad for their critical review and constructive comments, which significantly improved the paper. The senior author especially thanks Dr. Jose Fernandez-Triana (CNC) for his guidance and comments on earlier versions of this paper.

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REDIA, 102, 2019: 61-67 http://dx.doi.org/10.19263/REDIA-102.19.09

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AN ANNOTATED CHECKLIST OF SCIRTOIDEA (COLEOPTERA) OF IRAN

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Klausnitzer B., Ghahari H. – An annotated checklist of Scirtoidea (Coleoptera) of Iran.

The fauna of Iranian Scirtoidea (Coleoptera) is summarized in this paper. In total, three species of Clambidae (within the genus *Clambus* Fischer von Waldheim, 1821), one species of Eucinetidae (within single genus *Bisaya* Reitter, 1884), and 23 species of Scirtidae (within the genera *Contacyphon* Gozis, 1886, *Elodes* Latreille, 1797, *Hydrocyphon* Redtenbacher, 1858, *Microcara* C. G. Thomson, 1859, and *Scirtes* Illiger, 1807) are listed as members of the Iranian fauna.

KEY WORDS: Elateriformia, Clambidae, Eucinetidae, Scirtidae, distribution, new record, Iran.

INTRODUCTION

The superfamily Scirtoidea Fleming, 1821 (former Eucinetoidea) includes four families, Clambidae Fischer von Waldheim, 1821 (incl. Calyptomeridae), Decliniidae Nikitsky, Lawrence, Kirejtshuk & Gratshev, 1994, Eucinetidae Lacordaire, 1857, and Scirtidae Fleming, 1821 (= Cyphonidae, Helodidae, Elodidae) (LAWRENCE & NEWTON, 1995). Except for Decliniidae, the other families are represented in the fauna of Iran.

The family Clambidae (Minute Beetles) comprises about 150 species in five genera worldwide (LESCHEN, 2016). They vary in size from 0.7-2.0 mm, are convex and contractile, capable of deflecting their head and pronotum into a seed-like form (YOUNG, 2002b). Metacoxal plates enlarged and legs partly concealed; tarsi 4-4-4 or rarely 3-3-3; abdomen with five or six free ventrites; functional spiracles present on segment VIII (LESCHEN, 2016). They are found throughout the world on all continents (except for the Antarctic) and on many scattered and isolated islands in the Pacific and Indian Oceans (YOUNG, 2002b; MAJKA & LANGOR, 2009). Most adults are collected from decaying vegetation, leaf litter and rotten wood, and sometimes flying at dusk (YOUNG, 2002b). Most species are known to feed on microfungi or spores and hyphae on larger fruiting bodies of Ascomycetes, Myxomycetes, Basidiomycetes and Zygomycetes (LawrencE & NEWTON, 1980; WHEELER & HOEBEKE, 1984; LAWRENCE, 1991).

The family Eucinetidae (Plate-thigh Beetles) comprises eight genera and more than 40 valid species worldwide (VIT, 1999, 2000). Distinguishing features of adult Eucinetidae include a streamlined elliptical body and the ability to jump using their modified hind legs (KRYZHANOVSKIJ, 1989; MIFSUD, 2001; MAJKA, 2010; WEBSTER *et al*., 2012). The development of the two stages, the adults and the larvae, is strongly connected with the mould fungi, as the adults could be found also under bark or in wood of decayed trees (NIKITSKY, 1989; VIT, 2000). Little is known about the biology of these beetles, but most records result from collections made in leaf litter. In some cases it has been asserted that these insects feed on spores of slime moulds [Myxomycetes (=Mycetozoa)] or on the fruiting bodies of basidiomycete fungi (Agaricaceae, Bolitaceae, and Coniphoraceae) (WHEELER & HOEBEKE, 1984; YOUNG, 2002a; GUÉORGUIEV, 2004).

To date over than 1800 species of the family Scirtidae (Marsh Beetles) have been described. However, a total of at least 3000 species in all zoogeographic regions can be expected (KLAUSNITZER, 2016a, 2017). Important features for the specific identification of the adults (when examined externally) are: length ratios and form of the 1st-4th antennal segment, punctures of head and pronotum, shape of the pronotum, shape and hairiness of the 7th sternite, colouring (in some genera). Features which require special preparation: shape and fine structure of the 8th and 9th tergite and sternite, tegmen, penis (male), sclerites in bursa (prehensor and bursal sclerite) and the 8th sternite (female). For most species a genital preparation is necessary. Larvae are generally aquatic and frequent stagnant and flowing waters such as streams, lakes, ponds, swamps and sphagnum bogs (YOUNG, 2002c; WEBSTER *et al*., 2012), tolerating significant fluctuations of the water level, where they gather algae and other small particles from liquid substrates (MARINONI *et al*., 2001; YOUNG, 2002c). Sometimes larvae of some species develop in groundwater as deep as 10 meters (KLAUSNITZER & POSPISIL, 1991). Adults of some species can occur in many dry localities, including even arid places in deserts (KIREJTSHUK & NEL, 2013). Adults of some species can be predators, living under the vegetation and feeding on microinvertebrates (KLAUSNITZER, 2009b), and a few species are associated with decaying animal matter (PEREIRA FARIAS *et al*., 2013). Regarding the Iranian fauna, KLAUSNITZER (2016b, c) listed 18 species of Scirtidae for Iran including two new species.

Iran occupies a large part of the Iranian plateau, and covers an area of 1,623,779 km². Climatologically, Iran is a predominantly arid and semi-arid country, but the northern slopes of the Alburz ranges and the Caspian lowland receive 800 to 2000 mm annual rainfall, making them the most humid parts of the country. The Dasht-e Kavir and Dasht-e

– Received 7 January 2019 Accepted 22 May 2019

Lut deserts are the driest areas with an annual precipitation of less than 150 mm. The highlands receive between 250 and 800 mm (ZEHZAD *et al*., 2002).

MATERIAL AND METHODS

The published data on distribution of the families Clambidae, Eucinetidae and Scirtidae (Coleoptera: Scirtoidea) in Iran are summarized by province. Genera and species are listed alphabetically. The following data are included in the catalogue for each species: (1) valid name, (2) junior synonym(s) used in literature about Iran, (3) published Iranian records arranged by province (classified by alphabetical order of Iranian province names) and the relevant references (classified by chronological order), (4) synoptic information on general distribution on a world scale and feeding habits. Data about classification, nomenclature and distribution are according to LÖBL & LÖBL (2016 for Clambidae and Eucinetidae) and KLAUSNITZER (2016a for Scirtidae); in other situations, the related references are given. When accurate data about local distribution in Iran are lacking in a quoted reference, the mention "Iran (no locality cited)" is used. Additionally, the name "Persia" (as well as its equivalent German name "Persien") has been retained because this is traditional synonym for Iran. The provinces of Iran are represented in figure 1.

RESULTS

Totally 27 species of Scirtoidea (Clambidae: 3 species from one genus, Eucinetidae: one species, Scirtidae: 23 species from 5 genera) are represented in the Iranian fauna. *Clambus pilosellus* Reitter, 1876 (Clambidae) and *Contacyphon buceros* Nyholm, 1949 (Scirtidae) are new records for the fauna of Iran. The list of species is given below with distribution data.

Series Scirtiformia Fleming, 1821
Superfamily Scirtoidea Fleming, 1821
Family Clambidae Fischer von Waldheim, 1821
Subfamily Clambinae Fischer von Waldheim, 1821
Genus *Clambus* Fischer von Waldheim, 1821

Clambus caucasus Endrödy-Younga, 1960
Distribution in Iran: Qazvin (SAKENIN *et al*., 2018).

General distribution: Azerbaijan, Caucasus, Italy, Turkey.

Fig. I – Map of Iran with provincial boundaries.

Clambus dux xerxes Endrödy-Younga, 1960

Distribution in Iran: "Persien" (Endrödy-Younga 1960), Iran (no locality cited) (Klausnitzer & Merk,l 1987; Löbl & Löbl, 2016).

General distribution: Caucasus, Iran, Israel, Pakistan.

Clambus pilosellus Reitter, 1876 (Fig. II)

Material examined. East Azarbaijan province, Azarshahr, 20 km E Nadiloo, 37°43'N 45°54'E, 117 m, 1 ex, 10.viii.2004. *New record for Iran*.

General distribution: Austria, Azerbaijan, Bulgaria, Croatia, Georgia, Hungary, Italy, Nepal, Romania, Slovakia, Taiwan, Turkey.

Family Eucinetidae Lacordaire, 1857
Genus *Bisaya* Reitter, 1884

***Bisaya nossidiiformis* Reitter, 1884**

Distribution in Iran: Iran (no locality cited) (Löbl & Löbl 2016).

General distribution: Azerbaijan, Iran.

Family Scirtidae Fleming, 1821
Genus *Contacyphon* Gozis, 1886

Comments: All the below species have been recorded from Iran under *Cyphon* spp.

Contacyphon algenib (Klausnitzer, 2003)

Synonym: *Cyphon simulans* Klausnitzer, 1990: 160.

Distribution in Iran: Guilan (Klausnitzer 1990 as *C. simulans*; Klausnitzer 2003), Mazandaran (Klausnitzer, 2012b).

General distribution: Endemic to Iran.

Contacyphon arietizans (Nyholm, 1970)

Distribution in Iran: Iran (no locality cited) (Klausnitzer, 2009b).

General distribution: Armenia, Georgia, Iran, Turkey.

Contacyphon buceros (Nyholm, 1949) (Fig. II)

Material examined: Guilan province, Astara (in bogs of Mordab-Astara River), 38°20′N 48°46′E, 3 ex, 14.viii.2001. New record for Iran.

General distribution: Armenia, Azerbaijan, Georgia, Russia (South European Territory), Turkey.

Contacyphon caspicus (Klausnitzer, 1975)

Distribution in Iran: Iran (no locality cited) (Klausnitzer, 2006).

General distribution: Azerbaijan (Klausnitzer, 1975a, 1976), Iran (Klausnitzer, 2006).

Comments: Occurrence of *C. caspicus* in Iran is likely, although it has not been recorded so far from any precise locality.

Contacyphon laevipennis (Tournier, 1868)

Synonym: *Cyphon phragmiteticola* Nyholm, 1955.

Distribution in Iran: East Azarbaijan, Mazandaran (Klausnitzer, 1990 as *C. phragmiteticola*; Klausnitzer, 2012b), Guilan (Klausnitzer, 1981 as *C. phragmiteticola*; Klausnitzer, 2012b), Iran (no locality cited) (Klausnitzer, 2009b).

General distribution: Afghanistan, Albania, Armenia, Austria, Azerbaijan, Bulgaria, Byelorussia, Czech Republic, Denmark, Egypt, Estonia, Finland, France, Germany, Great Britain, Greece, Hungary, Iran, Iraq, Israel, Italy, Kazakhstan, Latvia, Lithuania, Luxembourg, Moldavia, Mongolia, Morocco, Netherlands, North Korea, Norway, Poland, Russia (Central European Territory, East Siberia,

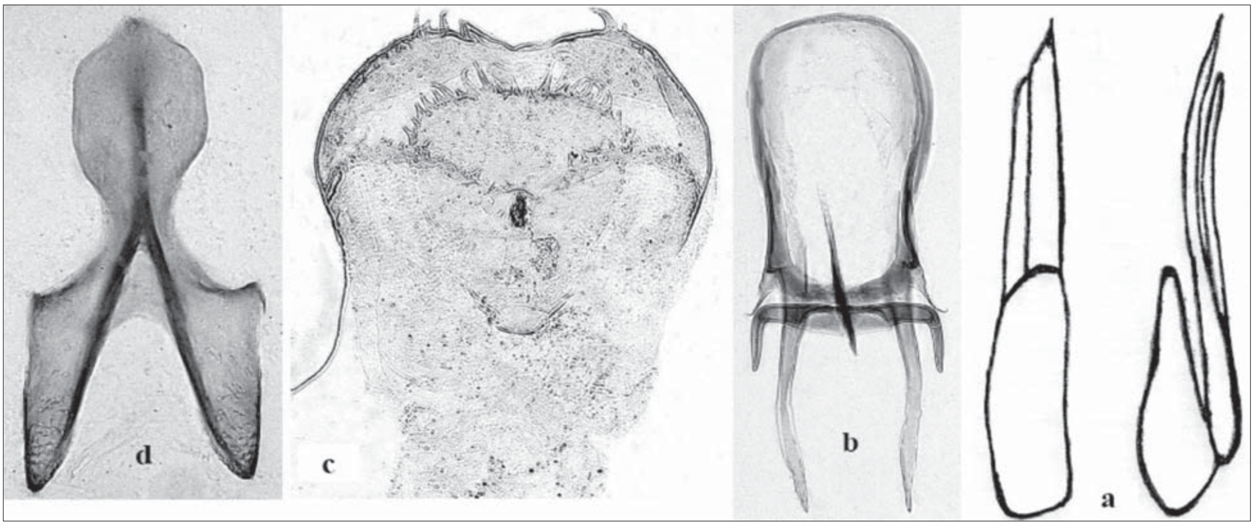

Fig. II – a: *Clambus pilosellus* Reitter, 1876: Penis (lateral and dorsal view). b-d: *Contacyphon buceros* Nyholm, 1949 (b: Penis, c: Prehensor, d: Tegmen).

North European Territory, South European Territory), Saudi Arabia, Slovakia, Spain, Sweden, Switzerland, Syria, Tadzhikistan, Tunisia, Turkey, Turkmenistan, Ukraine.

Contacyphon lepidulus (Nyholm, 1968)
Distribution in Iran: Guilan, Kordestan (KLAUSNITZER, 1990, 2012b), Iran (no locality cited) (KLAUSNITZER, 2009b; ZWICK *et al*., 2013).

General distribution: Azerbaijan, Cyprus, Greece, Iran, Iraq, Syria, Turkey.

Contacyphon longiusculus (Nyholm, 1970)
Distribution in Iran: Mazandaran (KLAUSNITZER, 2012b), Iran (no locality cited) (KLAUSNITZER, 2009b).

General distribution: Azerbaijan, Georgia, Greece, Iran, Israel, Turkey.

Contacyphon ochraceus ochraceus (Stephens, 1830)
Distribution in Iran: Iran (no locality cited) (SHAVRIN *et al*., 2015).

General distribution: Austria, Byelorussia, Czech Republic, Denmark, Finland, France, Germany, Great Britain, Hungary, Iran, Ireland, Italy, Kazakhstan, Latvia, Lichtenstein, Luxembourg, Netherlands, Norway, Poland, Russia (Central European Territory, North European Territory, South European Territory), Slovakia, Spain, Sweden, Switzerland, Turkey, Ukraine.

Contacyphon palustris (C. G. Thomson, 1855)
Distribution in Iran: East Azarbaijan, Golestan, Kordestan (KLAUSNITZER, 1990, 2012b, c).

General distribution: Albania, Algeria, Austria, Azerbaijan, Bulgaria, Byelorussia, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Great Britain, Greece, Hungary, Iran, Ireland, Israel, Italy, Kazakhstan, Latvia, Lebanon, Liechtenstein, Lithuania, Luxembourg, Netherlands, North Korea, Norway, Poland, Romania, Russia (Central European Territory, Far East, North European Territory, South European Territory), Slovakia, Spain, Sweden, Switzerland, Syria, Turkey, Ukraine.

Contacyphon pareuoplus (Klausnitzer, 2012)
Distribution in Iran: Mazandaran (KLAUSNITZER, 2012b), Iran (no locality cited) (ZWICK *et al*., 2013).

General distribution: Endemic to Iran.

Contacyphon perses (Nyholm, 1976)
Distribution in Iran: Guilan (Assalem - Holotype) (NYHOLM, 1976; KLAUSNITZER, 1990), Mazandaran (KLAUSNITZER, 1990, 2012b, c), Iran (no locality cited) (ZWICK *et al*., 2013).

General distribution: Endemic to Iran.

Contacyphon reitteri (Klausnitzer, 1976)
Distribution in Iran: Iran (no locality cited) (KLAUSNITZER, 2006).

General distribution: Azerbaijan (Lerik) (KLAUSNITZER, 1976), Iran (KLAUSNITZER, 2006).

Comments: Lerik is located near the Iranian border in southern Azerbaijan, between the tall mountains of the Talysh Mountains and the agricultural plain of the Lankaran Lowland.

Contacyphon simplex (Klausnitzer, 1981)
Distribution in Iran: Hamadan, Kermanshah, Kordestan, Mazandaran (KLAUSNITZER, 1990, 2012b, c), Tehran (Emameh - Holotype) (KLAUSNITZER, 1981), Iran (no locality cited) (ZWICK *et al*., 2013).

General distribution: Endemic to Iran.

Contacyphon sp.
Distribution in Iran: Fars (OSTOVAN & NIAKAN, 2005, 2006).

Genus *Elodes* Latreille, 1797

Elodes eberti Klausnitzer, 1970
Distribution in Iran: Fars, Zanjan (KLAUSNITZER, 1970, 1972, 2009b), Tehran (KLAUSNITZER, 1990, 2000, 2009b, 2012b), Iran (no locality cited) (KLAUSNITZER, 2001, 2009a, 2012a, 2016a).

General distribution: Azerbaijan, Iran, Turkey, Ukraine.

Elodes frenzeli Klausnitzer, 2016
Distribution in Iran: Isfahan (Quamsar, 10 km SW, Kamoo) (KLAUSNITZER, 2016c).

General distribution: Endemic to Iran.

Elodes orientalis Iablokoff-Khnzorian, 1973
Distribution in Iran: Hamadan (KLAUSNITZER, 2012b, c).

General distribution: Iran, Tadzhikistan.

Elodes persicus Klausnitzer, 1975
Distribution in Iran: Golestan (Gole Lovae) (KLAUSNITZER, 1975b, 2000, 2009a, 2012a, b), Semnan (KLAUSNITZER, 2012b, c), Iran (no locality cited) (KLAUSNITZER, 2001, 2016a).

General distribution: Endemic to Iran.

Elodes unicolor Klausnitzer, 2012
Distribution in Iran: Kerman, Yazd (KLAUSNITZER, 2012b).

General distribution: Endemic to Iran.

***Elodes* sp.**
Distribution in Iran: Qazvin (Klausnitzer 1990 as *Helodes* sp.).

Genus *Hydrocyphon* Redtenbacher, 1858

Hydrocyphon consolatorius Klausnitzer, 1990
Distribution in Iran: Fars (KLAUSNITZER, 1990, 2012b), Iran (no locality cited) (YOSHITOMI & KLAUSNITZER, 2003; YOSHITOMI, 2012; KLAUSNITZER, 2016a).

General distribution: Endemic to Iran.

Hydrocyphon keili Klausnitzer, 2016
Distribution in Iran: Hormozgan (Sikhoran) (KLAUSNITZER, 2016b).

General distribution: Endemic to Iran.

Hydrocyphon segrex Nyholm, 1972
Distribution in Iran: Lorestan (KLAUSNITZER, 1981, 2009b, 2012b), Iran (no locality cited) (YOSHITOMI & KLAUSNITZER, 2003; YOSHITOMI, 2012; KLAUSNITZER, 2016a).

General distribution: Caspian Sea (YOSHITOMI, 2012), Greece, Azerbaijan, Iran, Turkey (YOSHITOMI & KLAUSNITZER, 2003; KLAUSNITZER, 2016a).

Genus *Microcara* C. G. Thomson, 1859

Microcara luteicornis Reitter, 1888
Distribution in Iran: Mazandaran (KLAUSNITZER, 2012b, c).

General distribution: Azerbaijan, Georgia, Iran, Russia (South European Territory), Turkey.

Genus *Scirtes* Illiger, 1807

Scirtes orbicularis Panzer, 1793
Distribution in Iran: Guilan (KLAUSNITZER, 2012b, c).
General distribution: Austria, Azerbaijan, Belgium, Byelorussia, Czech Republic, Denmark, France, Georgia, Germany, Great Britain, Hungary, Ireland, Italy, Latvia, Netherlands, Poland, Russia (Central European Territory, South European Territory), Slovakia, Sweden, Switzerland, Turkey, Ukraine.

DISCUSSION

This checklist comprises 27 species within seven genera among which the genus *Contacyphon* with 13 recorded species is the most species-rich in Iran. Thus, of the 410 recorded species within 29 genera of Scirtoidea in the Palaearctic Region (LÖBL & LÖBL 2016; KLAUSNITZER, 2016a), just ~6.6% have so far been recorded from Iran. Also, nine species are endemic to Iran. Iran comprises 31 provinces and Scirtoidea have been collected from just 17 of them. The highest diversity is found in the provinces Mazandaran and Guilan with seven and six species, respectively (Fig. I). No species are known from other 14 provinces lack of sampling. The exact localities of six species are unknown (Iran - no locality cited). The checklist indicates that most species have been recorded from northern parts of Iran (Guilan and Mazandaran provinces), comprising various aquatic ecosystems. Additionally, samplings on Iranian Scirtoidea, allowing a probably better knowledge of the regional fauna, have not been so far organized systematically. Among the neighbouring countries of Iran, the fauna of Turkey, with 54 recorded species has been studied better than others, followed by Russia (Central European Territory, East Siberia, Far East, North European Territory, South European Territory, West Siberia) and Azerbaijan with 49 and 14 species, respectively (LÖBL & LÖBL, 2016; KLAUSNITZER, 2016a) (Fig. I). Additionally, Turkey and Azerbaijan both with 14 species, share the highest number of species with Iran than any other countries, followed by Russia (6), Armenia (3), Iraq (2), Afghanistan (1), Pakistan (1), Saudi Arabia (1), and Turkmenistan (1). However, since Iran is a large country with highly heterogeneous geographical regions and climates, surely there remain many further species to be discovered. Upon the several samplings and the fauna of areas adjacent Iran and other Palaearctic regions, this list is far from being complete. It is likely that the actual number of species of Scirtoidea occurring in Iran would probably exceed 50 species; further faunistic surveys systematically organized in several provinces of the country are strongly needed, as well as a more complete study of the available unidentified material deposited in different museums.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Rafal Ruta (University of Wroclaw, Poland), Dr. Petr Kment (National Museum of Prague, Czech Republic), Dr. Kee-Jeong Ahn and Dr. Jeong-Hun Song (Chungnam National University, South Korea), Dr. Csaba Csuzdi (Eszterházy Károly University, Hungary), and Dr. Peter Zwick (Schlitz, Germany) for contributions to this project. The research was supported by Islamic Azad University (Yadegar-e- Imam Khomeini (RAH) Shahre Rey Branch) and Senckenberg Deutsches Entomologisches Institut.

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REDIA, 102, 2019: 69-72 http://dx.doi.org/10.19263/REDIA-102.19.10

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FIRST RECORD OF *BLATTISOCIUS MALI* (OUDEMANS, 1929) IN HUNGARY ASSOCIATED WITH THE DROSOPHILID FLY *PHORTICA SEMIVIRGO* (MÁCA, 1977)

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Kerezsi V., Kiss B., Deutsch F., Kontschan J. – First record of *Blattisocius mali* (Oudemans, 1929) in Hungary associated with the drosophilid fly *Phortica semivirgo* (Máca, 1977).

The mite *Blattisocius mali* (Oudemans, 1929), as a new phoretic associate of drosophilid fly *Phortica semivirgo* (Máca, 1977), is firstly reported from Hungary. The host is known as a potential vector of nematode eyeworm infestation, *Thelazia callipaeda* Railliet & Henry, 1910.

Key Words: Mites, phoresis, fly.

INTRODUCTION

The males of drosophilid fruitflies of *Phortica* genus usually feed on the lacrimal secretions of animals and humans and play a role in the transmission of the canine thelaziosis nematode (*Thelazia callipaeda* Railliet & Henry, 1910), which parasitizes the conjunctival sac of dogs, cats, wild carnivores and sometimes even humans (Máca and Otranto, 2014). This canine thelaziosis nematode has been known as oriental eyeworm since its original geographical distribution is in the Far East where it is widely distributed. For now, *T. callipeada* is in expansion in large parts of Europe from Spain to Hungary (Marino *et al.,* 2018, Farkas *et al.,* 2018). In Europe, *Phortica variegata* (Fallén, 1823) is the only confirmed vector of the canine thelaziosis nematode, however, the other *Phortica* species, *Ph. semivirgo* (Máca, 1977), is also mentioned as a potential vector (Máca and Otranto, 2014).

Till today several mite species are recorded from different groups of flies (Samšiňák, 1979, 1989, Perotti and Braig, 2009, Mumcuoglu and Braverman, 2010, Perez-Leanos *et al.,* 2017), but the mites associated with drosophilids are scarcely investigated. Despite the fact that the first co-occurrence of mites with drosophilid flies are known from the Miocene and Upper Eocene (25-40 MYA) (Poinar and Grimaldi, 1990, Ross 1998), only a few recent cases of associations are presented (Poinar and Grimaldi, 1990, Lehtinen and Aspi, 1992, Perez-Leanos *et al.,* 2017).

In the framework of a local faunistic study on drosophilids, some mite specimens were found on the body of *Phortica semivirgo*. This mite species was the blattisociid *Blattisocius mali* (Oudemans, 1929) which was not reported from Hungary till today. The association of *B. mali* with drosophilid flies is poorly-known, only Lehtinen and Aspi (1992) presented some records from Finland, but nobody found the association of this mite with the drosophilid fly *Ph. semivirgo*.

MATERIALS AND METHODS

Two of the three investigated female mite specimens were found attached to two females of *Phortica semivirgo* collected by bottle traps containing red wine and apple vinegar. The traps were installed at 56 localities in Hungary. In the faunistic survey 250 *Phortica* individuals (88 females and 73 males of *Ph. semivirgo* respectively) were caught and examined. The third mite specimens were attached to a male of *Ph. semivirgo*, which was caught by an insect net when approaching to human eye. Collecting dates and localities of the three mite specimens: 1) from female host, 08/05/2018, Budapest (Hungary), 47°22'45"N, 19°03'05E" (motorway rest area); 2) from female host, 07/28/2018, Pilisvörösvár (Hungary), 47°37'58N", 18°52'55E" (forest edge); 3) from male host, 09/20/2018, Budapest (Hungary), 47°32'31"N, 18°56'58E" (forested area).

The collected drosophilids and mites were placed in 96% ethanol. Before examination, the collected mites were separated from the flies and they were placed to a slide into lactic-acid for a few days to clear them properly for identification. The investigated specimens were studied under scientific light microscope (Leica 1000DM). The drawings were made by using drawing tube of Leica DM1000. The investigated specimens are deposited in the Department of Zoology, Plant Protection Institute, Centre for Agricultural Researches of Hungarian Academy of Sciences. Measurements are given in μm.

RESULTS

***Blattisocius mali* (Oudemans, 1929)**
(Fig. I).

Diagnosis – Idiosoma: Oval, color yellowish-brown, length 500-510, width 352-355. Length of legs: I 390-450, II 358-392, III 352-361, IV 474-480, chelicera: 134-136. Dorsal shield undivided and without midlateral incisions. Dorsal setae ca 48-60, smooth and needle-like, sculptural pattern on dorsal shield reticulate (Fig. I, 1). Sternal shield with 3 pairs of setae, st4 situated on soft cuticle. Metasternal shields absent. Ventrianal shield subtriangular, its anterior margin concave, bearing 3-4 pairs of ventral setae

– Received 19 October 2018 Accepted 27 May 2019

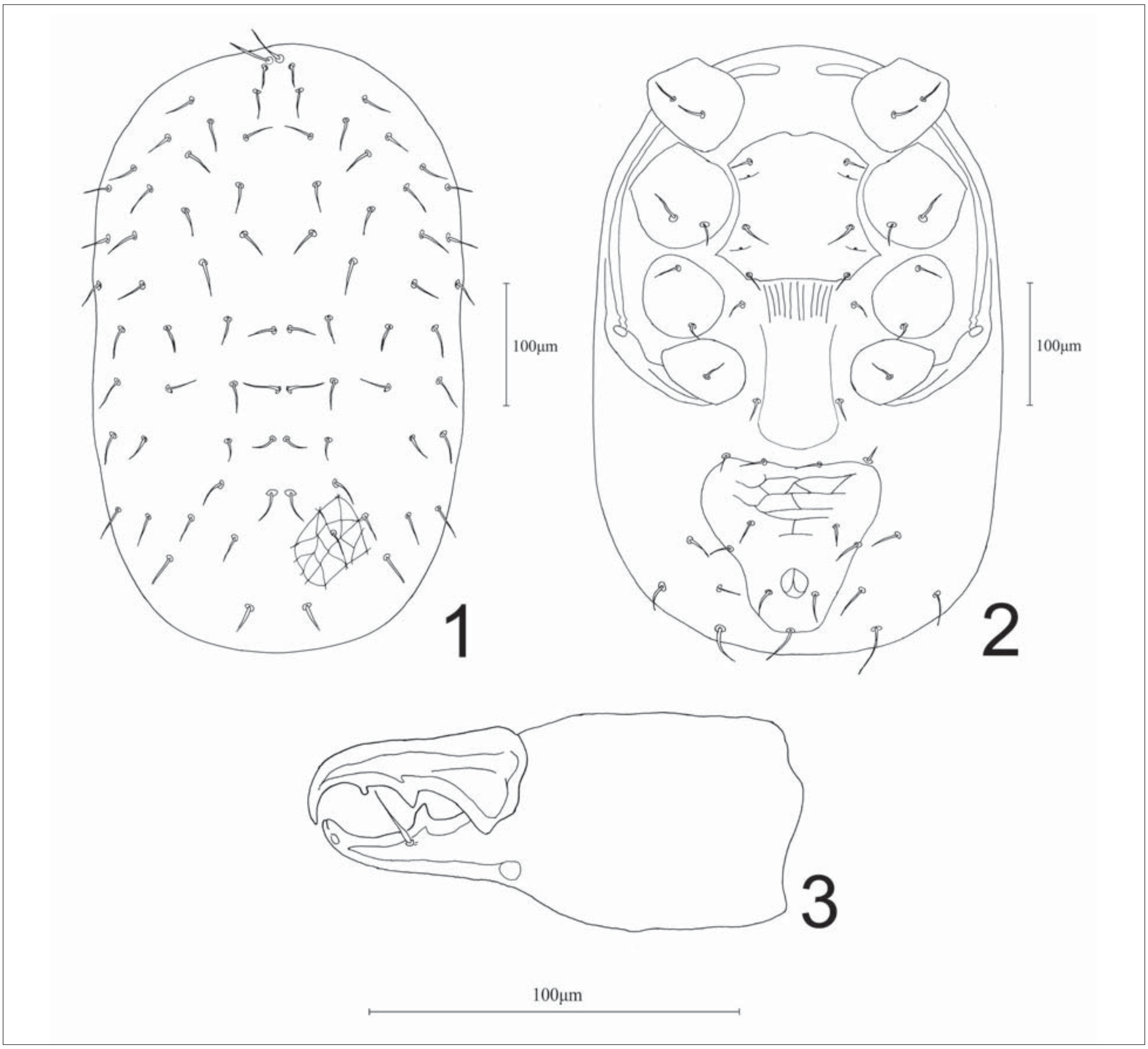

Fig. I – *Blattisocius mali*, from Hungary, female, 1. dorsal view of idiosoma, 2. ventral view of idiosoma, 3. lateral view of chelicera.

and with one pair of adanal and one postanal setae. Adanal setae inserted at level of posterior margin of anal opening. Peritrematic shield slender, posterior part curved and reaching to posterior margin of coxae IV (Fig. I, 2). Chelicera robust, movable digit with a bigger and a smaller tooth, fixed digit with a central tooth and a filiform pilus dentilis (Fig. I, 3).

DISTRIBUTION – Europe and North-Africa (KARG, 1993), China and Taiwan (ZHANG and FAN, 2010), India (MODAK *et al.,* 2004) and Turkey (ÇAMAK *et al.,* 2011). This species is reported from North-America as well (FARRIER and HENNESSEY, 1993), originally described as *Lasioseius (Paragarmania) bakeri* Chant, 1958 and synonymized with *Blattisocius mali* by HAINES (1978), which was also collected in an association with the North-American drosophilid, *Drosophila hexastigma* Patterson & Mainland, 1944 (PEREZ-LEANOS *et al.,* 2017).

BIOLOGY – This species occurs in agricultural areas (on plants and in soil) and in natural habitats as well (KARG, 1993). Very often occurs in stored product as well (e.g. apricot, fig, corn, wheat, sunflower, oat, bran, raisin, hazelnut) (ÇAMAK *et al.,* 2011), where it is an important predator of pest astigmatid mites (DE MORAES *et al.*, 2015). However, it was rarely found in association with drosophilid flies (LEHTINEN and ASPI, 1992; CHANT, 1958, PEREZ-LEANOS *et al.,* 2017).

INFESTATION RATE – Considering all examined *Phortica* specimens, the infestation rate (2/250) was less than 1 %. Both female mite specimens were attached to the leg in the case of female host, which is similar to observation of LEHTINEN and ASPI (1992) in other drosophilid flies, but the male host specimen carried its phoront on the ventral part of thorax (Fig. II).

DISCUSSION

The mite species, *Blattisocius mali* was collected on the body of the drosophilid flies, namely *Drosophila littoralis*

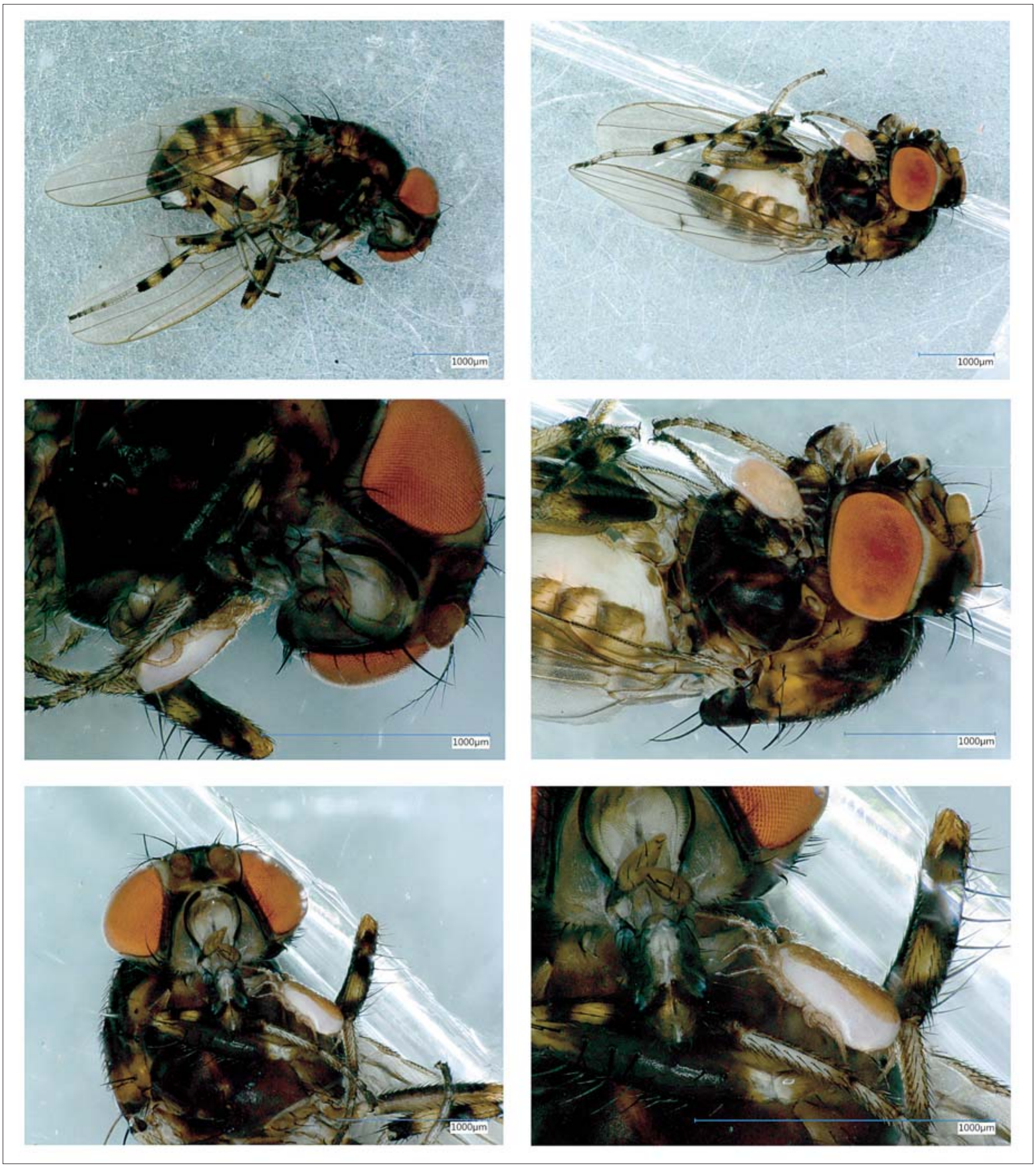

Fig. II – *Blattisocius mali* attached to the drosophilid fly *Phortica.*

Meigen, 1830; *D. montana* Stone, Griffen & Patterson, 1941; *D. lummei* Hackman, 1972; *D. ezoana* Takada & Okada, 1958; *D. transversa* Fallén, 1823 and *D. subarctica* Hackman, 1969 (Lehtinen and Aspi, 1992). These drosophilids belong to the subfamily Drosophilinae, contrary with the Hungarian host *Phortica semivirgo* which belongs to subfamily Steganinae. Our record is the first confirmed occurrence of this mite on drosophilid fly *Ph. semivirgo* and it is the first record of the *B. mali* associated with a member of the subfamily Steganinae. The three incidences of these mites collected on *Ph. semivirgo,* in contrast with the lock of such observations on thousands of *Drosophila suzukii* (Matsumura, 1931) and *D. melanogaster* Meigen, 1830 examined under microscope in the framework of the same projects (Kiss B., personal communication), may suggest that some factor specifically predisposes *Ph. semivirgo* for this type of acarian infestation. Unfortunately, the biology of *Ph. semivirgo* and *B. mali* are both poorly known for forming more concrete hypothesis on the nature of such potential predisposition.

ACKNOWLEDGEMENTS

This study was supported by: OTKA 108663 and NKFI-6 K119844.

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ABUNDANCE AND DIVERSITY OF MACRO-MOTHS IN AN *ACER*-DOMINATED FOREST OF THE POLLINO NATIONAL PARK, SOUTHERN ITALY (LEPIDOPTERA MACROHETEROCERA)

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Greco S., Ienco A., Scalercio S. – Abundance and diversity of macro-moths in an *Acer*-dominated forest of the Pollino National Park, southern Italy (Lepidoptera Macroheterocera).

Very few data are available on the biodiversity hosted by *Acer*-dominated forests in Europe, despite their importance for biodiversity conservation. In this paper we describe the moth assemblage of the *Acer*-dominated forest of the Special Area of Conservation (SAC) Monte Sparviere, southern Italy, where probably the highest diversity of maple species (6) is hosted at national level. We settled up ten monitoring sites, representative of the habitat complexity of the area, where moths were sampled monthly from March to November 2017. We found 371 species of Macroheterocera, among which tree-feeding species (in the larval stage) were particularly abundant and the presumably *Acer*-feeding *Nothocasis rosariae* was dominant. Despite a great homogeneity among individual species assemblages (the 26 most abundant species shared by all sites), the quantitative analysis of samples clearly separated pure *Acer* forests from mixed and *Alnus* forests, highlighting the importance of quantitative data for improving the use of nocturnal Lepidoptera as bioindicators. The richness of species at their boundary range and of conservation concern, make this area one of the most important for the conservation of Lepidoptera in southern Italy.

KEY WORDS: maple trees, Natura 2000 Network, Habitat Directive, species assemblage.

INTRODUCTION

Acer species are recognized to characterize two European forest types (EFTs): 5.4 Maple-oak forest and 5.6 Maple-lime forest, both Mesophytic deciduous forest (BARBATI *et al.*, 2014). In Italy they are largely distributed and present in several forest types as accessory species, composing characteristics forests on a surface of 177,504 ha only, of which 153,904 as maple-lime mountain forests and woods with ash tree and other species, and 23,600 ha as Apennine maple forests (GASPARINI and TABACCHI, 2011), for a total of the 1.7% of the Italian forested areas.

Despite the small surfaces occupied by these forests, they have a great importance from a conservation point of view as the Tilio-Acerion forests of slopes, screes and ravines (Code: 9180), the most widespread *Acer*-dominated forest, is of priority importance in the Habitat Directive 92/43/CEE. The importance of maple species for biodiversity increases in southern Italy where the endemic *Acer cappadocicum lobelia* (Ten.) A.E. Murray is present. One of the most important Italian *Acer*-dominated forest is on the north-eastern slope of the Sparviere Mount, in the Pollino National Park, Calabria, the southernmost region of the Italian peninsula. It is included within the Special Area of Conservation (SAC) Monte Sparviere (Natura2000 site code: IT9310019), where probably the highest diversity of maple species is hosted at national level. In detail, *Acer pseudoplatanus* L. is the dominant species, associated, according to local edaphic conditions, to *Acer opalus* Mill., *Acer monspessulanum* L., *Acer campestre* L., *Acer cappadocicum lobelia* (Ten.) A.E. Murray, and *Acer platanoides* L. (AVOLIO, 1993). In the next future, this forest can provide important genetic resources for the conservation of more than one *Acer* species as marginal and peripheral populations are present (DUCCI, 2015).

Studies concerning the insects living in maple forests are mainly addressed to their role as pests, such as the beetle *Glycobius speciosus* (Say) (HORSLEY *et al.*, 2002; DUKES *et al.*, 2009) and the moth *Paraclemensia acerifoliella* (Fitch) (PARKER *et al.*, 1983; DUKES *et al.*, 2009). The insect diversity of maple forests is studied and reported mostly for North America habitats (TERRIEN *et al.*, 1999; GERING and CRIST, 2000; BENTZ and TOWSEND, 2005; SUMMERVILLE and CRIST, 2005; MAJKA, 2010; MAGUIRE *et al.*, 2016; MLYNAREK *et al.*, 2018). In Europe few data are available (LESLIE, 2005; WOJTERSKA *et al.*, 2012), mostly for the forests belonging to the Rete Natura 2000 sites, and limited to the species included in the Annex II and IV of the Habitat Directive. Sporadic data can be gathered from faunistic papers, but these are usually hardly attributable to sites located within *Acer*-dominated forests.

Although several Lepidoptera species are trophically linked to *Acer* species, few data on the abundance of non-pest species with larvae feeding on *Acer* or living in maple-dominated forest ecosystems are available. This tree genus appears to be of particular importance in southern Italy as some moths of great biogeographic interest have larvae supposedly feeding on *Acer*, such as the notodontid *Ptilophora variabilis* Hartig, 1968 and *P. nebrodensis* Infusino & Scalercio, 2018, recently recognized as Italian endemic species (INFUSINO *et al.*, 2018a), and the geometrid *Nothocasis rosariae* Scalercio, Infusino & Hausmann, 2016, described few years ago, known for southern Italy and Greece only and likely by error related to *Fagus sylvatica* as hostplant (SCALERCIO *et al.*, 2016).

Recently, the most interesting faunistic findings obtained

– Received 7 January 2019 Accepted 28 May 2019

during a standardized survey of nocturnal Lepidoptera carried out in the Monte Sparviere *Acer* forest were published (GRECO *et al.*, 2018a,b). Most of the species treated in this paper have there their southern range limit, elucidating the importance of this forest as a reservoir of a portion of biodiversity usually distributed at higher latitudes. In this paper we provide the complete dataset gathered during this survey describing for the first time a complete taxocoenosis for this forest type. Furthermore, we highlight the importance of abundance data as a measure of functional relationships between phytophagous Lepidoptera and their foodplants and for the use of this taxon as a bioindicator of forest ecosystems.

MATERIAL AND METHODS

Ten light traps were positioned in the Special Area of Conservation (SAC) Monte Sparviere (Natura2000 site code: IT9310019), in the eastern part of the Pollino National Park, southern Italy (Fig. I). All surveyed localities are included in the municipality of Alessandria del Carretto, at the northern administrative border of Calabria region (Fig. I).

Sampling sites were chosen in order to cover the habitat complexity of the *Acer* forest, from pure and mature stands with trees older than 100 years to stands mostly covered by young renewal of maple trees, from a clearing with *Pyrus* trees (*Pyrus pyraster* (L.) Burgsd.) to a pure stand of alder (*Alnus cordata* (Loisel.) Desf.) at the margin of the *Acer* forest, from stands in the dry facies of the forest to stands along water courses (Table 1).

Sampling was carried out from late March to mid-November 2017 (23rd March, 18th April, 19th May, 21st June, 19th July, 17th August, 18th September, 12th October, 12th November). *UV*-LED light traps (see INFUSINO et al., 2017a) were settled-up during nights favorable to the activity of moth and to the attractive power of traps, i.e. low wind speed, temperature not lower than the average of the period, no rain, no moonlighting. Traps were turned on at dusk and moths were collected the morning after. Specimens were counted and identified at species level using, in most difficult cases, the morphology of genitalia mounted on slides. Voucher specimens are deposited in the collection of the Council for Agricultural Research and Economics, Research Centre for Forestry and Wood (CREA-FL), Rende (Cosenza), Italy. Nomenclature follows the most updated version of Fauna Europaea (KARSHOLT and NIEUKERKEN, 2013). Species are listed in alphabetical order within any family. In the faunistic list (Appendix 1) we report for any species the number of individuals collected in each stand, the total abundance, the number of sites where they were found, and the phenology indicated with the months of sampling in roman numbers.

The stand/species matrix with abundance data was submitted to Cluster Analysis (Past, version 2.17c) in order to group species assemblages according to their similarity. We used paired groups as algorithm and the abundance-based Morisita index as similarity measure.

RESULTS

We collected 12,007 specimens belonging to 371 species (see Appendix 1). Individual stands showed a richness

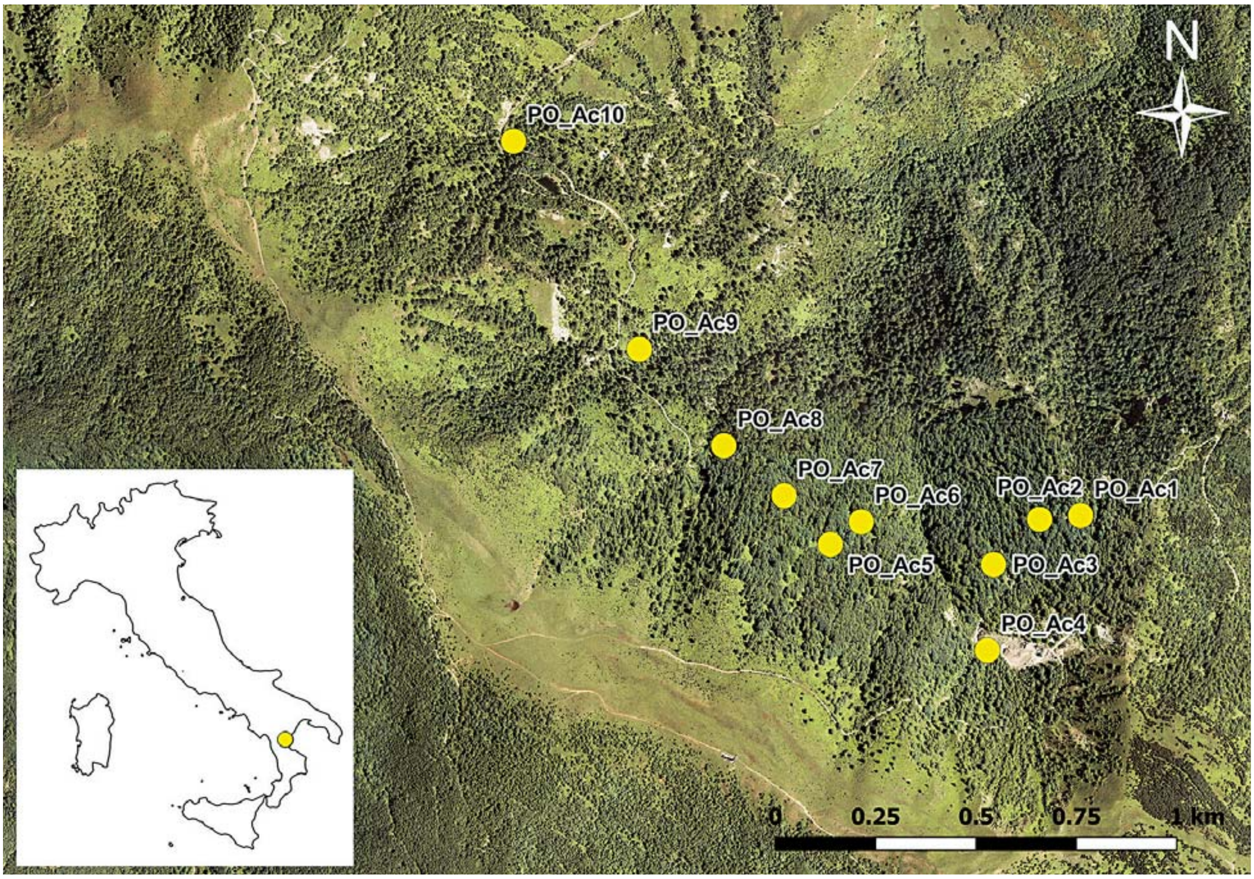

Fig. I – Location of study area (from GRECO et al., 2018a, modified).

Table 1 – List of sampled sites and their description.

Site Code	Coordinate (lat-long)	Altitude (m a.s.l.)	Locality	Short description
PO_Ac1	39.9275; 16.3636	1371	Destra Balestrieri	Mature forest with maple trees older than 100 years
PO_Ac2	39.9275; 16.3624	1337	Destra Balestrieri	Forest with unevenly aged maple trees
PO_Ac3	39.9259; 16.3603	1305	Vallone Lupara	Mixed forest of broadleaves with unevenly aged trees
PO_Ac4	39.9245; 16.3609	1345	Vallone Lupara	Young mixed forest of alder and maple along a water course
PO_Ac5	39.9269; 16.3563	1314	Difesa di Privitera	Mature forest with maple trees older than 100 years
PO_Ac6	39.9274; 16.3572	1285	Difesa di Privitera	Small clearing with *Pyrus* trees in mature maple forest
PO_Ac7	39.9280; 16.3550	1291	Difesa di Privitera	Mature forest with maple trees older than 100 years
PO_Ac8	39.9291; 16.3532	1253	Sciortaglie	Sparse old maple trees in a *Pteridium aquilinum*-dominated area
PO_Ac9	39.9313; 16.3508	1246	Sciortaglie	Mixed stand of young alder and maple trees
PO_Ac10	39.9358; 16.3471	1253	Tappaiolo	Stand of unevenly aged alders

varying from 151 species found in the alder woodlot at the margin of the study area (PO_Ac10), to 209 found in the stand nearest to it (PO_Ac9) (Table 2). Many species (104) were collected at least in eight stands showing a relative homogeneity of species assemblages, as confirmed by the low number of exclusive species, ranging from two to 13, and their very low abundance (not more than 3 individuals) (Table 2). The 26 most abundant species are present in all sampled sites and cover 58.3% of the entire sample.

The most abundant species was *Nothocasis rosariae* (Geometridae) (n=1263), followed by *Alcis repandata* (n=574), and *Cabera pusaria* (n=456). Represented by more than 100 individuals were also the tree-feeding *Campaea margaritaria*, *Opisthograptis luteolata*, *Epirrita christyi*, *Allophyes corsica*, *Colotois pennaria*, *Ptilophora variabilis*, *Asteroscopus sphinx*, *Diloba caeruleocephala*, *Poecilocampa alpina*, *Cosmia trapezina*, and *Ptilodon cucullina*, and other 12 species feeding mainly on herbs and vegetal debris.

Among the three most abundant species within individual stands, *Nothocasis rosariae* was the most recurrent (eight out of ten stands), followed by *Alcis repandata* (5/10), *Xestia stigmatica* (3/10), *Cabera pusaria* and *Hypena proboscidalis* (2/10), and ten more species recurring only once (Table 2). Consistently with this observation, the first dichotomy on the tree obtained by Cluster Analysis (cophenetic correlation: 0.8868) separated the two stands without *N. rosariae* among dominant species from the others (Fig. II). This grouping is also consistent with the different composition of the tree cover, being the two separately grouped stands dominated by the alder *Alnus cordata* (Loisel.) Duby. The mixed composition of the tree cover in PO_Ac4 (see Table 2) determined the secondary separation of this species assemblage from the other maple woodlots. An important role in separating species assemblages was played by *Cabera pusaria* of which larvae feed primarily on alders.

At the beginning of spring, the species assemblage was characterized by species belonging to the genus *Orthosia*, mainly *O. gothica*, *O. incerta*, *O. cruda*, *O. cerasi*, and with few individuals *O. populeti* and *O. rorida* (Fig. III). Also the overwintering adults of *Conistra vaccinii* and *C. rubiginea* were abundant. Significant changes occurred only in May, when few individuals of *O. gothica* are still on flight and the assemblage was dominated by *Colocasia coryli* and *Peribatodes rhomboidaria*. From June to September four species, namely *Campaea margaritaria*, *Peribatodes rhomboidaria*, *Hoplodrina ambigua* and *Hypena proboscidalis*, were constantly among the most abundant, accompanied by different species as the season proceeded (Fig. III). The beginning of the summer is characterized by *Xanthorhoe montanata*, *Calliteara pudibunda* and *Charanyca apfelbecki*, whilst later the following species became more abundant: *Cabera pusaria* in July and August, *Alcis repandata*, *Xestia triangulum*, and *Eilema lurideola* in July only, *X. rhomboidea*, *Opistograptis luteolata*, and *E. complana* in August only. Late-summer assemblage was mainly characterized by *Eugnorisma depuncta*, accompanied by the first individuals of autumnal species. In September we observed the peak of abundance for *Nothocasis rosariae* that was the most abundant species until November. The first part of the autumn was characterized by *Trigonophora flammea*, *Mesotype parallelolineata* and *Tiliacea sulphurago*, later accompanied by *Allophyes corsica* and *Diloba caeruleocephala*. November was characterized by the abundance peak of *Epirrita christyi*, *Colotois pennaria*, *Ptilophora variabilis*, *Asteroscopus sphinx* and *Poecilocampa alpina*, found in October with only very few individuals.

DISCUSSION

The *Acer*-dominated forest of the Monte Sparviere is inhabited by a specialized moth species assemblage, dominated by *Nothocasis rosariae*, recently recognized as bona species. The larva of this species feeds on *Acer* trees,

Table 2 – Sampling results in the investigated maple stands. The number of species (S), the number of exclusive species (Sexcl), the number of individuals (N), the dominant species and the incidence of dominance species (%) are reported for each stand.

Stand	S	Sexcl	N	Dominant species	Incidence of dominant species (%)
PO_Ac1	166	11	805	*Nothocasis rosariae*	18.5
				Opistograptis luteolata	
				Eilema lurideola	
PO_Ac2	187	4	1248	*Xestia triangulum*	17.4
				Alcis repandata	
				Nothocasis rosariae	
PO_Ac3	199	13	1408	*Nothocasis rosariae*	28.1
				Alcis repandata	
				Epirrita christyi	
PO_Ac4	176	6	2127	*Nothocasis rosariae*	41.0
				Cabera pusaria	
				Alcis repandata	
PO_Ac5	160	2	829	*Nothocasis rosariae*	18.6
				Xestia stigmatica	
				Alcis repandata	
PO_Ac6	201	9	1399	*Nothocasis rosariae*	19.7
				Othosia gothica	
				Alcis repandata	
PO_Ac7	182	5	1102	*Nothocasis rosariae*	16.6
				Eugnorisma depuncta	
				Colocasia coryli	
PO_Ac8	161	9	1080	*Nothocasis rosariae*	19.2
				Hypena proboscidalis	
				Ptilophora variabilis	
PO_Ac9	209	13	1236	*Eilema complana*	12.4
				Allophyes corsica	
				Xestia stigmatica	
PO_Ac10	151	6	773	*Cabera pusaria*	23.8
				Hypena proboscidalis	
				Xestia stigmatica	

as known for the congeneric *N. sertata*. Scalercio *et al.* (2016) supposed this species feeding on *Fagus sylvatica* L., but its high abundance in this *Acer*-dominated forest, where no *Fagus* trees were observed, leave little doubt about its larval foodplant. Among other top-scoring species in abundance forest species the presence of *Ptilophora variabilis* is remarkable, another species supposed to have larvae feeding on *Acer*, an endemic of the Italian Peninsula (Infusino *et al.*, 2018a). Very interesting is also the presence of *Eupithecia inturbata* and *Cyclophora albiocellaria*, two species with monophagous larvae on *Acer* (Mironov, 2003; Hausmann, 2004). These species are here at their southern range border in the Italian Peninsula (Greco *et al.*, 2018b), whilst *N. rosariae* and *P. variabilis* were also found southwards in other forest types where *Acer* trees are accessory species only (Infusino *et al.*, 2017b, 2018b; Scalercio and Greco, 2018). Compared to the species assemblage sampled in the beech forest of the same Massif by Infusino and Scalercio (2018), the main difference of the *Acer*-dominated forest was the rarity of *Operophtera fagata*, very abundant in the beech forest and here represented with just two individuals, whilst most of the other species with larvae feeding on broadleaved trees are similarly abundant.

Quantitative similarity analysis showed a clear difference of species assemblages sampled within alder-dominated forests from those of maple-dominated forests. Although a great number of species is shared between these broadleaved forests, moth assemblages are clearly separated by the mean of the relative abundance of *N. rosariae* and *Cabera pusaria*, the latter having larvae mainly feeding on *Alnus* (Flamigni *et al.*, 2016). This result underlined the

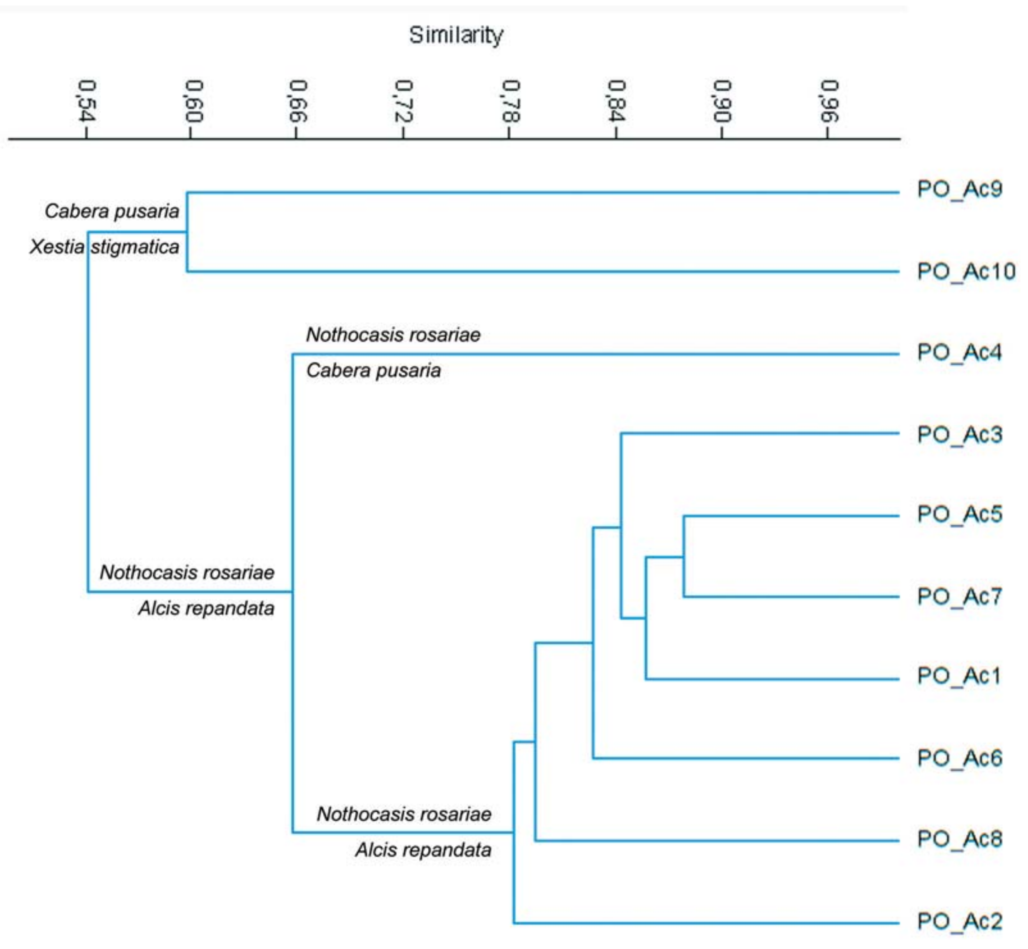

Fig. II – Ordination of stands obtained by Cluster analysis (Algorithm: paired groups; Similarity measure: Morisita).

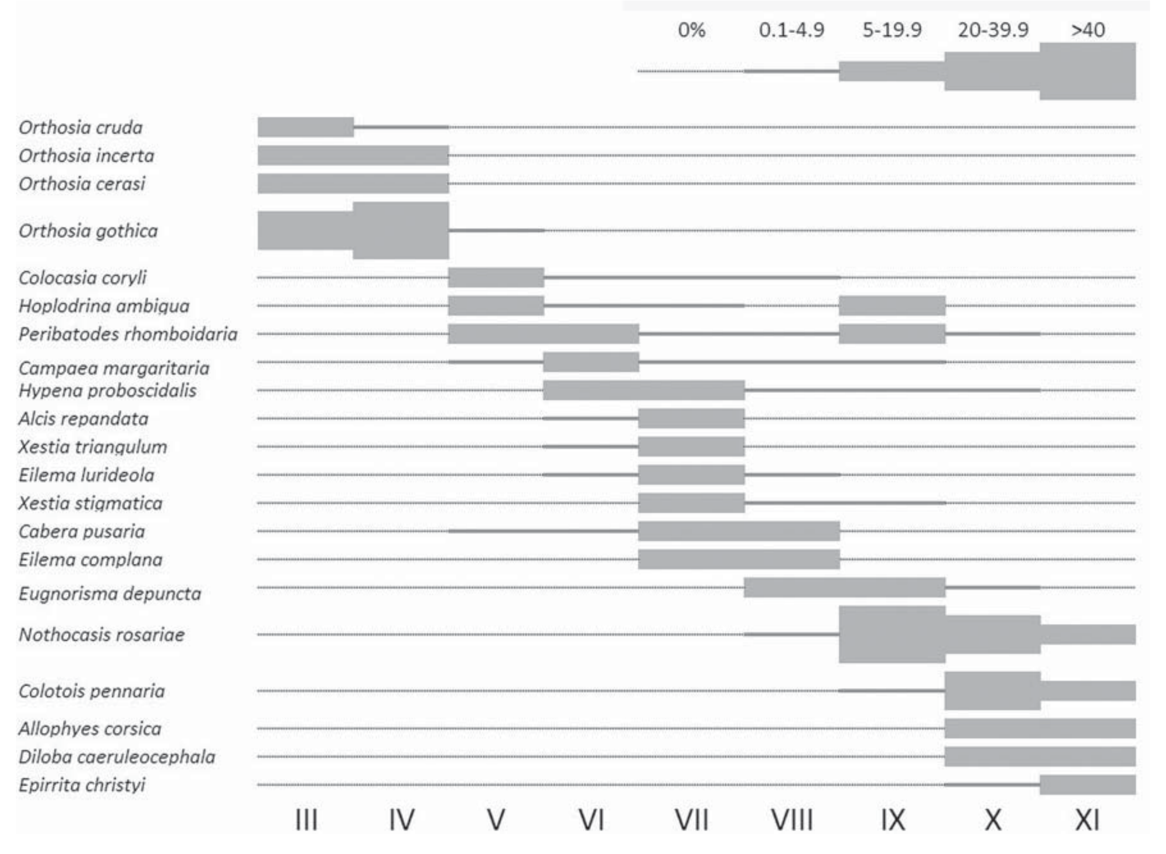

Fig. III – Phenological diagram of the three most abundant species during each sampling session.

habitat fidelity of moths which, although dispersing easily thanks to their high mobility, kept their highest abundance within the habitat of origin. As a consequence, the use of moths as bioindicators is greatly improved using quantitative data.

From a conservation point of view the Special Area of Conservation Monte Sparviere has a great importance for the presence of several species of biogeographic interest (Greco *et al.*, 2018a,b), and *Euplagia quadripunctaria*, the only moth of priority importance in the annexes of Habitat Directive 92/43/CEE. Furthermore, during our surveys we also occasionally observed the diurnal *Zerynthia cassandra* (Geyer, 1828), *Parnassius mnemosyne* (Linnaeus, 1758), *Phengaris arion* (Linnaeus, 1758), *Euphydryas aurinia* (Rottemburg, 1775), and *Melanargia arge* (Sulzer, 1776), also included in the Habitat Directive. In definitive, this site appears to be one of the most important for the conservation of Lepidoptera within the Natura2000 Network in southern Italy and further studies will likely increase its value.

ACKNOWLEDGEMENTS

We thank Marco Infusino for the help in field work and the Pollino National Park for the financial support of this study (Project: Studio pilota per il monitoraggio della biodiversità dei Lepidotteri in SIC forestali del Parco Nazionale del Pollino).

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REDIA, 102, 2019: 81-88 http://dx.doi.org/10.19263/REDIA-102.19.12

FABIO CIANFERONI [a, b]

REVIEW OF *KERMES ROBORIS* (GEOFFROY, 1785), WITH NEW RECORDS FOR HOST PLANTS, ASSOCIATED ANTS, OCCURRENCE, ITALIAN DISTRIBUTION, AND NOMENCLATURAL HISTORY (HEMIPTERA COCCOIDEA KERMESIDAE)

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Cianferoni F. – Review of *Kermes roboris* (Geoffroy, 1785), with new records for host plants, associated ants, occurrence, italian distribution, and nomenclatural history (Hemiptera Coccoidea Kermesidae).

The nomenclatural history of the scale insect *Kermes roboris* (Geoffroy, 1785) is reviewed. The authorship of this species is corrected in Geoffroy in Fourcroy, 1785 (or simply Geoffroy, 1785) and the synonymy of *Lecanium quercus* Burmeister, 1835 with *Kermes roboris* (Geoffroy in Fourcroy, 1785) is discussed and confirmed.

Two new host plants of *K. roboris* are recorded from Spain: *Quercus faginea* Lam. and *Q. pyrenaica* Willd. First records of ant species associated to this scale insect are also given, namely *Formica pratensis* Retzius, 1783 and *Lasius fuliginosus* (Latreille, 1798), in addition to others Formicinae identified at genus level.

The general and Italian distribution of *K. roboris* are reviewed, and the first record for the region Trentino-Alto Adige is given. Further new Spanish records from the web are also reported.

KEY WORDS: Sternorrhyncha, Kermesidae, scale insects, Trentino, Italy, Spain, *Quercus faginea*, *Quercus pyrenaica*, *Formica pratensis*, *Lasius fuliginosus*

INTRODUCTION

Kermes roboris (Geoffroy, 1785) is a species of scale insect (Hemiptera: Sternorrhyncha: Coccoidea) belonging to the family Kermesidae.

All Kermesidae species develop exclusively on Fagaceae, with the only exception of the species belonging to the genus *Eriokermes* Miller & Miller, 1993, associated with Cupressaceae. *Quercus* spp. are the only host plants of European Kermesidae, whereas a few Asiatic *Kermes* have been collected also on other Fagaceae (MILLER and MILLER, 1993).

Kermes roboris is one of the largest and colorful scale insect of Europe. The adult females show a globose body, nearly spherical, reaching 8.5 mm in width, deep yellow in colour, with transverse bands and oval spots blackish brown (Fig. I-II); males are still unknown (TARGIONI TOZZETTI, 1888; CECCONI, 1915; LEONARDI, 1920; KOSZTARAB and KOZÁR, 1988).

The species is widely distributed in Europe south of 60° North latitude and it is recorded for Portugal, Spain, France, United Kingdom (England), the Netherlands, Germany, Italy, Austria, Czech Republic, Hungary, Croatia, Greece, Poland, Belarus, Ukraine, Moldova, Romania, Bulgaria. It occurs also in Russia, Morocco, Turkey, Georgia, and China (SZULCZEWSKI, 1926; BALACHOWSKY, 1950; HOY, 1963; KOZÁR and OSTAFICHUK, 1987; KOSZTARAB and KOZÁR, 1988; TAO, 1999; FOLDI, 2001; JANSEN, 2001; FUSU and POPESCU, 2003; PELLIZZARI and RUSSO, 2004; MASTEN MILEK and ŠIMALA, 2008; FRANCO *et al.*, 2011; MALUMPHY and BADMIN, 2012; MASTEN MILEK *et al.*, 2016; SCHMUTTERER and HOFFMANN, 2016; BATSANKALASHVILI *et al.*, 2017; SZITA *et al.*, 2017).

In Italy it has been generically recorded for the northern, central, and southern regions (including Sicily) by LEONARDI (1920), BARBAGALLO *et al.* (1995), LONGO *et al.* (1995) and PELLIZZARI and RUSSO (2004); general Italian quotations were provided, for example, also by TARGIONI TOZZETTI (1868), DEL GUERCIO (1900), and SILVESTRI (1939); no records instead in TARGIONI TOZZETTI (1867). However, more precise records (some very old) seem to exist only for Piedmont (RIDOLFI, 1831; see also LEONARDI, 1922 and GOIDANICH, 1975: 861), Lombardy (CAVANNA, 1879, 1881; TARGIONI TOZZETTI, 1888), Veneto (CECCONI, 1915), Friuli-Venezia Giulia (MELIS, 1949); Campania (TRANFAGLIA, 1981), Basilicata (MAROTTA *et al.*, 1998), Apulia (CECCONI, 1915), and Sicily (LONGO *et al.*, 1991).

Although widespread, it is usually found in small numbers (JANSEN, 2009), although sometimes it was reported as common (see e.g. CECCONI, 1915).

Kermes roboris develops on thin branches of some species of *Quercus* L.; so far it has been reported from the following species: *Q. ilex* L., *Q. petraea* (Matt.) Liebl., *Q. pubescens* Willd., and *Q. robur* L. (e.g., MELIS, 1949; HOY, 1963; KOSZTARAB and KOZÁR, 1988; LONGO *et al.*, 1991; ŁAGOWSKA and GOLAN, 2002; JANSEN, 2009). In Italy it has been recorded on *Q. robur*, *Q. petraea*, and *Q. ilex* (CECCONI, 1915; LEONARDI, 1920; MELIS, 1949; GOIDANICH, 1975: 860; LONGO *et al.*, 1991; PELLIZZARI *et al.*, 2012). TARGIONI TOZZETTI (1888) listed it also for *Populus* sp. (Salicaceae).

According to TZALEV (1968), in Bulgaria, this species overwinters as second instar nymph on the woody parts of the host plant. The eggs are laid in spring, between May and June, and only a generation per year is observed (DEL GUERCIO, 1900; TZALEV, 1968; SCHMUTTERER, 1972).

– Received 16 April 2019 Accepted 5 September 2019

Fig. I – Adult females of *Kermes roboris* (Geoffroy, 1785) attended by ants of the species *Lasius fuliginosus* (Latreille, 1798) on a twig of *Quercus pubescens* Willd. s.l. (photo by Marco Floriani).

Fig. II – Adult female of *Kermes roboris* (Geoffroy, 1785) attended by ants of the species *Lasius fuliginosus* (Latreille, 1798) on a twig of *Quercus pubescens* Willd. s.l. (photo by Marco Floriani).

Often the females of *K. roboris* are attended by ants (see e.g. Réamur, 1738: 61; Kosztarab and Kozár, 1988).

Several parasitoids have been recorded as associated to this species (e.g., Fulmek, 1943; Borchsenius, 1960; Tsalev, 1972; Tudor, 1982; Graham, 1987; Kosztarab and Kozár, 1988; see also García Morales *et al.*, 2016 and Noyes, 2019), they mostly belong to Hymenoptera Chalcidoidea, families Encyrtidae: *Blastothrix erythrostetha* (Walker, 1847), *B. ilicicola* Mercet, 1921, *B. longipennis* Howard, 1881, *B. sericea* (Dalman, 1820), *Cheiloneurus paralia* (Walker, 1837), *Discodes aeneus* (Dalman, 1820), *Microterys chalcostomus* (Dalman, 1820), *M. ferrugineus* (Nees, 1834), *M. lunatus* (Dalman, 1820), *Phaenodiscus aeneus* (Dalman, 1820), *Psilophrys tenuicornis* Graham, 1969, *Tetrastichus pachycerus* Graham, 1991, and Eulophidae: *Aprostocetus pachyneuros* (Ratzeburg, 1844), *Quadrastichus thysanotus* (Foerster, 1861). Also a beetle, *Anthribus nebulosus* Forster, 1770 (Coleoptera: Curculionoidea: Anthribidae), was recorded in association with *K. roboris* (Cecconi, 1915), as well as with other species of scale insects (Kosztarab and Kozár, 1988; Dervisevic and Graora, 2019).

The fortuitous record of this interesting species of scale insect in the Italian region Trentino-Alto Adige stimulated a detailed research on its distribution, host plants and nomenclatural history.

MATERIAL AND METHODS

For each record of *K. roboris*, the following information is given: country, region, localities, elevation, coordinates, plant species, date, collector or photographer, number of specimens, sex (although all are females) and development stage, repository, identifier of the scale insect, ant taxon found in association. In the verbatim transcription of original labels, vertical bars "|" separate lines and double vertical bars "‖" separate labels.

Ants were identified by the myrmecologist Fabrizio Rigato (Natural History Museum of Milan, Italy). Geographical coordinates are in decimal degrees (datum WGS84). The uncertainty of data (in metres) was indicated according to the point-radius method (Wieczorek *et al.*, 2004).

Only synonyms and change of combinations, based on Fernald (1903), Kozár and Drozdiák (1998), Miller *et al.* (2005), and García Morales *et al.* (2016), were listed under the accepted name of the insect. All references, especially those concerning nomenclature, have been checked by reading the original sources and translating them from Latin, French, German, Italian, and Russian to English.

Acronyms:
BV = www.biodiversidadvirtual.org
CAM = Alessandro Mascagni collection - Scandicci, Florence (Italy)
CFC = Fabio Cianferoni collection - Florence (Italy)
MSNM = Museo di Storia Naturale di Milano [Natural History Museum of Milan] - Milan (Italy)
MZUF = Museo di Storia Naturale dell'Università degli Studi di Firenze, Zoologia [Natural History Museum of the University of Florence, Zoology] - Florence (Italy)

***Kermes roboris* (Geoffroy in Fourcroy, 1785)**

Chermes roboris Geoffroy in Fourcroy, 1785.
Original combination (Geoffroy, 1785: 229).

Coccus variegatus Gmelin, 1790.
Junior synonym (Gmelin, 1790: 2221)
Synonymized by Bodenheimer (1931: 243).

Chermes variegatus (Gmelin, 1790).
Change of combination (Olivier, 1792: 440).

Lecanium quercus Burmeister, 1835.
Junior synonym (Burmeister, 1835: 71)
Synonymized by Targioni Tozzetti (1868: 733).
Confirmed (present work).

Kermes variegatus (Gmelin, 1790).
Change of combination (Targioni Tozzetti, 1868: 733).

Kermes pallidus Signoret, 1875.
Junior synonym (Signoret, 1875: 553).
Synonymized by Balachowsky (1950: 743).
Needing confirmation.

Kermes roboris (Geoffroy in Fourcroy, 1785).
Change of combination (Fernald, 1903: 65).

Kermes variegatus corticalis Nassonow, 1909.
Junior synonym (Nassonow, 1909: 490).
Synonymized by Balachowsky (1950: 743).

Kermococcus roboris (Geoffroy in Fourcroy, 1785).
Change of combination (Leonardi, 1918: 212).

Coccus pallidus (Signoret, 1875).
Change of combination (Cockerell, 1929: 150).

Coccus roboris (Geoffroy in Fourcroy, 1785).
Change of combination (Cockerell, 1929: 150).

Talla pallida (Signoret, 1875).
Change of combination (Lindinger, 1933: 143).

Talla roboris (Geoffroy in Fourcroy, 1785).
Change of combination (Kawecki, 1936: 322).

Talla roboris simplex Lindinger 1949.
Junior synonym (Lindinger, 1949: 213).
Synonymized by Kosztarab and Kozár (1988: 267).

Material examined. Italy: Trentino-Alto Adige: Trentino, Pergine Valsugana (Trento), ca. 800 m a.s.l., 46.039100° N 11.213649° E (WGS84, uncertainty = 10 m), on *Quercus pubescens* Willd. s.l., 25.VI.2018, Marco Floriani legit, 4 adult females (CFC), 4 adult females (CAM), det. Fabio Cianferoni, in association with *Lasius fuliginosus* (Latreille, 1798) (Fig. I and II); Apulia: Mottola (Taranto), on *Quercus pubescens* Willd. s.l., 23.V.1896, [collector non specified], "Kermes variegatus SK | (su quercus) | 23.V.1896 Mottola [probably not original label written with typewriter] || "La Specola – Firenze | Mag 1623 | Kermes variegatus | Puglia: Mottola (TA) | 29/V/1896 ? [subsequent transcription]", 16 adult females (MZUF), revid. Fabio Cianferoni. Spain: Castilla–La Mancha: Guadalajara, on *Quercus faginea* Lam., 3.VI.2017, photo by Jesús Carrizo (BV), 1 adult female, det. Ferran Garcia, in association with *Formica* sp.; Madrid: Madrid, on *Quercus pyrenaica* Willd., 19.VI.2015, photo by Diego Gil (BV), 1 adult female, det. Diego Gil; Aragon: Teruel, on *Quercus faginea* Lam., 2.VI.2015, photo by José-Antonio Sánchez (BV), 1 adult female, det. Ferran Garcia; Castile and León: Ávila, on *Quercus* sp., 18.VI.2012, photo by Yvonne Duchene (BV), 2 adult females, det. Ferran Garcia, in association with *Formica pratensis* Retzius, 1783; Ávila, Sierra de Gredos, on *Quercus* sp., 30.VI.2012, photo by Yvonne Duchene (BV), 2 adult females, det. Ferran Garcia; *idem*, 11.X.2011, photo by Yvonne Duchene (BV), 4 adult females, det. Ferran Garcia; *idem*, 29.V.2011, photo by Yvonne Duchene (BV), 1 adult female, det. Ferran Garcia, in association with *Formica pratensis* Retzius, 1783; Extremadura: Badajoz, on *Quercus pyrenaica* Willd., 6.VI.2010, photo by Arturo López (BV), 2 adult females, det. Ferran Garcia.

Published record of *K. roboris* with new ant record. Netherlands: Limburg: Lottum, on *Quercus robur* L., 20.VI.2008, G. Vuillings legit, 1 adult female, det. Maurice Jansen (photo from Janssen, 2009), in association with *Lasius* sp.

Nomenclatural history of *Kermes roboris*

The nomenclatural history of *Kermes roboris* is entangled by so many synonyms by different authors that it can be difficult understanding the whole situation.

The synonymy of *Lecanium quercus* Burmeister, 1835 under *Chermes roboris* Geoffroy in Fourcroy, 1785, was suggested by Targioni Tozzetti (1868: 733), and it was accepted by several subsequent authors (see e.g. Fernald, 1903; Kozár and Drozdiák, 1998). Miller *et al.* (2005: 187) cast doubt about the validity of the synonymy of *L. quercus* with *Kermes roboris*, and in fact *Lecanium quercus* Burmeister is not reported among their list of *K. roboris* synonyms (Miller *et al.*, 2005: 186; see also Garcia Morales *et al.*, 2016).

In the attempt to clarify this situation, the nomenclatural history of *K. roboris* is reported below in chronological order.

René Antoine Ferchault de Réamur described "on oak, a scale insect almost spherical as big as a small cherry, whose skin has the polish and the light of the grains of which the rosaries are made. If one looks at it with a magnifying glass, its polish seems even greater, and such as that of ice. The background of its color is a yellowish white, on which are three black stripes; black dots are distributed in intervals who are between the lines; ants like this species as the others [scale insects], and they made me discover it" [translated from French] (Réamur, 1738: 61), and he illustrated in a plate (Réamur, 1738: pl. 5, figs 3-4 – see Fig. III) this scale insect as "of a different species [referring to a previous species; see fig. 2 of plate 5] […] and one of the most spherical and largest" [translated from French] (Réamur, 1738: 78). However, the work by

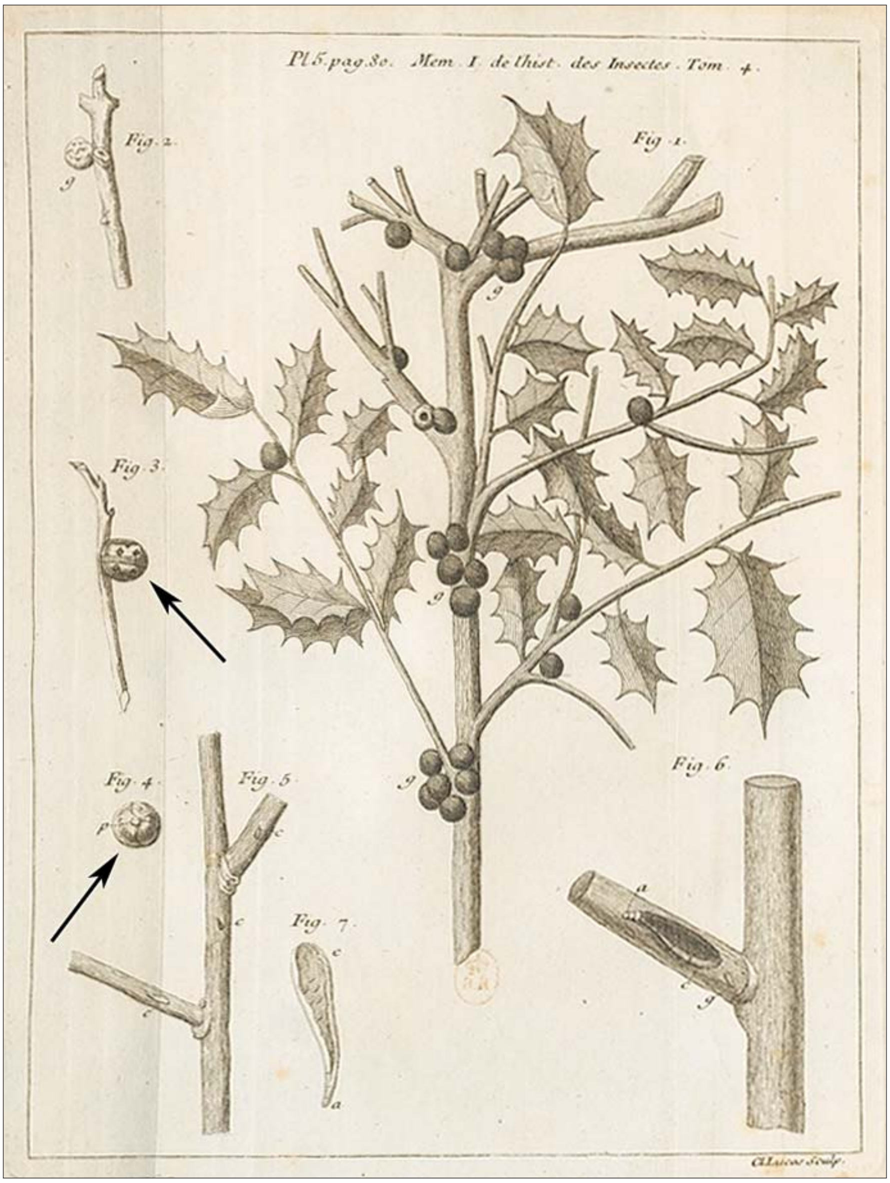

Fig. III – Original plate from the "Mémoires pour servir à l'histoire des insectes" by Réamur (1738) with the drawing of different scale insect species. The scale insect which will be named *Kermes roboris* (Geoffroy, 1785), corresponds to the drawing numbers 3 and 4 (indicated by arrows). (Bibliothèque nationale de France - gallica.bnf.fr).

Réamur (1738) is pre-Linnean and published before the starting point (1st January 1758) fixed by the International Code of Zoological Nomenclature (ICZN, 1999).

After Réamur (1738), Étienne Louis Geoffroy listed this species as "*Chermes quercûs rotundus, ex albo flavescente nigroque variegatus*", quoting also the work by Réamur (Geoffroy, 1762: 508). Geoffroy's description, although unofficial according to the ICZN (1999) since it is not associated with a binomial, is however also unambiguous: "oak kermes spherical and variegated […] background yellowish white, on which are three transverse black lines. Between these lines, in the intervals, there are black dots distributed also transversally" [translated from French] (Geoffroy, 1762: 508), and clearly refers to the same species.

Several years later, Fourcroy (1785) resumed the work by Geoffroy (1762), replacing the long descriptions of this author with binomial names and short diagnostic sentences, as well as hundreds of new species (see Boisduval and Lacordaire, 1835; Ganglbauer and von Heyden, 1906; Hemming, 1954); in particular it was Geoffroy (1785: 229) himself who named this species *Chermes roboris* in the work by Fourcroy (1785). In fact, Fourcroy (1785) stated in the preface to his book that all the binomina had been proposed by E.L. Geoffroy, and all the diagnoses had been either provided by that author or borrowed word for word from his earlier publication (Geoffroy, 1762). Correspondingly, the authorship of all the new names should read "Geoffroy in Fourcroy" (or simply "Geoffroy"), but not "Fourcroy" as reported so far for this species (see Kerzhner, 2008).

Gmelin (1790: 2221), in a new edition of the Linnaeus' "Systema Naturae", repeated a description similar to that of Geoffroy (1762), quoting also Réamur (1738), and named the species *Coccus variegatus*. Subsequently, *C. variegatus* was synonymized by Bodenheimer (1931: 243).

Burmeister (1835: 71) in his description of *Lecanium quercus* wrote [translated from German]: "on the branches of common oak; pea-sized balls of yellowish gray color with brown marks" and quoted after the specific name *L. quercus* the work by RÉAMUR (1738) who clearly described and illustrated the same species (see RÉAMUR, 1738: 61, 78, and pl. 5, figs 3-4 – see Fig. III). Thus, no particular doubts seem to arise about the identity of the taxon named by BURMEISTER (1835). BOUCHÉ (1851: 112) reported *L. quercus* quoting both RÉAMUR (1738: pl. 5, but including also the fig. 2, considered as a different species of scale insect by Réamur himself, in addition to the figs 3-4) and BURMEISTER (1835). Eventually, TARGIONI TOZZETTI (1868: 733) listed the names *Lecanium quercus* under the new proposed combination *Kermes variegatus* Gmelin as synonym.

SIGNORET (1875), quoting another insect scale described and figured by RÉAMUR (1738: pl. 5, fig. 1) (see Fig. III) from *Quercus ilex*, described it as *Kermes pallidus* Signoret, 1875 (SIGNORET, 1875: 553). Subsequent authors (e.g., FERNALD, 1903; COCKERELL, 1929; LINDINGER, 1933) listed *K. pallidus* as a separate species, until BALACHOWSKY (1950: 743) synonymized it with *Kermes roboris* (BALACHOWSKY 1950: 743; HOY, 1963: 159). Both in MILLER *et al.* (2005) and in GARCIA MORALES *et al.* (2016) this synonymy is ascribed to BORCHSENIUS (1960: 46), who simply quoted the paper by BALACHOWSKY (1950).

RESULTS

According to the study of the original papers by RÉAMUR (1738), GEOFFROY (1762), FOURCROY (1785), and BURMEISTER (1835) the authorship for *Chermes roboris* is corrected in Geoffroy in Fourcroy, 1785 (or simply Geoffroy, 1785) and the synonymy of *Lecanium quercus* Burnmeister, 1835 with *Chermes roboris* Geoffroy in Fourcroy, 1785, first proposed by TARGIONI TOZZETTI (1868: 733, under *K. variegatus*) and discussed above, is here confirmed.

Moreover, the synonymy of *Kermes pallidus* Signoret, 1875 with *C. roboris*, erroneously ascribed to BORCHSENIUS (1960) (MILLER *et al.*, 2005; GARCIA *et al.*, 2016), is due to BALACHOWSKY (1950: 743) as correctly reported by BORCHSENIUS (1960) and HOY (1960).

Two new species of *Quercus* L. from Spain are reported as host *K. roboris*, namely *Q. faginea* Lam. and *Q. pyrenaica* Willd.

The females of *K. roboris* are often visited by ants (KOSZTARAB and KOZÁR, 1988) but no more precise information has been published. From the material reported above, two genera of Formicinae are recorded as attending *K. roboris*: *Formica* Linnaeus and *Lasius* Fabricius, respectively with the species *F. pratensis* Retzius, 1783 and *L. fuliginosus* (Latreille, 1798); some other unidentifiable ants from the available photos could very likely belong to different species (F. Rigato, pers. com.), however they fall into the same genera.

DISCUSSION

The synonymy of *Kermes pallidus* Signoret, 1875 with *Chermes roboris* Geoffroy, 1785 proposed by BALACHOWSKY (1950) would need verification, since in this case is not possible to confirm it directly from the plate by RÉAMUR (1738: pl. 5, fig. 1) (see Fig. III) who clearly treated these specimens as different from those which subsequently will be named as *C. roboris* by GEOFFROY in FOURCROY (1785). GARCIA *et. al.* (2016: Matile-Ferrero, pers. comm., 2 December 1999) wrote that not type material of the species described by SIGNORET (1875) seems to be present in the Muséum national d'Histoire naturelle (Paris, France). However, Victor Antoine Signoret's collection was deposited in the Naturhistorisches Museum Wien (Vienna, Austria) (FAIRMAIRE, 1889: 505; GOUILLARD, 2004: 74).

The taxon *Kermes muhlisi* described by BODENHEIMER (1941: 76) from Turkey has been suspected to be a synonym of *K. roboris* by BALACHOWSKY (1950: 745), whilst BORCHSENIUS (1960: 50) treated it as a separate species (see also GARCIA *et. al.* 2016). I did not include this taxon among the synonymic list of *K. roboris* but its status still needs to be clarified.

Although in BALACHOWSKY (1950) and LONGO *et al.* (1999) *K. roboris* is considered an Eurosiberian element, this species seems to occur also in Maghreb (HOY, 1963) and China (TAO, 1999), but these data need confirmation; if correct, the chorotype should be more extensive (see VIGNA TAGLIANTI *et al.*, 1999). However, current data still lack to be able to definitively assign it.

The species has been always reported from oaks (*Quercus* spp. - Fagaceae), only TARGIONI TOZZETTI (1888) reported it from *Populus* spp. (Salicaceae), writing: "our specimens were taken from poplar" [translated from Italian]. However it does not seem that he had personally collected these specimens, since he received them from a forest inspector from northern Italy ten years before (TARGIONI TOZZETTI, 1888; see also CAVANNA, 1879, 1881). As such, the record for this plant can be considered doubtful (very likely erroneous) and need confirmation.

The new record of *K. roboris* for Trentino (Trentino-Alto Adige region) and the old specimen from Apulia preserved in the MZUF are the first ones giving with certainty *Q. pubescens* Willd. sensu lato as host of *K. roboris* in Italy (see Introduction).

Concerning the Italian distribution, the occurrence records are very scattered and located only in northern and southern regions; indeed no published records seem to exist for the regions of Central Italy. Only LEONARDI (1920) clearly indicated "central regions" of Italy in addition to the northern and southern ones. However, the lack of records in Central Italy is probably due only to undersampling of this uncommon species.

CECCONI (1915) reported also several specimens of *Anthribus nebulosus* Forster, 1770 (Coleoptera: Anthribidae) emerging from females of *K. roboris*. The record was given under the synonym *A. variegatus* Fourcroy, although is to be attributed to E.L. Geoffroy: *A. variegatus* Geoffroy, 1785 (see also KERZHNER, 2008), as correctly indicated by TRÝZNA and VALENTINE (2011).

Further research will certainly add to these first data also more ant taxa as attending *K. roboris*.

ACKNOWLEDGEMENTS

I am grateful to Marco Floriani (Serso, Pergine Valsugana - Italy) who collected the specimens of the scale insect in Trentino and to Alessandro Mascagni (Scandicci, Florence - Italy) for providing me some of the specimens and information, to Giuseppina Pellizzari (University of Padua - Italy), Santi Longo and Gaetana Mazzeo (University of Catania - Italy) for providing useful information. A special thanks to Fabrizio Rigato (Natural History Museum of Milan - Italy) for his kindness in having identified the ants associated to the scale insects, to Francesca Graziani (Florence - Italy) for help with a bibliographic reference, to Saulo Bambi (Natural History Museum, University of

Florence - Italy) for allowing me to use the microscope under his care for photos, to Jean-François Elder (La Meauffe - France) for confirming a translation from French, to Luca Bartolozzi (Natural History Museum of the University of Florence - Italy) for allowing me to study the entomological collection of the museum, to Filippo Ceccolini (Florence - Italy) for useful advices, and to Lorenzo Cecchi (Natural History Museum of the University of Florence - Italy) for information on a botanical species. I would like to thank also an anonymous reviewer who provided very useful information and comments which improved the article.

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PREDATION BY NYMPHS OF *PICROMERUS BIDENS* (HETEROPTERA PENTATOMIDAE ASOPINAE) ON CATERPILLARS OF *EUPHYDRYAS AURINIA PROVINCIALIS* (LEPIDOPTERA NYMPHALIDAE) IN ITALY

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Pinzari M., Cianferoni F., Fabiani A., Dioli P. – Predation by nymphs of *Picromerus bidens* (Heteroptera Pentatomidae Asopinae) on caterpillars of *Euphydryas aurinia provincialis* (Lepidoptera Nymphalidae) in Italy.

Euphydryas aurinia (Rottemburg 1775) is one of the most seriously threatened European butterflies, as it suffered a severe decline in most countries and it is today listed in the European Community Habitats and Species Directive (92/43/EEC). Although its ecology and biology have been increasingly studied during the 20th Century, the factors affecting its mortality have not been described in detail, and the role of natural enemies on its population dynamics is largely unknown.

In this paper, we present new data on predation events of *P. bidens* (Linnaeus, 1758) on caterpillars of *Euphydryas aurinia* spp. *provincialis* (Boisduval, 1828) in Central Italy, and discuss the stages during which predation could have a stronger impact. Our results show that II to IV instar nymphs of *P. bidens* attack the pre-diapause 3rd instar caterpillars when they live in a communal silken nest, while adults were found on butterfly larval webs, but never preying on caterpillars. The bugs visited a larval group more than once a day and at different times, and showed preference for cooler habitats (i.e., they were not observed on webs of warmer habitats). All together, our observations point out to the potential significant impact that gregarious nymphal instars can have on the population dynamics of *E. aurinia* in cooler habitats, where predator and prey coexist.

KEY WORDS: *Euphydryas aurinia provincialis,* larvae, predator, *Picromerus bidens.*

INTRODUCTION

The ecology and biology of *Euphydryas aurinia aurinia* (Rottemburg, 1775) has been intensively studied in Europe (PORTER, 1981; WARREN, 1993; 1994; 1996; WARREN *et al*., 1994; KLEMETTI & WAHLBERG, 1997; WAHLBERG, 2001; HULA *et al*., 2004; SAARINEN *et al*., 2005; MAZEL, 2006; SVITRA & SIELEZNIEW, 2010; PORTER & ELLIS, 2011; CASACCI *et al*., 2014) especially owing to its severe decline in most European countries during the 20th century (VAN SWAAY *et al*., 2010). However, the natural history of its enemies and their role in its population dynamics are scarcely unknown (VAN NAUHUYS & HANSKI, 2004).

Caterpillars of *E. aurinia* sp. *provincialis* show six larval stages; during the first four, they are gregarious and live in a communal silken web (PINZARI *et al*., 2010, 2016, 2017) on a variety of host plants, likely playing an important role in this complex multitrophic community. In Europe, arthropods and small mammals have been reported as parasitoids or predators of *E. aurinia* (VAN NAUHUYS & HANSKI, 2004). Among insect parasitoids, the impact of braconid wasps on *E. a. aurinia* populations has been studied in detail since the '80s (PORTER, 1981; KANKARE *et al*., 2005; SHAW *et al*., 2009), while a more recently study showed the relationship between tachinids and *E. a. provincialis*, as their host (PINZARI *et al*., 2017). On the contrary, scarce information is available on predation by heteropterans. In a review by VAN NAUHUYS & HANSKI (2004) only an old report by WILKINSON (1907) is mentioned, on unidentified heteropterans predating *E. a. aurinia* caterpillars. In addition, two recent notes described predation on pre-hibernation caterpillars of *E. a. aurinia* and *E. a. provincialis* by true bugs *Picromerus bidens* (Linnaeus, 1758) *Deraeocoris schach* (Fabricius, 1781) respectively (KONVICKA *et al*., 2005; PINZARI, 2016b). In these notes, while *D. schach* seems to be an occasional predator of pre-hibernation caterpillars*, P. bidens* looks like a regular and efficient predator, as it was seen at adult stage attacking both butterflies and caterpillars at their fourth larval-instar stage.

Pre-hibernation caterpillars of *E. a. provincialis* feed gregariously and move from plant to plant (PINZARI *et al*., 2016), representing an important food source for *P. bidens*, especially when they are also in a gregarious phase. In fact, gregarious behaviour is typical of their I-II instar nymphs (LARIVIÈRE & LAROCHELLE, 1989; DE CLERCQ, 2000) and, according to KONVICKA *et al*. (2005), this species could represent a substantial mortality factor in populations of butterflies.

Within this framework, in the context of an ongoing survey of the Lepidoptera fauna in central Italy (PINZARI *et al*., 2010; PINZARI & SBORDONI, 2013; PINZARI, 2016a; PINZARI *et al.,* 2018a, 2018b, 2019a, PINZARI & PINZARI, 2019a, 2019b), in 2010 we started a study on several aspects of the biology of *E. aurinia* sp. *provincialis* in

– Received 11 February 2019 Accepted 10 September 2019

Central Italy; we described its larval host plants preference (PINZARI *et al.,* 2016), adult population dynamics (MANUELA PINZARI, unpublished data), mating behaviour (PINZARI *et al*., 2019), and, finally, we identified its main predators (PINZARI, 2016; PINZARI, 2019) and parasitoids (PINZARI *et al*., 2017). Here, we report our observations on bug nymphs of *P. bidens* predating the caterpillars of *E. a. provincialis*.

MATERIALS AND METHODS

STUDY SPECIES

Euphydryas aurinia provincialis (Lepidoptera: Nymphalidae) are widespread over the Central Apennines and, in particular, on Monti Reatini (data from Osservatorio per la Biodiversità del Lazio). In the study area (see below), they occupy habitat patches in montane grassland and slopes, within a mosaic of different habitats including wooded and rocky areas, hedgerows, fields and less steep areas which are occasionally ploughed and cultivated.

They have one generation per year: they lay their eggs in large clumps under plant leaves in May-June, and develop into six larval instars. The first three instars are gregarious: they spin a substantial nest between the leaves of the food plants and live together from May to August within the communal silken web; the IV instar-larvae enter diapause in August-September and overwinter in a larval web (hibernaculum) close to the ground. By February, caterpillars are again active, they complete their life-cycle and pupate by the end of April. Adults start to emerge in May (PINZARI *et al.*, 2016).

Picromerus bidens (Hemiptera: Heteroptera: Pentatomidae, Asopinae) is distributed in continental Italy and in Sicily (FARACI *et al*., 1995). It is associated with a wide range of habitats, mainly on shrubs and forests of open forest edges, meadows and gardens, preferring fresh, shaded and humid areas (LARIVIÈRE & LAROCHELLE, 1989). The proximity of fresh vegetation is essential to the successful development of nymphs and adults (MAYNÈ & BRENY, 1948) and for reproductive activity (MAHDIAN *et al*., 2008). Females deposit egg batches (typically 35 to 45 eggs), as many as five and varying in size (SCHUMAKER, 1911; MAYNÉ & BRENY, 1948b; JAVAHERY, 1986 LARIVIÈRE & LAROCHELLE, 1989), and the nymphs hatch in spring (MAYNÉ & BRENY, 1948a; 1948b). There are five nymphal instars which, depending on ecological conditions, take from 25 to 60 days to complete development (STRAWINSKI, 1927; SOUTHWOOD & LESTON, 1959; JAVAHERY, 1986; DE CLERCQ, 2000; MUSOLIN & SAULICH, 2000; SAULICH & MUSOLIN, 2014). During the I instar, the nymphs live close together in aggregations that vary in size in relationship to the number of eggs for individual batches. The II instar nymphs also tend to be gregarious, living in groups of 3-56 individuals, although they start to disperse intermittently to find prey. Activity and mobility increases from the III to the V nymphal instar (JAVAHERY, 1986).

Concerning feeding behaviour, the I instar-nymphs do not feed but only take up water (DE CLERCQ, 2000) or suck liquids from a wide diversity of plants (MAYNÉ & BRENY, 1948b; SOUTHWOOD & LESTON, 1959). Animal food is required from the II or III nymphal instars (MAYNÉ & BRENY, 1948b). *Picromerus bidens* is a highly polyphagous bug that preys on larvae, pupae and adults of more than 250 species from several insect orders (LARIVIÈRE & LAROCHELLE, 1989). It is often found on plants that are attacked by leaf-eating larvae, particularly of Lepidoptera and Coleoptera (STRAWINSKY, 1927; MAYNÉ & BRENY, 1948a, 1948b). It is a little vagile and disperses mainly by walking (MAYNÉ & BRENY, 1948b; JAVAHERY, 1986; LARIVIÈRE & LAROCHELLE, 1989).

STUDY AREA AND LARVAL WEBS

Our observations were carried out in 2015-2016 in an area (2 ha)) near the "crossroads Santa Maria del Monte - Fonte Brignola" (1300 m). Here, we monitored 115 larval webs of *E. a. provincialis* (62 in 2015 and 53 in 2016).

To daily (and more easily) observe the interaction between predators and prey, we also collected an egg batch of *E. a. provincialis* and established it on a host plant of *Scabiosa columbaria* L. (Dipsacaceae) in a clearing between trees and shrubs (*Fraxinus ornus* L., *Fagus sylvestris* Gaertn., *Quercus* sp., *Salix caprea* L., *Malus sp., Acer* sp., *Juniperus* sp., *Cytisus* sp., *Prunus* sp., *Lonicera caprifolium* L., *Clematis vitalba* L.*, Rubus* sp.*, Abies alba* Mill.). The area was close to our private laboratory in Colle Marcone (1121 m a.s.l.) (PINZARI *et al*., 2010). In this "artificial" larval web, eggs hatched successfully and larvae developed naturally. We visited this larval web during day light, as (or even more) often than the natural larval webs (i.e., every 2-3 hours per day), and also at night time, to ascertain possible nocturnal activities of the predators.

Over the two years, a total of 50 days were spent monitoring all egg batches and larval webs of *E. a. provincialis* focusing on: (i) the eggs (from May to the end of June), (ii) the pre-diapause gregarious phase (I-III instar on larval webs, from May to September), (iii) the diapause phase (IV instar in larval webs, from September to mid-February), and (iv) the post-diapause gregarious phase (IV instar, from mid-February to mid-March). We counted and collected heteropterans on butterfly larval webs. Sampling was carried out every 7-15 days throughout the year, except during winter (November to February), in all localities, visiting the larval webs along the same transect path. The path for monitoring the "natural" webs was marked by numbered flags, located at each of the host plants.

For each bug observed, we recorded the date and time of the observation, the exposure of the predator to sunlight (shade or sun), the position of the predator (plant or larval web), the behaviour of the predator (predation or not), and established the prey attacked by the predator. To identify the species of predators, some individuals were collected as vouchers and preserved dried (private collection, Pinzari - Rome, Italy), while others were only photographed.

RESULTS

OBSERVATIONS ON *PICROMERUS BIDENS*

During our observations on the 115 larval webs, we did not record the presence of *P. bidens*, which was instead observed on the "artificial" web in Colle Marcone, while it was preying on caterpillars. In particular, we sampled six nymphs and three adults in 2015, and observed 13 individuals without collecting them. Only a single nymph was collected in 2016 as voucher.

Twice in June 2015 and once in July 2016 (Fig. I), we observed II to IV instar nymphs of *P. bidens* on a larval web of *E. a. provincialis,* while preying on the pre-diapause III instar caterpillars (Fig. II, 1-4). No V instar nymphs were observed. Nymphs and adults of *P. bidens* were not seen in August; on the contrary, adults were found on larval webs or in their vicinity, on three occasions. In particular, we found: (i) a female at a distance of ca. 1 m from the larval web, on 26th September

2015; (ii) a male attacking a larva of an unidentified sawfly (Hymenoptera, Symphyta) (Fig. II, 5) near a larval web, on 29th September 2015; (iii) an adult (sex not identified) on the larval web, but not attacking the caterpillars, on 4th October 2015 (Fig. II, 5-6).

As far as sunlight exposure is concerned, the larval web in Colle Marcone was located within woodland and shrubs. It was in shade until 11 AM; during the following hours it was in sunlight until 3 PM and then in moderate-shade until sunset. We found *P. bidens* on host larval webs or nearby throughout the day (Fig. III). Our data show that different stages of this bug occurred at the larval webs at different times of a day. In particular, the II instar nymph visited the larval webs of *E. a. provincialis* mainly during the morning, while the III instar nymph stayed on the nest longer into the afternoon (Fig. III). During nocturnal observations, we did not find any predators either on larval web or in their vicinity.

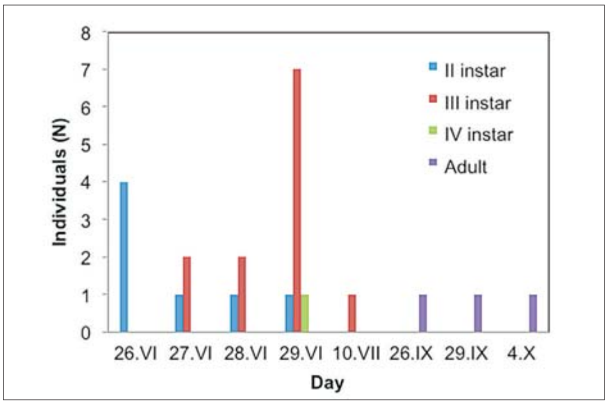

Fig. I – Occurrence of nymphs and adults of *Picromerus bidens* on the larval web of *E. a. provincialis* (Loc. Colle Marcone, 1121 m a.s.l.) in 2015-2016. Some records could involve individuals that have been observed repeatedly.

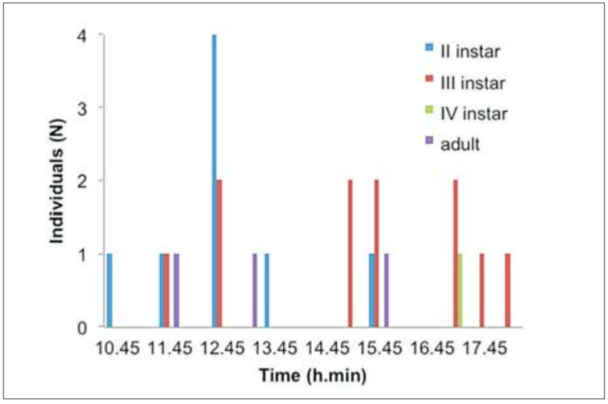

Fig. III – *Picromerus bidens*: clock time of the observations of nymphs and adults in 2015-2016 at Loc. Colle Marcone.

NOTES ON PREDATION BEHAVIOUR

We observed seven nymphs of *P. bidens* feeding on the caterpillars of *E. a. provincialis*.

During predation, the nymphs remained at the external edge of larval web and explored only the web surface of the older webbing of the nest. When they found a potential prey (i.e., the nearest caterpillar), they attacked the larva by piercing it with its stylet: they raised it with their beak, and moved away; then, they hid between leaves at a distance of ca. 20-30 cm from the nest and consumed the prey (Fig. II, 1-3).

On the 29th of June 2015, a II instar nymph approached the larval mass only when the nest was in sunlight and larvae were still inside the silk web (Fig. II, 1).

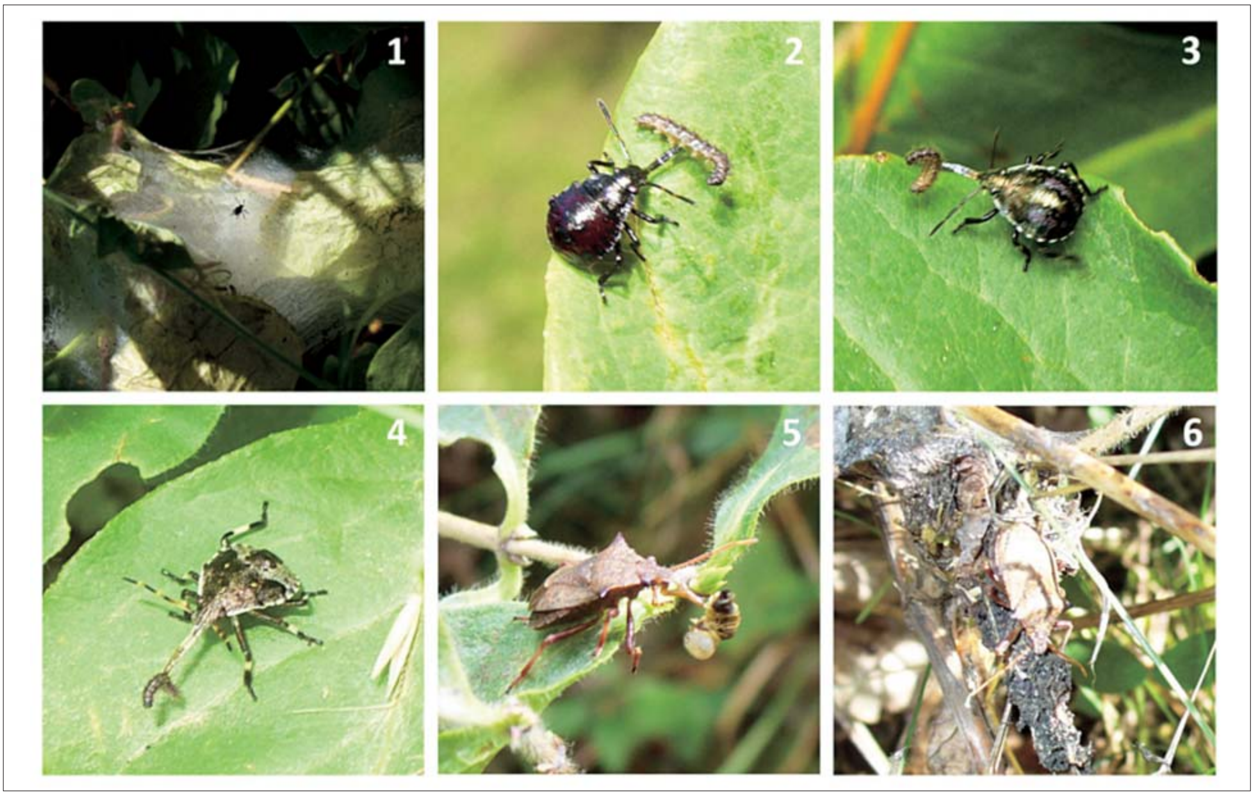

Fig. II – Predation by *P. bidens* on gregarious larvae of *E. a. provincialis* in a larval web on *L. caprifolium*: 1) a II instar nymph (29.VI.2015); 2) a II instar nymph (27.VI.2015); 3) a III instar nymph (29.VI.2015); 4) a IV instar nymph (29.VI.2015); 5) an adult while preying on a larva of unidentified sawfly (Symphyta) close to a larval web (27.IX.2015); 6) an adult on a larval web (4.X.2015).

We observed adults of *P. bidens* on a larval web of *E. a. provincialis* but we never recorded their predation events on caterpillars. On the larval web surface, we photographed only one adult preying, but it fed on a larva of unidentified sawfly (Hymenoptera, Symphyta) (Fig. II, 5).

DISCUSSION

Picromerus bidens is a generalist predator of a wide range of insects, including moths and butterflies (LARIVIÈRE & LAROCHELLE, 1989). For such a generalist, the pre-hibernation caterpillars of *E. a. provincialis* might represent an important food source for adult bugs and, even more, for their gregarious nymphs.

Gregarious behaviour is typical of the I-II instar nymphs of *P. bidens* (LARIVIÈRE & LAROCHELLE, 1989; DE CLERCQ, 2000), whenever the nymphs discover a large source of food (i.e., a butterfly larval web) they remain around it, even if they are no longer gregarious (III and IV nymphal instars). As they grow into the V instar stage, they may need larger preys and, therefore, abandon the butterfly larval webs (JAVAHERY, 1986).

During our observations, nymphs of *P. bidens* attacked caterpillars of *E. a. provincialis* quite efficiently and visited a single larval group repeatedly through time (from II to IV nymphal instars, Fig. III), acting as a potential and substantial mortality factor for our endangered study species.

Similar prey behaviour was, in fact, described for the V nymphal instar of *P. bidens* on the endangered *E. maturna* in Germany (DOLEK *et al*., 2007), and authors raised their worries on its potential impact on this species.

The absence of I instar nymphs *P. bidens* on larval webs of *E. a. provincialis* collates with the fact that they are not predators at this age (SOUTHWOOD & LESTON, 1959). Adults can instead attend larval webs and then, we surmise, feed on butterfly larvae, as already observed for *E. a. aurinia* by KONVICKA *et al.* (2005) in western Bohemia, Czech Republic. Unfortunately, we never recorded predation events on caterpillars by adult bugs.

To evaluate the predatory impact on *E. a. provincialis,* two major factors need to be taken into account: the habitat of the interacting organisms and the population size of predators and prey.

In the study area, *E. a. provincialis* can be found in both "cool and humid" and "warm and arid" habitat patches in montane grassland. On the contrary, according to JAVAHERY (1986), *P. bidens* in our study area prefers "cool and humid" patches and does not tolerate high temperatures. During our observations, when the larval web was in sunlight, both adult and nymph bugs used to hide in the vegetation in shaded areas, similarly to its prey; the caterpillars of *E. a. provincialis* also remained in the shaded parts of their host plant and retreated into the larval webs, when the temperature was getting higher. We therefore recorded the coexistence of *P. bidens* and *E. a. provincialis* only in Colle Marcone, which is characterized by cool and damp microclimate, while we never observed them together in warmer areas of our study area (such as "crossroads Santa Maria del Monte - Fonte Brignola"). As we did not observed *P. bidens* on any of the 115 webs in the field, we can state with some certainty that *P. bidens* was (and it is) absent in those warmer areas; nevertheless, we were not able to quantify the exact population size of bugs in the cool site where it occurred together with our butterfly. For this reason, we believe that a dedicated study carried out in the cooler habitats of *E. a. provincialis* would be needed, to ascertain the occurrence of *P. bidens*, to estimate the abundance of bugs and butterflies and, therefore, the impact of this predator on butterfly mortality.

It is also important to underline that consumption and developmental duration of the predatory stages of *P. bidens* is affected by temperature (MAHDIAN *et al*., 2006a, 2006b, 2008), which means that the species could adopt different developmental and feeding strategies in different habitats and the predatory effect on the butterfly population size could vary seasonally.

The predatory effect on larvae of *E. a. provincialis* by *P. bidens* was difficult to gauge, as we could not use permanent marking on the nymphs (because of their moulting), and individuals on larval webs could have been observed and counted repeatedly. However, we could estimate the minimum and maximum numbers of nymphs, considering that we captured six individual nymphs and observed 13 predator events (see Results). If the individuals observed were different, the total number of nymphs on our plant would be 19 (i.e., six captured plus 13 observed); if instead we observed only one individual, our total number would be 7 (i.e., six captured plus 1 observed). This range of 7-13 nymphs is consistent with that found by JAVAHERY (1986), who observed aggregations of 3-56 individuals.

The number of caterpillars in larval webs greatly varies depending on the host plant: from the few dozen larvae on *Scabiosa columbaria* to hundreds on *Gentiana cruciata* L. (Gentianaceae) (PINZARI *et al*., 2017). If we consider that nymphs return several times on the same plant to eat, and gravitate around the larval web at the III-IV instar stages, it is clear that they can represent an important threat for the caterpillar survival. Moreover, due to the gregarious behaviour of predators and preys, *P. bidens* nymphs of only a single egg batch could easily annihilate an entire larval web of caterpillars, with serious consequences on the survival of butterflies in that target area.

CONCLUDING REMARKS

In *Euphydryas* butterflies, larval mortality caused by generalist predators as *P. bidens* does occur and is occasionally severe (EHRLICH & HANSKI, 2004). *Picromerus bidens* could represent an important regulatory factor influencing the population of *E. a. provincialis,* as suggested for the rare butterfly *E. maturna* (VRABEC & JINDRA, 1998; VOINOT & ESSAYAN, 2015). The gregarious predatory behaviour displayed by early nymphal stages of *P. bidens* could strongly affect butterfly population size, especially in the case of small larval webs. However, the predatory impact of *P. bidens* on *E. a. provincialis* should be further evaluated, within a larger study including the effect of also other predators – among which, the true bug *Deraeocoris schach* (PINZARI, 2016), the theridiid spider *Phylloneta sisyphia* (Clerck, 1757) (PINZARI, 2019), the specialized parasitoids, tachinid fly *Erycia furibunda* (PINZARI *et al*., 2016) and likely many other unidentified species.

ACKNOWLEDGMENTS

We would like to thank Mario Pinzari (Rome, Italy) for his technical support during the field work and Prof. Valerio Sbordoni (Department of Biology, Tor Vergata University of Rome, Italy) for his general support during the study; special thanks go to Prof. Roger L.H. Dennis (Centre for

Ecology, Environment and Conservation, Oxford Brookes University), for his comments and suggestions on the final version of the manuscript.

The present study is a part of the project of the Osservatorio per la Biodiversità del Lazio (OBL), coordinated by Prof. Valerio Sbordoni, on behalf of "Assessorato Ambiente" e "Cooperazione tra i Popoli della Regione Lazio" aiming at providing guidelines to monitor those species protected under the Habitat Directive 92/43/CEE (Art. 17, par. 1). Because *E. aurinia* is a protected species under the Natura 2000 network, capture, manipulation and sample collecting of this butterfly was performed under permission of The Ministry of Environment (Prot.0032150 - 23/04/2013 - PNM-II).

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REDIA, 102, 2019: 95-98 http://dx.doi.org/10.19263/REDIA-102.19.14

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FIRST RECORD OF *MONOTOMA CONICICOLLIS* CHEVROLAT, 1837 (COLEOPTERA MONOTOMIDAE) IN THE APENNINES: INTRODUCTION OR SIMPLE OVERLOOKING?

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Cianferoni F., Frizzi F., Santini G. – First record of *Monotoma conicicollis* Chevrolat, 1837 (Coleoptera Monotomidae) in the Apennines: introduction or simple overlooking?

The myrmecophilous beetle *Monotoma conicicollis* Chevrolat, 1837 (Cucujoidea: Monotomidae) is recorded for the first time in the Apennines in a nest of *Formica paralugubris* Seifert, 1996 (Formicidae).

Since nests of this ant species were translocated from Alps in the past, two possible scenarios about the occurrence of this beetle are discussed: a simple overlooking of *M. conicicollis*, already occurring (in association with other *Formica* spp.) in the Apennines prior the introduction of *F. paralugubris*, or its introduction together with the ant.

KEY WORDS: Cucujoidea, Monotominae, *Formica rufa* group, *Formica paralugubris*, introduction.

INTRODUCTION

The beetle genus *Monotoma* Herbst, 1793 (Cucujoidea: Monotomidae: Monotominae) includes 23 spp. in the Palaearctic Region (JELÍNEK, 2007) and 15 spp. in Italy (ANGELINI *et al.*, 2018), of which one, *M. americana* Aubé, 1837, is introduced (see JELÍNEK, 2007; ANGELINI *et al.*, 2018).

Monotoma conicicollis Chevrolat, 1837 is a species widespread from Europe to Far East (see JELÍNEK, 2007). In Italy this taxon was known so far only for Northern Italy, with generic records from Piedmont and Trentino-Alto Adige (LUIGIONI, 1929; PORTA, 1929).

Monotoma conicicollis is a myrmecophilous beetle associated with ants belonging to the *Formica rufa* group (subgenus *Formica* s.s.), e.g. *F. rufa* Linnaeus, 1761, *F. pratensis* Retzius, 1783, *F. lugubris* Zetterstedt, 1840, *F. polyctena* Förster, 1850, *F. uralensis* Ruzsky, 1895, *F. aquilonia* Yarrow, 1955, and to *F. exsecta* Nylander, 1846 belonging to the subgenus *Coptoformica* Müller, 1923 (PÄIVINEN *et al.*, 2002; SOLODOVNIKOV and PLISKEVICH, 2014). *Monotoma conicicollis* was detected sometimes as the most abundant beetle species in Finnish nest samples of mound-building wood ants (e.g. PÄIVINEN *et al.*, 2004 with *F. aquilonia*) or as a quite rare myrmecophilous (e.g. HÄRKÖNEN and SORVARI, 2014 with *F. polyctena*).

In Italy *M. conicicollis* was reported in association with *F. rufa* and *F. pratensis* (PORTA, 1929).

Together with *M. angusticollis* Gyllenhal, 1827, *M. conicicollis* has been included in the subgenus *Gyroceris* Thomson, 1863 because of the association of the two species with nests of *Formica* s.s. or *F. rufa* group (see THOMSON, 1863; OTERO, 1991 as *F. rufa* Linnaeus).

The species is listed in the IUCN Red List of the Italian saproxylic beetles (AUDISIO *et al.*, 2014) as Data Deficient (DD).

MATERIALS AND METHODS

The specimen of *M. conicicollis* was recently sampled in a nest of *F. paralugubris* Seifert, 1996 (Formicidae) in the northern Apennines, in the context of a project dealing with the comparative analysis of the symbiont communities inhabiting the nest mounds of this species between Alpine (native) and Apennine (imported) populations, headed by the Department of Biology, University of Florence (Italy).

Collecting information is ordered as follows: country, region, municipality and province (in brackets), locality, coordinates (geographical: degrees, minutes, seconds; datum: WGS84), elevation (a.s.l.), date, collectors (leg. = legerunt).

The specimen, dry mounted, is preserved in the collection of the first author (Florence, Italy).

Dorsal habitus and details of the front and middle legs, with the tibial spines indicated, are figured. The photos were taken by the first author with a Leica M205 C stereomicroscope at the Natural History Museum of the University of Florence.

Nomenclature follows JELÍNEK (2007).

RESULTS AND DISCUSSION

A single specimen of *Monotoma conicicollis* (Fig. I) was recently sampled in a nest of *F. paralugubris*:

MATERIAL EXAMINED - Italy: Tuscany: Abetone Cutigliano (Pistoia), Abetone, 44°08'42.3" N 10°40'23.2" E, ab. 1320 m, 15.VII.2017, F. Frizzi & S. Giannotti leg.

NOMENCLATURAL NOTES - The name "*Monotoma conicicollis*" has been published for the first time by

– Received 16 April 2019 Accepted 16 September 2019

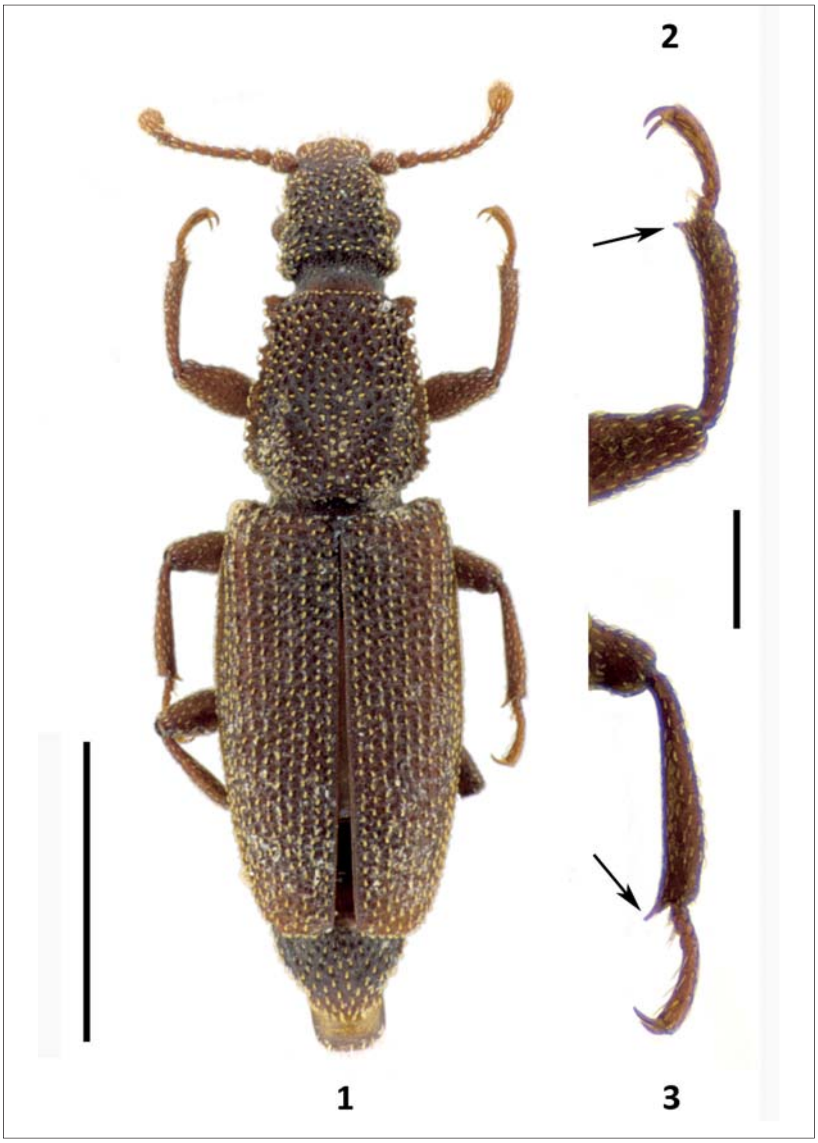

Fig. I – *Monotoma conicicollis* Chevrolat, 1837. 1) Habitus, dorsal view. Scale bar = 1 mm. 2) Detail of a front leg. 3) Detail of a middle leg. The arrows indicate the tibial spines. Scale bar = 200 µm (photo by F. Cianferoni).

Chevrolat (1835: 264) but without giving a description; as such it must be treated as a *nomen nudum*.

The name "*Monotoma Conicicolle*", attributed to L.A.A. Chevrolat, was then used by Dejean (1835: 312) in his catalogue but still not associated to any description (*nomen nudum*).

Guérin-Mèneville (1837: 190, pl. 41, fig. 2 a) briefly described and figured some features of the species and attributed it, as "*Monotoma conicicollis*", to Chevrolat who communicated the information to him (see Cowan, 1971 for datation).

In the same year, Aubé (1837: 455, pl. 17, fig. 1) gave a complete description and a figure of the same taxon. However he mistyped the genus, "*Monomotoma* [sic!] *Conicicollis*", but correctly including it in the context of the genus *Monotoma* Herbst, 1793 and quoted it as "*Monotoma Conicicollis*" in the plate.

The authorship attribution has had also a troubled history: currently the species is listed as "Chevrolat, 1837" (e.g., Jelínek, 2007; Angelini *et al.*, 2018) but in the past it was attributed also to Guérin-Mèneville (e.g., Reitter, 1911; Porta, 1929; Otero, 1991; Bousquet and Laplante S., 1999). The attribution to Aubé (1837) it is instead incorrect, since the work of Guérin-Mèneville (1837) has priority. Thus, the correct authorship attribution is "Chevrolat in Guérin-Mèneville, 1837" or more briefly "Chevrolat, 1837".

Nests of *F. paralugubris*, a native Alpine species (see Seifert, 1996; 2016), were translocated from the 50s of the XX century mainly from the Alps (particularly from Brescia and Bergamo provinces) to the Apennines for "biological control" of some native phytophagous species considered "harmful for the forest" (Pavan, 1959; Adlung, 1966; see Baroni Urbani, 1971; Eichorn, 1981; Ronchetti *et al.*, 1987; Frizzi *et al.*, 2018; Masoni *et al.*, 2018) (Fig. II).

The only species of the *Formica rufa* group (subgenus *Formica* s.s.) native of Tuscan Apennines is *F. pratensis*, where however it seems to be very rare and scattered (see Baroni Urbani, 1971; Pavan *et al.*, 1971). Another species of *Formica* (subgenus *Coptoformica*) also occurring in the Tuscan Apennines and associated to *M. conicicollis* (see Solodovnikov and Pliskevich, 2014) is *F. exsecta* (see Baroni Urbani, 1971).

The record of *M. conicicollis* is the first one for the Apennines and Tuscany, since this species was known so far only for the Alps (Piedmont and Trentino-Alto Adige).

Monotoma conicicollis shows a Sibero-European distribution (see Jelínek, 2007) and in the southern parts of its range it seems limited to mountains areas. According

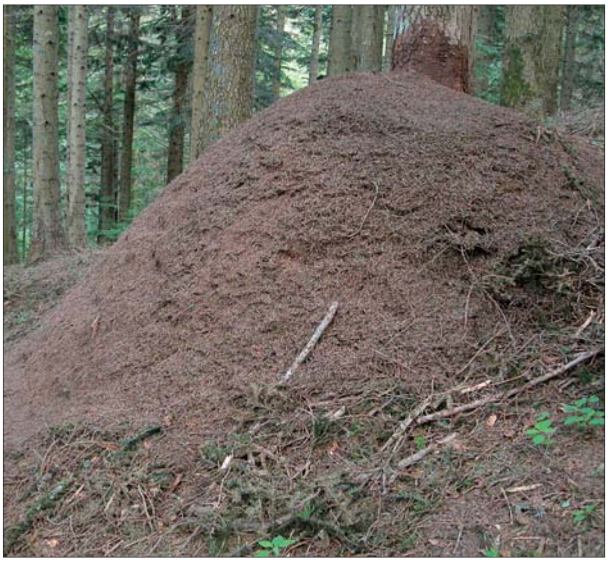

Fig. II – Nest of *Formica paralugubris* Seifert, 1996 in the Apennines (photo by F. Frizzi)

to the current knowledge, it represents the southernmost record for the whole range of the species (see Jelínek, 2007).

Monotoma conicicollis has not been formally reported in association with *F. paralugubris*; however the Italian records of the beetle with *F. lugubris* (Porta, 1929, without exact localities) could be possible misidentifications with this sibling species (see Seifert, 1996; 2016).

Essentially two scenarios seem possible concerning the Apennine record: 1) *Monotoma conicicollis* already occurred in the Apennines prior the introduction of *F. paralugubris*, in association with other native species belonging to the genus *Formica*, like *F. pratensis* or *F. exsecta* (see Porta, 1929; Baroni Urbani, 1971; Seifert, 1992; Solodovnikov and Pliskevich, 2014); 2) *M. conicicollis* was not native from the Apennines and has been moved there from the Alps, following the introductions programs of *F. paralugubris* (see Pavan, 1959).

Both hypotheses seem possible. Several species of insects, previously known only for the Alps, were often overlooked and their populations only recently recorded also in the northern Apennines and surrounding areas (e.g. Cianferoni and Terzani, 2013; Cianferoni *et al.*, 2015). However, the second scenario could be also plausible since the translocations of *F. paralugubris* were made by transferring the whole nests, possibly including also specimens of myrmecophilous taxa (presumably together with several other organisms occurring in soil). Big trucks delivered their load with ant-filled barrels all over Italy (Seifert, 2016).

Further research involving molecular tools could shed light on the origin of the Apennine specimens of *M. conicicollis* and establishing which of the two hypotheses is the correct one. This could lead to very different managing options, which could range from conservation (in case the species is native) up to containment together with *F. paralugubris* (in case of a proven introduction).

ACKNOWLEDGMENTS

The authors are grateful to Alberto Masoni, Stefano Giannotti, and Paride Balzani (Department of Biology, University of Florence - Italy) who helped us during the sampling activities. We would also like to thank the staff of the Reparto Carabinieri Biodiversità of Pistoia (Italy) and the unit of the “Riserva Naturale Biogenetica” of Abetone (Pistoia - Italy) for supporting us during all stages of the project, and Saulo Bambi (Natural History Museum, University of Florence - Italy) for allowing FC to use the microscope under his care for photos.

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REDIA, 102, 2019: 99-106 http://dx.doi.org/10.19263/REDIA-102.19.15

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INVENTORY OF ORNAMENTAL PLANT MEALYBUG (HEMIPTERA PSEUDOCOCCIDAE) IN TUNISIA: SPECIES, HOST PLANTS AND DISTRIBUTION

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Mdellel L., Adouani R., Zouari S., Ben Halima M.K., Germain J.F. – Inventory of ornamental plant mealybugs (Hemiptera Pseudococcidae) in Tunisia: species, host plants and distribution.

In recent years, mealybugs (Hemiptera, Pseudococcidae) have become an important pest of ornamental plant in Tunisia due to their accident introduction and invasiveness. Awareness, early knowledge of invasive species, their host plant and geographical distribution could help to implement control methods which minimize the economic losses caused by them. A survey was conducted during 2013-2018 period to identify the mealybug species on ornamental plant, their host plant and document their geographic and periodic distribution. Seven species belonging to five genera were identified: *Maconellicoccus hirsutus* Green, *Phenacoccus peruvianus* Granara de Willink, *Phenacoccus madeirensis* Green, *Planococcus vovae* Nassonov, *Planococcus citri* Risso, *Ferrisia virgata* Cockerelland *Pseudococcus longispinus* Targioni-Tozzetti. *M. hirsutus*was the most abundant mealybug and found in twenty-one sites on *Hibiscus rosa-sinensis* and *Hibiscus mutabilis*. *P. peruvianus* was the most polyphagous specie found on five host plants belonging to four families in eleven sites. *P. citri* was the single specie observed during wet season, therefore, all the rest species were found during dry season (summer, beginning autumn). These finding provide primary baseline knowledge of the mealybug fauna on ornamental plant in Tunisia and contribute information for use in the development of sustainable ornamental pest management strategies in the country.

KEY WORDS: Mealybug, ornamental plant, invasive species, distribution, Tunisia.

INTRODUCTION

Tunisia, located on the shores of the Mediterranean, is a rich repository of various plant resources. There are 22500 species of vascular plants in the Mediterranean (MYERS *et al.,* 2000), with approximately 11700 found in the 'hotspot region' including Tunisia (THOMPSON *et al.,* 2005). Part of them were having potential worth as medicinal or ornamental plants which play roles connected with positive shade, protection, and recreation both in private and in public areas as well as to complement and decorate the urban environment (MAZZEO *et al.,* 2014). Ornamental trees, shrubs and flowers have always been extremely popular and there is large demand. Thus, the increase demand of ornamental plant and commercial exchange has provided pests which cause several damages (MAZZEO *et al.,* 2014). Indeed, a great many species of insects attack ornamental plants. Among them, aphids, scales, whiteflies, lacebugs and mealybug (YOVKOVA *et al.,* 2013; MAZZEO *et al.,*2014; BEN-HALIMA, 2009; BEN-HALIMA *et al.,* 2014). Mealybug colonize vegetative organs and lives under bark, on leaf, inside bunches and occasionally on berries (BECERRA *et al.,* 2006). Moreover, they have been introduced into different countries in the Mediterranean Region and other areas of the Palaearctic Region (KAYDAN *et al.,* 2012). Cryptic habits and tiny size makes mealybug usually settle in small depressions or protected areas of plants which explain the fast spread throughout the Mediterranean (MARTÍNEZ-FERRER *et al.,* 2003). In Tunisia, literature provides little information about invasive mealybugs on ornamental plants, their geographic repartition and period abundance. In order to understand which mealybugs are most likely to invade Tunisia in the future and to implement control methods which minimize their economic losses, this study aimed at showing ornamental plant invasives mealybugs species in Tunisia, their geographical distribution and determining period of their abundance.

MATERIALS AND METHODS

STUDY SITES

This work was conducted during 2013-2018 period in ten Tunisian coastal governorates (Fig. I). A total of twenty three sites were surveyed (Table 1). Sites were chosen defined on the basis of presence of multitude varieties/species of ornamental plant and different climate.

COLLECTION OF MEALYBUGS

Infested leaves and inflorescences with mealybugs have first been photographed. Mealybugs were then collected and kept in alcohol 70° and sent to ANSES-Plant Health Laboratory/France for identification.

MORPHOLOGICAL IDENTIFICATION

Mealybugs were prepared by a five-stage process before mounting: i) the specimen was heated gently (<40°C) in 10% KOH for 20 min; ii) the specimen was washed in distilled

– Received 26 March 2019 Accepted 18 September 2019

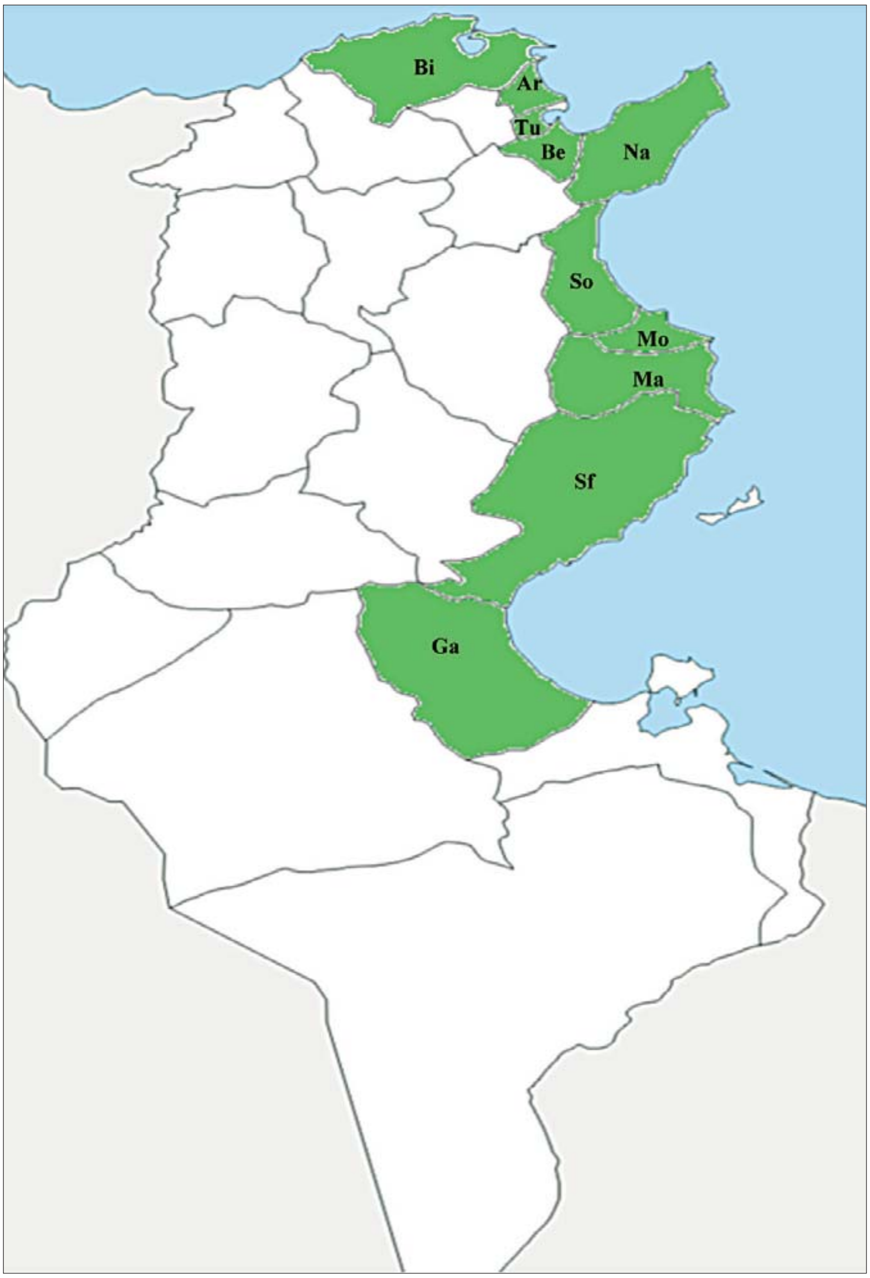

Fig I. Survey sites of ornamental plants mealybugs in Tunisia.

Ar: Ariana
Be: Ben-Arous
Bi: Bizerte
Ga: Gabes
Ma: Mahdia
Mo: Mounastir
Na: Nabeul
Sf: Sfax
So: Sousse
Tu: Tunis

Table 1 – Inventory sites and monitoring of ornamental plants mealybugs in Tunisia.

Governorate	Site/Zone	Climate*	Survey period
Bizerte	Bizerte City	Warm temperate	2016
Ariana	Ariana city, El-aouina, Sidi Thabet	Warm temperate	2016 - 2017
Tunis	Tunis city, El-marsa, Goulette Sidi Bou-saiid, Carthage	Warm temperate	2015 - 2017
Ben-Arous	Bou-mhel	Warm temperate	2016
Sousse	Sousse city, Msaken, Hammem-Sousse, Akouda, Chott Mariem, Kalaa Kbira, khezama, Port Kantaoui	Hot semi arid	2013 - 2018
Monastir	Monastir city	Hot semi arid	2016
Mahdia	Mahdia city	Hot semi arid	2016
Sfax	Sfax city	arid	2016
Gabes	Gabes city, Merth	Hot desert	2015 - 2016

Climate*: Koppen Geiger classification

water for 20 min; iii) the specimen was stained by incubation for 1 h in a stored solution of fuchsine in a 1:1:1 mixture of distilled water, lactic acid and glycerol; iv) the specimen was washed in glacial acetic acid for 1 h to stabilize the staining; v) the specimen was transferred to lavender oil for at least 1 h, placed in a drop of Canada balsamon a slide and covered with a cover slip. The slide was then labelled and observed immediately under amicroscope or after drying (40°C for 4-6 weeks).In most cases, identification was based on the keys of Cox (1989), Williams & Granara de Willink(1992) and Williams (2004); Kaydan & Gullan(2012), Danzig & Gavrilov-Zimin (2014; 2015).

RESULTS

Mealybug Species Identified

Seven mealybug species have been identified on ornamental plant in 23 surveyed sites. These are: *Maconellicoccus hirsutus* Green (Fig. II, 1-4), *Phenacoccus peruvianus* Granara de Willink (Fig. III, 1-3), *Phenacoccus madeirensis* Green (Fig. IV, 1-3), *Planococcus vovae* Nasonov (Fig. V, 1-2), *Ferrisia virgata* Cockerell (Fig. VI), *Planococcus citri* Risso (Fig. VII) and *Pseudococcus longispinus* Targioni-Tozzetti.

Host Plant and Geographical Distribution

The seven mealybugs were found on twelve ornamental plants belonging to nine families (Table 2). *Phenacoccus peruvianus* was the most polyphagous mealybugs recorded on four host plants (*Myoporum* sp., *Citharexylum quadrangularis*, *Bougainvillea glabra*) belonging to three families (Scrophulariacae, Verbenaceae, Nyctaginaceae). It's recorded on leaves, shoots and trunk (Fig. II, 4). *Ferrisia virgata*, *Planococcus vovae* and *Pseudococcus longispinus* were monophagous and recorded on leaves of *Lantana camara (*Verbenacae*)*, *Cupressus mactocarpum* (Cupressecae) and *Jasminum grandiflorum* (Oleaceae) respectively. *Maconellicoccus hirsutus*was the most relevant mealybug recorded in 19 sites in the north, middle and south of Tunisia. *Phenacoccus peruvianus* was the second relevant specie recorded in 12 sites. *Ferrisia virgata*, *P. vovae* and *P. longispinus*were the less relevant species and recorded on *L. camara, C. macrocarpum*and *J. grandiflorum* respectively.

Mealybug Species and Seasonal Abundance

Seasonal abundance of mealybugs on ornamental plants in 23 sites was shown in Table 3. During survey period (2013-2018), all species were recorded during dry season (June, July, August). *P. citri* was the single specie recorded in April (wet season). *P. peruvianus* and *M. hirsutus*were the relevant species and observed during four months (June, July, August, and September). *F. virigata,* observed on *Lantana camara*, was the single specie whose period attack spreads from dry season (August, September) to wet season (October). *P. longispinus*was a less prelevent specie recorded on *Jasminum grandiflorum* during short period (July) in one site/zone (Kantaoui Port).

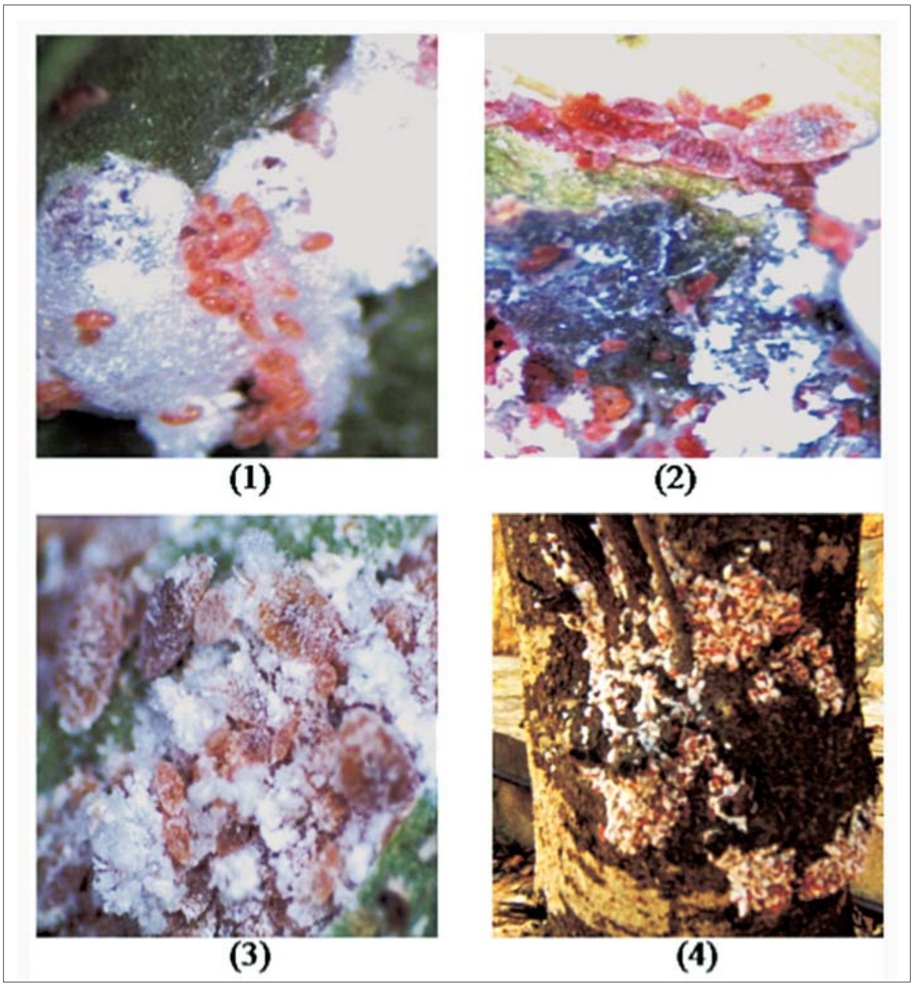

Fig. II – *Maconellicoccus hirsutus* on *Hibuscus rosacinensis*

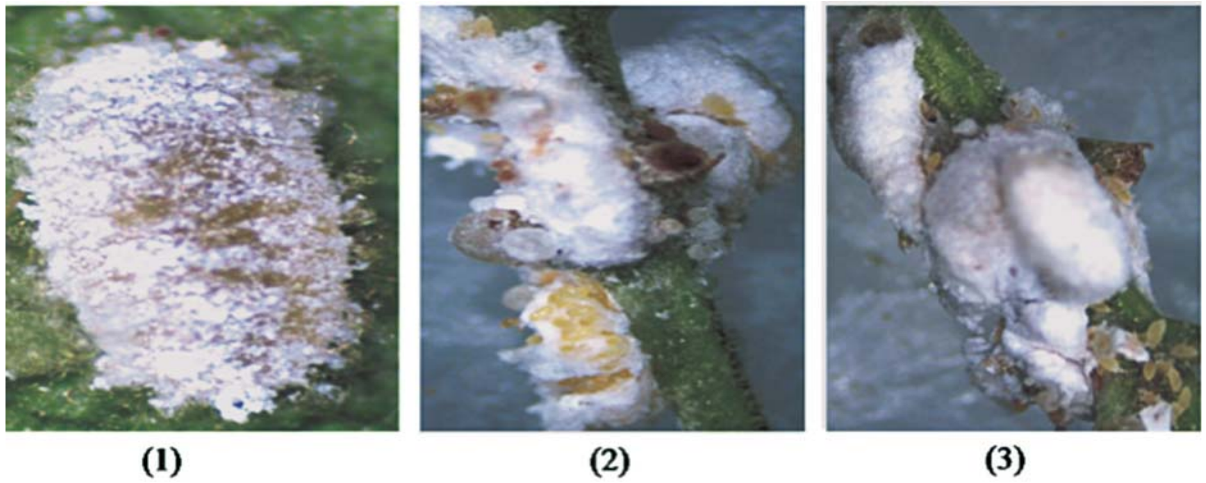

Fig. III – *Phenacoccus. peruvianus* on *Citharexylum quadrangularis*

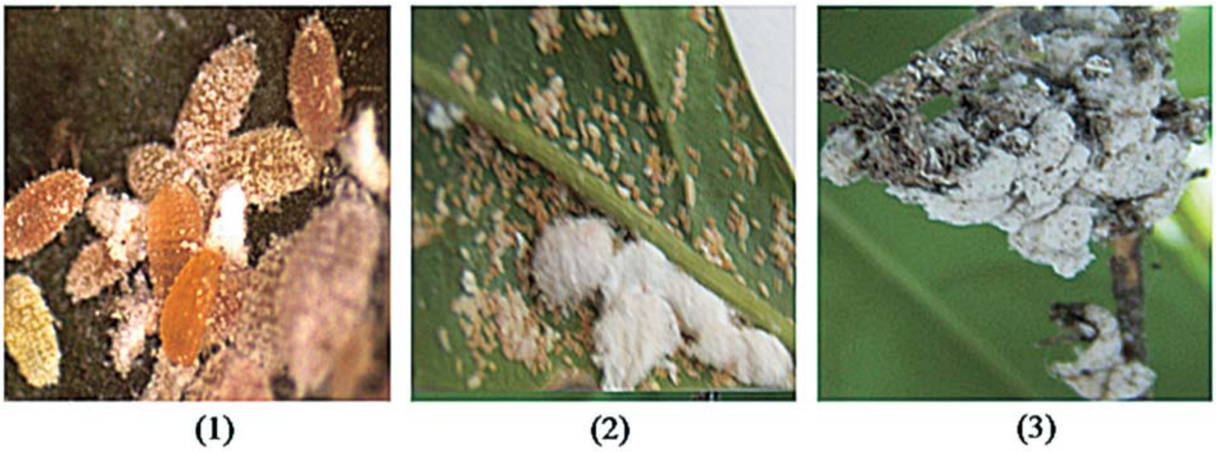

Fig. IV – *Phenacoccus madeirensis* on *Cestrum nocturnum*.

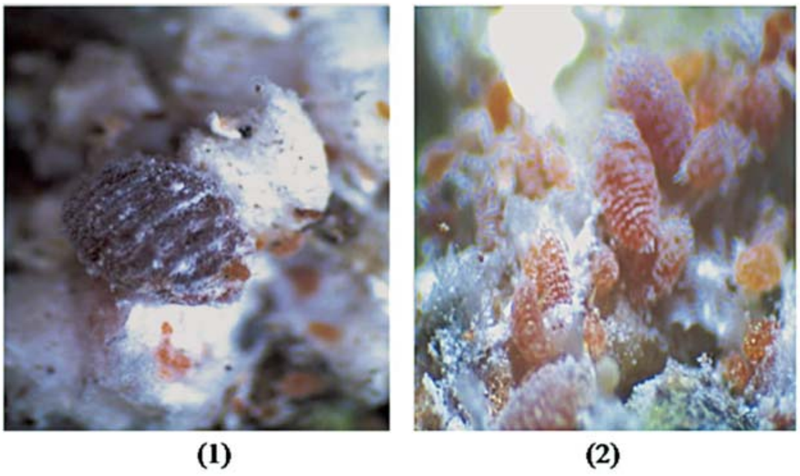

Fig. V – *Planococcus vovae* on *Cupressus mactocarpum*.

Fig. VI – *Ferrisia virgata* on *Lantana camara.*

Fig. VII. *Planococcus citri* on *Tradescantia fluminensis*

Table 2 – Mealybug species, host plant and geographical distribution in survey sites.

Mealybug specie	Ornamental plant/ Family	Site/Governorate
Maconellicoccus hirsitus	*Hibiscus rosa-sinensis*/Malvaceae	– Tunis City, El-Marsa, Goulette, Sidi-Bou Saiid, Carthage/Tunis. – Port Kantaoui, Akouda, Chott Mariem, Hammem-Sousse, Msaken/Sousse. – Ariana City, El-aouina/Ariana. – Bizerte City/Bizerte. – Monastir City/Monastir. – Boumhel/Ben-Arous. – Sfax City/Sfax – Gabes City, Merth/Gabes
	Hibiscus mutabilis/Malvaceae	– Chott Mariem/Sousse.
Phenacoccus peruvianus	*Myoporum sp*/ Scrophulariacae	– Sousse City, Hammem Sousse, Akouda, Kalaa Kbira/Sousse. – Monastir City/Monastir. – Tunis City/Tunis.
	Capsicum frutescens/Solanaceae	– Akouda/Sousse.
	*Citharexylum quadrangularis/*Verbenaceae	– Akouda/Souss
	Bougainvillea glabra/Nyctaginaceae	– Sousse City, Akouda, Hammem-Sousse, Msaken, Kantaoui-Port/Sousse. – Ariana City, El Aouina/Ariana. – Tunis City, El-Marsa/Tunis. – Monastir City/Monastir.
Phenacoccus madeirensis	*Cestrum nocturnum/*Solanaceae *Lantana camara*/Verbenaceae	– Akouda/Sousse. – Hammem-Sousse/Sousse.
Ferrisia virgata	*Lantana camara*/Verbenaceae	– Khezama/Sousse.
Planococcus vovae	*Cupressus macrocarpa*/Cuprussaceae	– Hammem-Sousse/Sousse.
Pseudococcus longispirus	*Jasminum grandiflorum*/Oleacae	– Kantaoui-Port/Sousse.
Planococcus citri	*Tradescantia fluminensis*/Commelinaceae *Thecoma smithi*/Bignoniaceae	– Sidi Thabet/Ariana. – Chott Mareim/Sousse.

DISCUSSION

The survey carried out in 23 sites/zones of 10 Tunisian coastal governorates allowed the discovery of seven mealybugs' species on 12 ornamental plants. These include *M. hirsutus*, *P. peruvianus*, *P. madeirensis*, *P. vovae* and *F. virgata*, *P. citri* and *P. longispinus*. So, this study reports for the first time on the existence of *M. hirsutus*, *P. peruvianus*, *P. madeirensis*, *P. vovae* and *F. virgata*in Tunisia and in the North of Africa (Ben-Halima *et al.,* 2014; 2015). *M. hirsutus*is probably from South-East Asia and has spread to Australia, Africa, the Middle East, Central America, the northern countries of South America with a recent report from Brazil (Marsaro Júnior *et al.,* 2013), and USA (Eppo, 2006; Bográn & Ludwig, 2007; Chong, 2009). In Europe, *M. hirsutus* was recorded only in Cyprus (Eppo, 2011). Recently, Spodek *et al.* (2016) reported the *M. hirsutus* in Israel. *P. peruvianus* recorded in *Bougainvillea glabra, Myoporum* sp., *Citharexylum quadrangularis* and *Capsicum frutescens* has a Neotropical origin and has been

Table 3 – Abundance of mealybugs according to the season.

Mealybugs	Wet season				Dry season					Wet season		
	January	February	March	April	May	June	July	August	September	October	November	December
Planococcus citri				+			+	+				
Planococcus vovae							+					
Pseudococcus longispinus							+					
Ferrisia virgata								+	+			
Phenacoccus madeirensis								+	+			
Phenacoccus peruvianus						+	+	+	+			
Maconellicoccus hirsutus						+	+	+	+			

recently recorded in Europe (Granara de Willink & Szumik, 2007). It was reported in southern Spain in 1999, subsequently, Sicily in 2002, U.K. and Corsica in 2005, Portugal in 2006, France mainland in 2008, Majorca Island in 2010 and Greece in 2013 (Beltrà *et al.,* 2010; Vasiliki & Panagiotis, 2013). *P. madeirensis* recorded in two sites on *Cestrum nocturnum* and *Lantana camara* was considered from Neotropical origin and now was widely distributed in Afrotropical, Australasian, Nearctic, Neotropical and Oriental zoogeographical regions (Ben-Dov *et al.,* 2013). In Europe, *P. madeirensis* was recorded in 1990 in Italy (Marotta & Tranfaglia, 1990), Crete (Jansen *et al.,* 2010), France (Matile-Ferrero & Germain, 2004), Greece (Papadopoulou & Chryssohoides, 2012), Portugal (Franco *et al.,* 2011), Spain (Beltrà & Soto, 2011) and Turkey (Kaydan *et al.,* 2012). There are also several records in the Far-East of the Palaearctic Region (Kondo *et al.,* 2001). In the last decade, the Madeira mealybug has continued to spread in the Mediterranean region and in other parts of the world, such as southern Asia and tropical Africa (Williams, 2004). As for host plant, *M. hirsutus* was relevant on leaves, shoots, trunk and collar of *H. rosa-sinensis* and seldom on *H. mutabilis*. Ben-Dov *et al.* (2015) reports the polyphagy of *M. hirsutus* which feeds on a wide range of ornamental and agricultural host plant species distributed in 218 genera of 76 botanic families. Fabaceae, Malvaceaeand Moraceae were the preference host plants and economical damages was on avocado, banana, citrus, cotton, grapevine and mulberry (Eppo, 2005; 2006). *P. peruvianus* was recorded on four ornamental plants belonging to four families. *Bougainvillea and Myoporum* were the most attacked plants. Beltrà *et al.* (2010) shows that *P. peruvianus* has been reported on Acanthaceae, Amaranthaceae, Asclepiadaceae, Asteraceae, Aucubaceae, Myoporaceae, Scrophulariaceae and Solanaceae. *Phenacoccus madeirensis, Ferrisia virgata, Planococcus vovae, Pseudococcus longispinus*and *Planococcus citri* were less relevant and monophagous recorded in limited sites. *F. virgata* was reported on cotton in India, Pakistan and Brazil (Oleiveira *et al.,* 2014). *P. citri* is a serious polyphagous species infesting a broad range of subtropical fruit plants and ornamentals worldwide (Mansour *et al.,* 2017). In Tunisia, *P. citri* was the most abundant and frequent scale insect species occurring on citrus trees (Jendoubi, 2007). As for abundant populations of recorded species, it was alternately depending on the season and the relevant population was during dry season. Indeed, influence of weather and ecosystems were among factors which affect mealybugs abundance and geographical repartition (Correa *et al.,* 2015). The climate as an extrinsic integrative factor plays a crucial role in determining the abundance and distribution of insect pest population. Singh *et al.* (2009) had observed that abiotic factors are believed to be responsible for pest population dynamics. Nébié *et al.* (2016) show the abundance of *F. virgata* during dry period and there are a significant and negative correlation between the populations of *F. virgata* with relative humidity and rainfall. Also, Katke (2008) showsthe incidence of *F. virgata* during dry season or prolonged period of drought. Therefore, Ghose (1972) was of the opinion that *M. hirsutus* was active during winter also, without hibernation but was most active during March-October on roselle around West Bengal. Azam (1983) found that the active period of *M. hirsutus* was during June-August and October-March on grapes around Hyderabad. These results demonstrate the necessity of several researches on impact of ecosystems and climate on abundance, distribution, biological parameters and abundance of their natural enemies in order to implement control methods which minimize their economic losses. In conclusion, during this study, seven mealybugs species were inventoried on ornamental plants in 23 sites. These include *M. hirsutus*, *P. peruvianus*, *P. madeirensis*, *P. vovae*, *F. virgata*, *P. citri* and *P. longispinus*, *M. hirsutus* and *P. peruvianus* were more abundant than *P. madeirensis*, *P. vovae*, *F. virgata*, *P. citri* and *P. longispinus* and their periodicity were related to geographic ornamental plant distribution and climate characteristic. Abundance of *M. hirsutus* and *P. peruvianus* may cause significant damages to ornamental plant. This shows the need to explore opportunities for sustainable control of this pest. The management of these mealybugs needs additional research on their host plants, damages, dispersion and their natural enemies.

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REDIA, 102, 2019: 107-111 http://dx.doi.org/10.19263/REDIA-102.19.16

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PUPA MORTALITY OF *ORGYIA TRIGOTEPHRAS* BOISDUVAL, 1829 (EREBIDAE LYMANTRIINAE) IN TUNISIA

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Hammami S., Ezzine O., Dhahri S., Villemant C., Schmidt S., Ben Jamâa M.L. – Pupa mortality of *Orgyia trigotephras* Boisduval, 1829 (Erebidae Lymantriinae) in Tunisia.

Orgyia trigotephras (Boisduval) is a polyphagous moth which is bivoltine in Tunisia, with two generations (spring and autumnal). This work, carried out in northeastern and northwestern Tunisia, aimed at studying pupal mortality of this pest on two host species, *Quercus coccifera* L. and *Pistacia lentiscus* L. Two types of antagonist factors were assessed: natural mortality (dried pupae) and mortality caused by parasitoids. Three pupal parasitoids species belonging to Hymenoptera order were identified: *Brachymeria tibialis* (Walker), *Pimpla rufipes* (Miller) and *Monodontomerus minor* (Ratzeburg).

KEY WORDS: *Orgyia trigotephras*, *Quercus coccifera*, *Pistacia lentiscus*, Parasitoids, Tunisia.

INTRODUCTION

Populations of forest pests often fluctuate, showing outbreaks and extended periods of low and endemic densities (BERRYMAN *et al*., 1987). Phytophagous insects may select plants or plant parts not only based upon nutritional content, but also on the intensity of predation and parasitism they may undergo (BERNAYS *et al*., 1988). During outbreaks, Lepidoptera defoliators of *Quercus* trees cause considerable damage, often leading to complete defoliation of trees. This defoliation could cause negative effects on their production and regeneration. Dynamic of lepidopteran insects is limited by the effect of the quality and the quantity of foliage of the host plant (SCHULTZ & BALDWIN, 1982; FRAVAL, 1984; MATTSON & HAACK, 1987), favorable climate (MARTINAT, 1987) and parasitoids (CANDAU, 2008) which are generally considered as key natural enemies (KIDD & JERVIS, 1997). These parasitoids have an important place in the ecosystems notably as naturals control agents of herbivorous insects (GODFRAY, 1994).

Orgyia trigotephras is a polyphagous moth widely distributed across the Mediterranean Basin (BASRI, 1994). It feeds on plants of the genus *Quercus*: *Q. suber* L., *Q. ilex* L. (VILLEMANT & FRAVAL, 1993; CHAKALI *et al*., 2002) and *Q. coccifera* L. (EZZINE *et al*., 2010), but also on shrub species, mainly: *Pistacia lentiscus* L. (EZZINE *et al*., 2010; BELLA *et al*., 2011) and *Retama monosperma* L. (DIONISIO, 2002).

Populations of oak lepidopteran pests are regulated by a variety of parasitoids that attacks every of their preimaginal instars (VILLEMANT & FRAVAL, 1991). In Morocco, in the Mamora cork oak forest, VILLEMANT (1989) reported the presence of five species of Hymenoptera parasitizing *Lymantria dispar* pupae: The Ichneumonidae *Pimpla rufipes* (F.), *P. turionellae moraguesi* (Schmiedecknecht) and *Vulgichneumon* sp., as well as two species of Chalcidoidea: the Chalcididae *Brachymeria tibialis* (Walker) and the Torymidae *Monodontomerus aereus* (Walker). Among them *P. turionellae moraguesi* and *M. aereus* were also reported as parasitoïds of *O. trigotephras* in the same forest (VILLEMANT & FRAVAL, 1991). BASRI (1994) also reported that *P. turionellae moraguesi* and *P. rufipes* attack both moth species in Morocco.

In a previous work (HAMMAMI *et al*., 2017) we showed that larval mortality of *O. trigotephras* in Tunisian oak forests has a significant impact on its population dynamics. In the present work, we combined field data and laboratory experiments, to evaluate the factors inducing pupal mortality of this pest: (i) dried pupae and (ii) mortality due to pupal parasitoids.

MATERIALS AND METHODS

STUDY AREA

The study was carried out in the northeastern (Jebel Abderrahmane, Cap Bon) in (Ftahiz; alt. 121m, 36°52’N, 10°45’E), (Delhiza; alt. 401 m, 36°51’N,10°47’E) and (Guitoun; alt. 136m, 36°83’N, 10°82’E) and the northwestern (Sejnane, Bizerte) (Dam Ziatine, alt. 48m, 37°11’N, 9°11’E) Tunisia.

SAMPLING AND HOST PLANT INFESTATION LEVEL

Pupae collection was made in 2013 from mid-May to mid-June, on 30 trees of each plant species: *Q. coccifera*

– Received 26 March 2019 Accepted 25 September 2019

(QC) and *P. lentiscus* (PL) in every station (Table 1). As a whole, 221 pupae were directly collected from the field. All were then placed individually in plastic boxes (8 cm height x 3cm Ø) at 25±2°C to follow mortality factors (dried pupae) and parasitism (parasitoid's emergence) (Table 1).

After two years of outbreak, in 2005 and 2009 (Ezzine *et al*., 2015a), the pest remained at low level of infestation in Jebel Abderrahmane since 2012 (Ezzine, 2016). In Sejnane, the pest population remained at low level since it has been first recorded in 2010 inducing with the Gelechiidae *Anacampsis scintillella* the defoliation of trees and shrubs (including QC and PL) of this cork oak forest (Ezzine *et al*., 2015b). In this work, host plant infestation was evaluated by counting the number of moth pupae on 30 trees of each plant species.

Table 1 – Number of pupae (N) collected from each station and on host plant species.

Station	Host plant	N
Ftahiz	*P. lentiscus*	68
	Q. coccifera	7
Delhiza	*P. lentiscus*	50
	Q. coccifera	38
Guitoun	*P. lentiscus*	26
	Q. coccifera	7
Dam Ziatine	*P. lentiscus*	8
	Q. coccifera	17

Parasitoid Identification

Emerged adults of parasitoids were preserved with ethanol (96%) in Eppendorf tubes (1.5 ml). Morphological identification was done at species level using keys: Bouček (1951) for *Brachymeria tibialis,* Fitton *et al*. (1988) for *Pimpla rufipes* and Peck *et al*. (1964) for *Monodontomerus minor*.

Statistical analysis

Generalized linear models (GLMs) were applied to the following dependent variables: (1) number of pupae on each host species; (2) total dead pupae; (3) dried pupae and (4) parasitized pupae. Two explanatory categorical variables were tested: host species and stations. The best distribution model was chosen according to the deviance to the degrees of freedom (df) criterion. A Poisson distribution model best fitted the number of pupae on each host species and total of *M. minor* in each station. For the other variables, the Negative Binomial distribution model best fitted the data. Results are presented in the form of the Wald's chi-square test value (χ^2), parameter estimates and the respective P value.

RESULTS

Host plant infestation level

There is a significant difference of infestation level (mean number of pupae/tree) between the two host plants (χ^2_1= 4.613; *P*=0.032) but not between stations. The interaction term was significant (χ^2_2= 7.623; *P*=0.022). Number of pupae observed on PL was the highest in Ftahiz and the lowest in Dam Ziatine (Table 2) while, on QC the number of observed pupae was the highest in Delhiza and the lowest in Ftahiz and Guitoun (Table 2).

Table 2 – Average number (±SE) of pupae in each station on both host plant species.

Station	Host plant	Number of pupae			
		mean/ tree	SE	min/ tree	max/ tree
Ftahiz	*P. lentiscus*	2.26	0.67	2	12
	Q. coccifera	0.23	0.1	1	2
Delhiza	*P. lentiscus*	1.66	0.48	2	10
	Q. coccifera	1.26	0.47	1	13
Guitoun	*P. lentiscus*	0.86	0.37	1	10
	Q. coccifera	0.23	0.12	1	3
Dam Ziatine	*P. lentiscus*	0.80	0.29	1	5
	Q. coccifera	1.1	0.3	1	6

General pupal mortality

Among the 221 studied pupae, 165 adult moths emerged (74.6%). Pupal mortality was 25.3% as a whole. There was no significant difference between stations and host plants. In Ftahiz (42%) and Dam Ziatine (23%) general mortality rate was higher on QC (42% and 23% respectively) than on PL (26% and 13% respectively). Contrariwise, in Delhiza, mortality rate was higher on PL (38%) than on QC (16%). In Guitoun, mortality reached 15% on both host plants (Fig. I).

Dried pupae

Statistical analysis showed that there is no significant difference of dried pupae between stations and host plants. In Ftahiz, dried pupae were observed only on PL (10%), while, in Delhiza and Dam Ziatine dried pupae were observed only on QC (24% and 16% respectively) (Fig. II). In Guitoun, percentage of dried pupae varied between 11% and 14% on both host plants.

Parasitism

Parasitoid adults emerged from June 2013 to September 2013. No significant difference of parasitized pupae numbers was observed between host plants, but a highly significant difference was observed between stations (χ^2_3=14.654, *P*=0.002). Parasitism was higher on QC in Ftahiz (43%) and Delhiza on PL (38%), whereas it

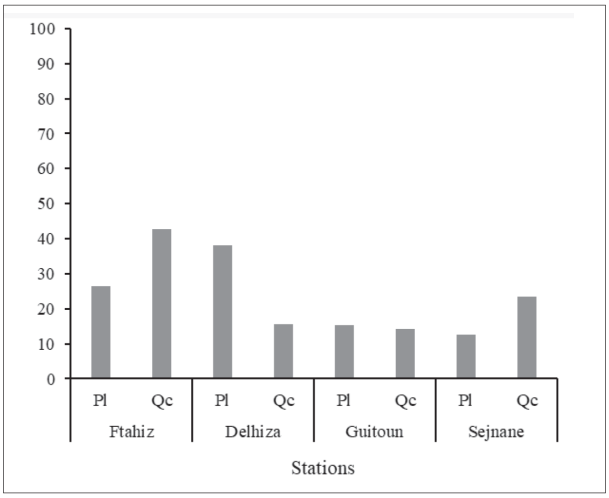

Fig. I – Global mortality rate of *O. trigotephras* pupae on *P. lentiscus* and Q. coccifera.

remained very low in Guitoun and Dam Ziatine (4% and 13% respectively) (Fig. II).

Three pupal parasitoid species belonging to the Hymenoptera order were identified (Fig. III): *Brachymeria tibialis* (Walker, 1834), *Pimpla rufipes* (Miller) and *Monodontomerus minor* (Ratzeburg).

Only one specimen of *B. tibialis* emerged in July 2013 from pupae collected on PL in Delhiza, also only one specimen of *P. rufipes* emerged from pupae collected on PL in Guitoun in June 2013 and, another one emerged from a pupa collected in Dam Ziatine in July 2013.

Monodontomerus minor was obtained from pupae collected on PL in both Delhiza and Ftahiz, without a significant difference of parasitism. Total number of *M. minor* reached 31 specimens in Delhiza and 10 in Ftahiz. Sex ratio was in favor of females in the two stations reaching 0.40 in Delhiza and 0.25 in Ftahiz.

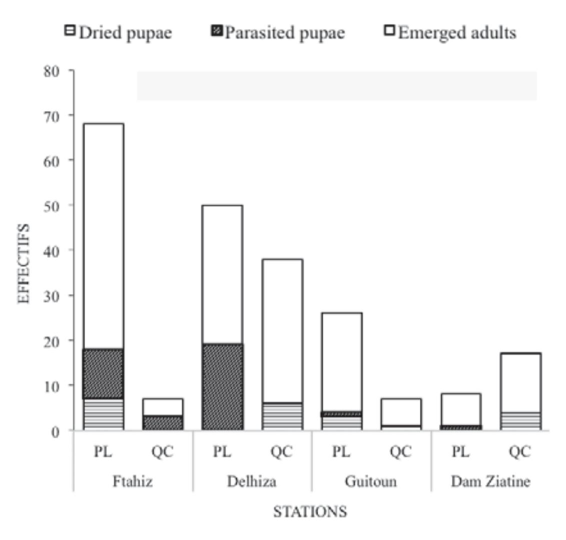

Fig. II – Rate of Parasitism and dried pupae of *O. trigotephras* on *P. lentiscus* and *Q. coccifera* in each stations.

DISCUSSION

In our study, as a whole, population density of *O. trigotephras* was low (average density =1.05 pupae/tree) and pupa mortality rather low (25%). We found that, except for Dam Ziatine (Sejnane) where the general infestation was the lowest, the number of pupae of *O. trigotephras* was higher on PL than on QC (Jbel Abderrhamane). Ezzine *et al*., (2015a) showed that during outbreak years (2005 and 2009) in Jebel Abderrahmane, density of egg masses of *O. trigotephras* was higher on PL than on QC, while, at low population density (2014), infestation on QC was slightly higher than on PL. Ezzine *et al*., (2015b) showed that in Sejnane and, during the 2010 outbreak, pupae of *O. trigotephras* were mainly observed on *Halimium halimifolium* (L.) Willk., *Q. coccifera* and *P. lentiscus*. However, larval parasitism was also higher on QC than on PL (Hammami *et al*., 2017) which can partly explain the inverse results we obtained for pupal numbers.

Host use for oviposition by *O. trigotephras* is ruled by resources exploitation in function of density dependent intraspecific competition (Ezzine *et al*., 2015a) and interspecific competition between *O. trigotephras* and *Acrobasis consociella* (Pyralidae) which also feed on QC (Ezzine *et al*., 2016). Our study (2013) coincides with the retrogradation phase of *O. trigotephras* population in all stations. The general mortality varied between stations but not significantly and was higher on PL than on QC in Delhiza. This variability may be due not only to the difference in the density of QC trees that was higher in Delhiza than in the other stations (Ezzine, 2016) but also to the effect of larval parasitoids (Hammami *et al*., 2017).

Natural mortality (dried pupae) rate may be determined by the pest gradation level, the quantity and the quality of available food that mature larvae need to pupate. Likewise, competition between *O. trigotephras* and *A. consociella* was higher in Delhiza than in the other stations (Ezzine, *pers. obs.*) since neonate larvae feed on QC (Ezzine *et al*., 2015a).

Fraval (1984) showed that the quality and the quantity

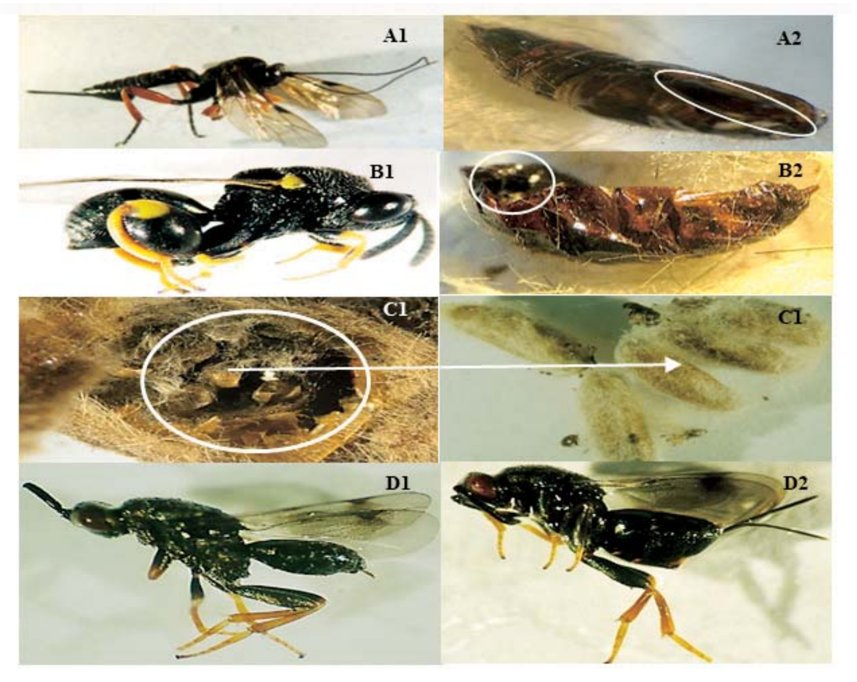

Fig. III – A. *Pimpla rufipes:* A1. Adult, A2. parazited pupa. B. *Brachymeria tibialis:* BI. Adult, B2. parazited pupa. CI. Parasitized pupa of *M. minor, D1.* Adult male of *M. minor, D2.* adult female of *M. minor.*

of food are among the most important factors in the regulation of *L. dispar* populations. HÉRARD (1984) showed that the old leaves of cork oak that persist are scorned as long as the new foliage is not exhausted, their consumption by the larvae of *L. dispar* causes high mortality and reduces the reproductive potential of the population.

General mortality caused by parasitism was low (5%). The pupal parasitism rate was significantly different between host species but not between stations. This may be due to the interaction between plant species. In fact, GRIPENBERG *et al*. (2010) showed that host plant selection and herbivore performance are correlated. Furthermore, CONNELL (1983) indicated that competition among different plant species includes all the limiting effects between plants (resources and allopathy) which reduce or prevent the growth and the survival of the plant.

Thus, the use of plants like PL that do not offer good food quality can be offset by an indirect positive effect "the escape from natural enemies" (EZZINE *et al*., 2015a). It should be the case of *M. minor* that was observed only on PL in Jebel Abderrahmane. Forty-one specimens emerged in the lab from pupae collected on PL from early June to the end of September 2013. This Torymidae is a gregarious parasitoid of lepidopteran pupae and often a hyperparasitoid of Hymenoptera and Diptera via their lepidopteran and Hymenopteran hosts (STEFFAN, 1952; KULMAN, 1965; IWATA & TACHIKAWA, 1966).

The other parasitoids collected are too few to draw the same hypothesis. Only one specimen of *B. tibialis* was observed on PL in only one station (Delhiza) where it was already observed during an outbreak of *O. trigotephras* in 2005 (Ezzine *pers. obs.*). *Brachymeria tibialis* is known to attack *L. dispar* during outbreak (VILLEMANT, 1989) in most European and North African countries.

Another common parasitoid of moths in Europe, *P. rufipes,* attacks a wide range of Lepidoptera species (VILLEMANT & FRAVAL, 1991), notably pupae of *L. dispar* and *O. trigotephras* (DAJOZ, 2010). In our study (2013) two specimens of this ichneumonid emerged from pupae collected on PL in Guitoun and Dam Ziatine, while in 2009, it was observed on QC in December in Delhiza and in June in Dam Ziatine (Ezzine *pers. observ.*).

These results highlight the complexity of mechanisms of pupa mortality involving the relation between their host plant use and parasitoids (higher trophic level) and the difficulty to highlight them at low pest population densities. The assembly of parasitoids in a particular habitat depends indeed on host and environmental factors and may result in different degrees of natural control of a given pest (MILLS, 1994).

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REDIA, 102, 2019: 113-119 http://dx.doi.org/10.19263/REDIA-102.19.17

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CONFIRMATION OF THE PRESENCE OF *PACHYPAPPA WARSHAVENSIS* (NASONOV, 1894) [HEMPITERA APHIDIDAE] IN THE IBERIAN PENINSULA

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Casiraghi A., Martínez-Torres D., Pérez Hidalgo N. – Confirmation of the presence of *Pachypappa warshavensis* (Nasonov, 1894) [Hempitera Aphididae] in the Iberian Peninsula.

The aphid *Pachypappa warshavensis* (Nasonov) (Aphididae: Eriosomatinae: Pemphigini) on its primary host *Populus alba* L. is properly confirmed in the Iberian Peninsula. The species was initially cited as *Asiphum varsoviensis* (Mordvilko, 1835) in Spain, but the absence of specimens in the entomological collections, as well as the alleged limited distribution to the countries of Northern and Eastern Europe, made it removed from the list of the Ibero-Balearic aphids. *P. warshavensis* is a Palaearctic species that is widely distributed in Europe (Belarus, Germany, Hungary, Poland, Romania, Serbia, Spain, Sweden and Ukraine) and Central Asia (Kazakhstan). The identification based on morphological and molecular characters (COI) has been confirmed and the fundatrix and its winged fundatrigenia are described. In addition, phylogenetic relationships based on COI sequences of *P. warshavensis* with other *Pachypappa* species available in the databases are presented.

Key Words: Aphids, COI, *Populus alba*, faunistic studies, Eriosomatinae, Pemphigini.

INTRODUCTION

The genus *Pachypappa* Koch 1856 corresponds to a little aphid taxon from the Pemphigini tribe (Hemiptera: Aphididae: Eriosomatinae), that host alternates between species of *Populus* L. (the primary host) and different secondary hosts, not always known, but frequently being different species of *Picea* Dietrich (five species) or *Salix* L. (two species) (Pike *et al.*, 2012; Blackman and Eastop, 2019).

Pachypappa groups 13 species with Holarctic distribution (Favret, 2019). Of these, three are Nearctic (Canada and United States), and the rest are widely distributed in the Palaearctic region, from Britain to Japan, or only in a part of it (Blackman and Eastop, 2019).

Currently, six species are present in several countries of Northern and Central Europe: *P. marsupialis* Koch 1856, *P. myrtilli* Börner 1950 (only in Germany), *P. populi* (Linnaeus 1758), *P. tremulae* (Linnaeus 1761), *P. vesicalis* Koch 1856 and *P. warshavensis* (Nasonov 1894) (Nieto Nafría *et al.*, 2011; Blackman and Eastop, 2019). The genus is characterized by large fundatrices without waxy plates and alatae spring migrants always having wax plates and forewings generally with a once-branched medial vein (Blackman and Eastop, 2019).

Like most species of Pemphigini and other Eriosomatinae, their full (holocyclic) life cycles are rather complex and their ability to develop paracycles on subterranean parts of their secondary hosts makes their taxonomy problematic in some species. However, nowadays some of these taxonomic problems can be solved using molecular studies (Pike *et al.*, 2012).

In early-June 2018 typical pseudogalls made by *Pachypappa* aphid colonies were found on several white poplars in the city of León (Fig. I). A detailed study of the fundatrix, and alatae viviparous females revealed that they belonged to the aphid genus *Pachypappa*, and more specifically to *Pachypappa warshavensis* (Nasonov), a species that was mentioned for the first time in the Iberian Peninsula by Gómez-Menor and Nieto (1977) as *Asiphum varsoviensis* (Mordvilko, 1835), currently synonym of *P. warshavensis* (Nasonov, 1894). However, the absence of specimens of this sample in the entomological collections that could confirm it, the null results after the efforts made for its location, as well as its limited distribution to the countries of northern and eastern Europe, made think to Pérez Hidalgo and Nieto Nafría (2003a) that this species was not present in Spain and that it could be an identification error.

Our records, therefore confirm definitively the presence of this species of Pemphigini in the Iberian Peninsula.

MATERIALS AND METHODS

Material collected

Aphids were collected on 4-vi-2018 from various plants of *Populus alba* L. located between coordinates 42.610522, -5.590680 and 42.605037, -5.586273, in a green area on the left bank of the Bernesga river, along a path in the city of León. Half of the sampled aphids were put in 100% ethanol for molecular analysis and the other half in 70% ethanol solution for morphological studies. The aphids (sample references: 1850 and 1779) and the DNA were deposited in the aphidological collection of the Institute for Integrative Systems Biology (Mixt Center of University of Valencia and CSIC).

Morphological study

Subsequently, the individuals in 70% ethanol were used to mount microscope slides following the methodology

– Received 31 May 2019 Accepted 8 October 2019

Fig. I – Several colonies of *Pachypappa warshavensis* (Nasonov) on *Populus alba*.

described by Nieto Nafría and Mier Durante (1998). Identification at the species-level was carried out using quantitative and qualitative characters, following the identification keys of Heie (1980) and Blackman and Eastop (2019). A camera lucida fitted to the microscope was used for the drawings and the microphotographs were taken with a Leica DC digital camera with IM 1000 version 1.10 software.

DNA extraction and PCR amplification

The specimens conserved in 100% ethanol were used in DNA extraction following the HotSHOT method (Hot Sodium Hydroxide and Tris) (Truett *et al.*, 2000). In a second step, a 710 bp fragment of the 5' region of the mitochondrial cytochrome c oxidase subunit 1 (COI) was amplified using primers LCO1490 and HCO2198 (Folmer *et al.*, 1994). 3 µl of the extracted DNA was used for every amplification. PCR conditions were as follows: 94°C for 1 min; 35 cycles of 94°C for 30 s, 48°C for 1 min and 68°C for 1 min; a final extension step of 7 min at 68°C was included after cycling.

Sequencing and analysis of DNA sequences

PCR products were purified by ammonium precipitation and reconstituted in 10 µL of LTE buffer (10mM Tris, 0,1mM EDTA). Sequencing of the amplified fragments was done using PCR primers and conducted using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) following the manufacturer's instructions. Samples were loaded onto an ABI 3700 automated sequencer. Sequences corresponding to each sample were assembled using Staden package v2.0.0 (Staden *et al.*, 1998). Multiple alignments were carried out with Clustal X v1.81 (Thompson *et al.*, 2002) with gap opening and gap extension penalties of, respectively, 10.0 and 0.2, and then manually revised. MEGA7 (Kumar *et al.*, 2016) was used to align sequences and to do the phylogenetic analysis. The Neighbor-joining algorithm on Kimura 2-parameters distances was used to build a phylogenetic tree including several *Pachypappa* sequences available at NCBI (accession numbers MG508635.1, MF830952.1, KR0 29847.1 and GU679671.1) along with the sequence of *P. warshavensis* here reported. Node support was obtained after 500 bootstrap replicates. The sequence of the species *Mordwilkoja vagabunda* (Walsh 1863) was used as outgroup (accession number KR043679.1).

RESULTS

Morphological data

The analysis of qualitative and quantitative (metric and meristic) characters of the specimens available (one fundatrix and hundreds of fundatrigeniae viviparous females) confirmed that they belong to the genus *Pachypappa*, because the fundatrix lacks waxy plates and the fundatrigeniae viviparous females have oval primary rhinaria surrounded by distinct rings of shorts hairs and small siphuncular pores, with a diameter shorter than 2.3 times the articular diameter of antennal segment III (Heie, 1980). The association with *Populus alba* and the presence of secondary sensoria with thick sclerotic rims on the distal half of the antennal segment III (Fig. II, 1), among other characters, identified it as *P. warshavensis* (Nasonov) (Heie, 1980; Blackman and Eastop, 2019).

The only one **fundatrix** caught is large (4.34 mm) but with short appendages (antenna of 0.6 mm and 0.14 times the body length and hind tibia 0.62 mm and 0.14 times the body length). In life, it is reddish-brown and lacks waxy plates and siphunculi. The antenna (Figs. II, 2; III, 2) has five segments without secondary sensoria and the antennal segment III is 0.64 times the rest of antennal flagellum and the terminal process is 0.09 times the part basal of the last antennal segment (*D*). The rostrum is around 0.77 mm and the apical rostral segment is 0.19 mm, 1.32 times its basal width and 0.9 times the segment of the hind tarsus. The cauda is rounded and short (0.09 mm and 0.38 times its width). The anal plate has a large number of setae (around 35) and the genital plate have 14 on the anterior part and 25 on posterior marge (Fig. III, 3). The setae on the vertex is around 0.09 mm and 2.73 times the articular diameter of antennal segment III. The spinal setae on abdominal segment III is 0.036 mm and the marginal of the same segment is 0.042 mm (1.09 and 1.27 times *D*, respectively) and the setae on abdominal segment VIII is 0.093 mm and 2.82 times *D*.

The **alate fundatrigenia females** (measurements based on 20 specimens) are large (2.9-3.84 mm), with wings of 3.69 mm and 2.88 times its maximal width and with the medial vein variable: unbranched (14% of 100 studied specimens) (Fig. IV, 1) or with a fork, short (7%) (Fig. IV, 2) or quite big (75%) (Fig. IV, 3), even different medial vein phenotypes can appear in the same individual. Colour in life

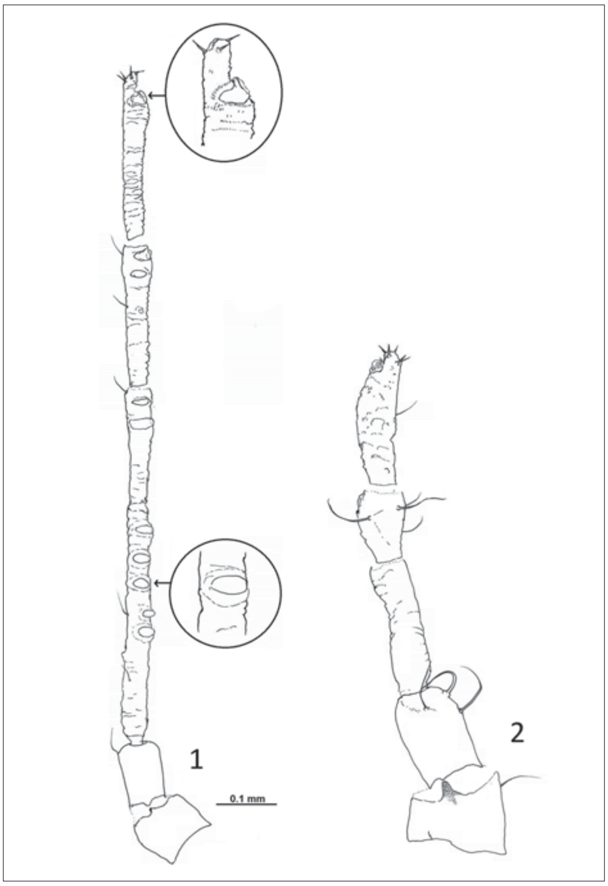

Fig. II – Antenna of the fundatrigenia alata (1), with detail of secondary sensoria on antennal segment III and primary sensoria of the last antennal segment, and antenna of the fundatrix (2) of *Pachypappa warshavensis* (Nasonov).

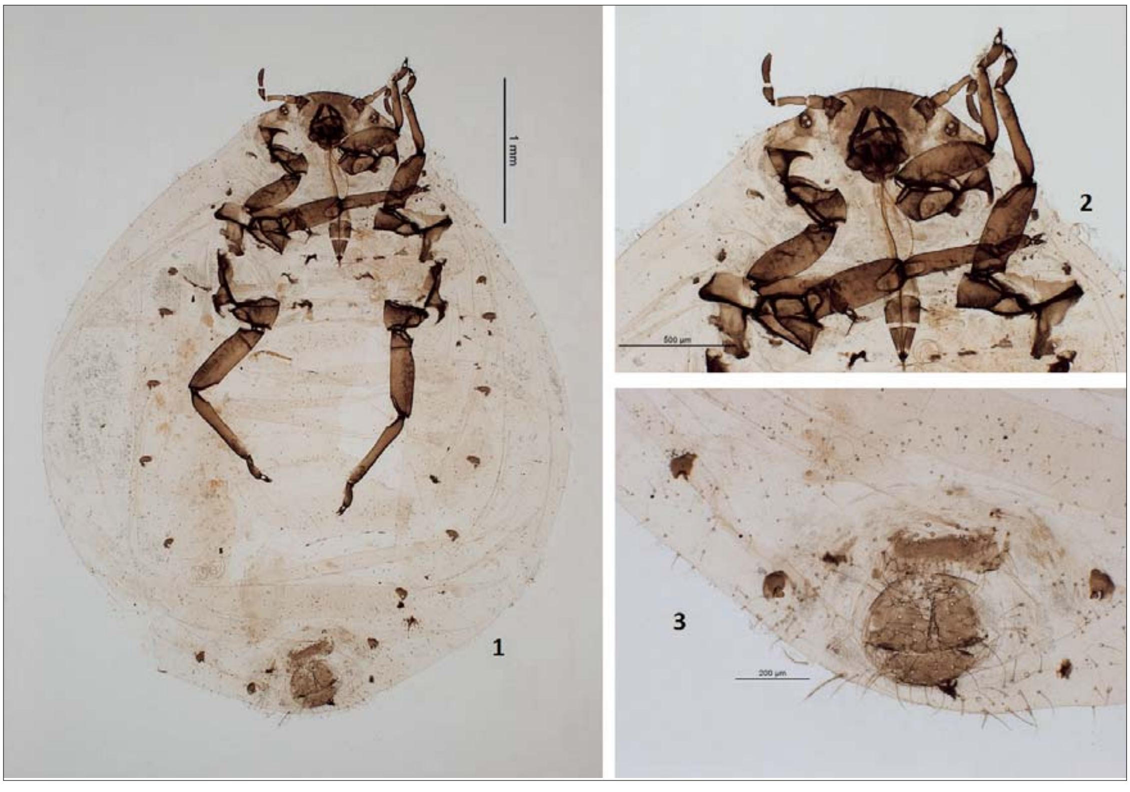

Fig. III – Fundatrix of *Pachypappa warshavensis* (Nasonov): habitus (1) and detail of the anterior (2) and posterior (3) part of the body.

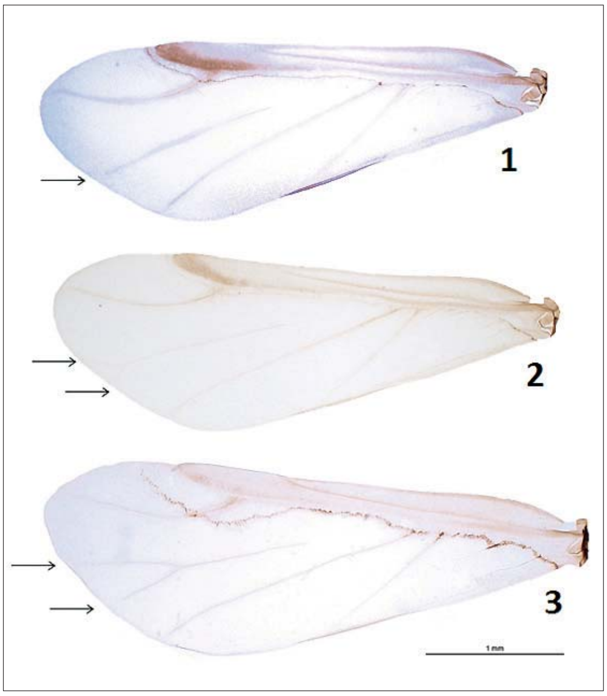

Fig. IV – Forewings of several fundatrigeniae alatae of *Pachypappa warshavensis* (Nasonov) with medial vein variable: unbranched (1), with a short (2) or big (3) fork (the arrows indicate the branches of the medial vein).

is reddish-brown (see also http://influentialpoints.com/Gallery/Pachypappa_aphids.htm) and in slide, they have brown head, thorax, legs, spiracular sclerites and cauda, anal and genital plates and spinal sclerotization on abdominal segments IV to VIII (Fig. V).

The **antennae** are 1.05-1.17 mm, and 0.30-0.37 times the body length (Fig. V, 1). The antennal segment III is 0.31-0.36 mm and have 3 to 7 secondary sensoria located in 68% of the distal part of the segment (Fig. II, 1), the IV is 0.16-0.19 mm and have from 1 to 4 secondary sensoria, the V is 0.18-0.23 mm and have until 3 secondary sensoria, and the VI is 0.22-0.26 mm and without secondary sensoria; the terminal process is 0.13-0.20 times the basal part of the last segment and the diameter of the primary sensoria on antennal segment VI is 0.02-0.05 mm. The antennal segment III have 5 to 12 setae that are 1.22-2.14 times the articular diameter of the same article (*D*). The rostrum is 0.69-0.77 mm and its apical rostral segment is 0.17-0.20 mm, 1.51-2.56 times its basal width and 0.59-0.68 times the segment of the hind tarsus; and have two accessory hairs. The siphunculi pores are very small, around 0.04-0.05 mm and 0.60-0.89 times the diameter in the middle of the hind tibia. The cauda is rounded and short (1.1-2.10 mm and 0.20-0.41 times its width) and have 16 to 20 setae, some of which are longer and thicker (Fig. V, 3). The anal plate shows a variable number of setae, from 28 to 48, while the genital plate has around 18-27 (with 6 to 11 on anterior half), with the setae of anterior part slightly longer and

Fig. V – Fundatrigenia alata of *Pachypappa warshavensis* (Nasonov): habitus (1), abdomen (2) and dorsal (3) and ventral (4) view of the posterior part of the body.

thicker (Fig. V, 4). The hind legs have femora of 0.32-0.45 mm and tibiae of 0.48-0.57 mm and 0.09-0.14 and 0.14-0.17 times the body length, respectively; and the setae of hind tibia are 0.05-0.08 mm and 2.00-2.35 times *D*. Formula tarsal 2.2.2. The wax plates are absent on the head and present on the prothorax and metathorax (a spinal pair and another marginal), mesothorax (one pair spinal) and on all abdominal segments (a spinal pair on abdominal segments I to VIII and a marginal pair on the segments I to VII, that in the latter can be divided) (Fig. V, 2). The body setae are pointed and large: on frons are 0.05-0.09 mm (2.25-3.43 times *D*), the spinal setae on the abdominal segment III are 0.11–0.15 mm (3.89-6.71 times *D*) and the marginal of the same segment are 0.10–0.13 mm (3.56-6.00 times *D*), while the ventral ones are more delicate and a little shorter (0.08-0.15 mm and 3.25-6.25 times *D*) and the setae on the abdominal segment VIII (4 to 10) are 0.18–0.21 mm and 6.67-8.71 times *D*. Nine specimens were dissected to count the embryos, finding a range between 105-269, with a mean value of 200 embryos.

MOLECULAR DATA

The phylogenetic tree including other *Pachypappa* species built using molecular data corresponding to a partial sequence of the mtDNA gene encoding the subunit I of the *cytochrome oxidase* (COI) confirmed that the specimens belong to the *Pachypappa* genus (Fig. VI).

BIOLOGY AND DISTRIBUTION

Pachypappa warshavensis shows mainly *P. alba* as primary host, although there are also records on *P. canescens, P. euphratica* and *P. pruinosa* (SHAPOSHNIKOV, 1964; CARTER and DANIELSSON, 1993) and there is a record on the Nearctic *Populus tremuloides* Michx. (POLJAKOVIĆ-PAJNIK, 2006) in Serbia, that must be confirmed. According to SZELEGIEWICZ (1982), apterae recorded on roots of *Salix caprea* L. could be assigned to this species. Also, CARTER and DANIELSSON (1993) tentatively associate *P. warshavensis* to adult apterae and alatoid nymphs found around roots of *Populus canescens* in Wales (in October) and on fine roots in England (in June), but these both relations need experimental transfers and molecular studies.

Pachypappa warshavensis is a Palaearctic species that is widely distributed in Europe (Belarus, Germany, Hungary, Poland, Romania, Serbia, Sweden and Ukraine) and more recently was recorded in Central Asia (Kazakhstan) (HOLMAN, 2009; KADYRBEKOV, 2011). Several authors (GÓMEZ-MENOR and NIETO, 1977) and later HEIE (1980) mentioned *P. warshavensis* in Spain but PÉREZ HIDALGO and NIETO NAFRÍA (2003a,b) not included this species in the review of the Iberian Eriosomatinae because they did not find specimens to corroborate this record in any aphidological collection. Our record confirms the presence of this species in the Northwest of Spain (province of León) and allows us to think that it is also present in the province of Madrid according GÓMEZ-MENOR and NIETO (1977).

DISCUSSION

The species of genus *Pachypappa* present complex cycles with up to six very different morphs (fundatrix, fundatrigenia alata, virginogenia aptera, sexupara, ovipara and male). As a result, not all morphs have been correctly described for every species and some of them are not even known (PIKE *et al*., 2011; BLACKMAN and EASTOP, 2019). In the case of *P. warshavensis* there are descriptions of fundatrix and fundatrigenia alata (HEIE, 1980) made with few specimens and characters (SZELEGIEWICZ, 1982), which sometimes generates problems when identifying the known forms of the different species.

Our morphological identification was carried out using the keys for fundatrix of HEIE (1980) and the keys to identify alatae of HEIE (1980) and BLACKMAN and EASTOP (2019). In these last keys, they use the medial vein parameter (once-branched or unbranched vein) to separate the genus *Gootiella* Tullgren and to discriminate *Pachypappa* at a specific level. In particular, the last operation results problematic, because the polymorphism of the medial vein (Fig. V), which can have a big branch, a small branch or be unbranched and also because every individual can show different polymorphism at the same time, one on each forewing (see description of fundatrigenia alata).

Also in the identification, we even considered the description of *P. warshavensis* of HEIE (1980) and we

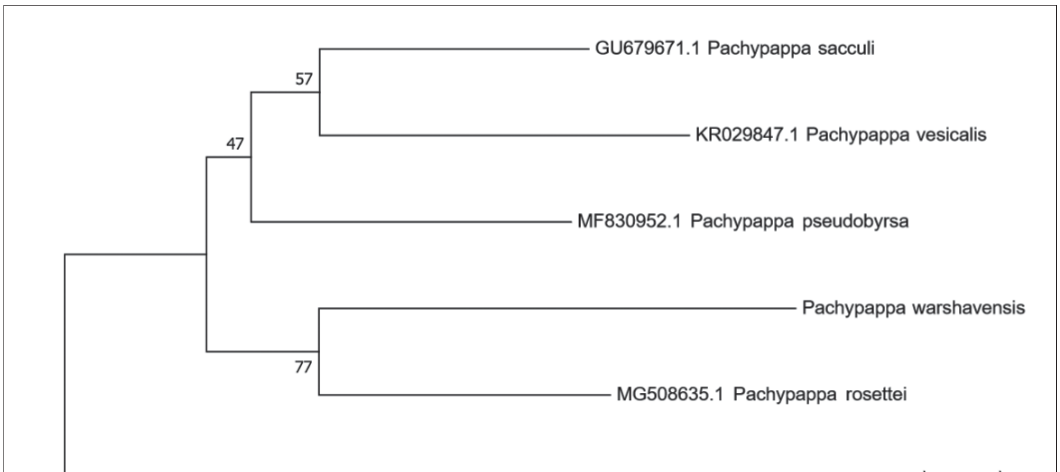

Fig. VI – Neighbour-joining phylogenetic tree (COI) from sequences of species of *Pachypappa*.

noticed that our fundatrix is slightly bigger (4.34 mm) than the fundatrix presented by him (4 mm). But, in any case, the number of the hairs can clearly separate the fundatrix of *P. warshavensis* (Fig. II) of the fundatrix of *P. tremulae* (see figure A in plate 1 in STROYAN, 1975).

Other problems showed up with the molecular analysis because sequences of *Pachypappa warshavensis* are not present in NCBI and we did not find specific matches using BLAST.

Although the species of the subfamily Eriosomatinae are among the first species of aphids mentioned in the Iberian Peninsula (PÉREZ HIDALGO and NIETO NAFRÍA, 2003b), some of them are difficult to locate although they live on very abundant and frequent plants, such as different species of *Populus*, or because they form colonies in roots of their secondary hosts.

Currently, the lack of aphidologists and faunistic studies means that there are large gaps in the known distribution of many species. However, the recent fondness for photography has helped in meeting and citing some species of aphids, apparently scarce, by naturalists or photography enthusiasts, as is the case of *Plocamaphis flocculosa* (Weed) a boreal species detected recently in the Iberian Peninsula (PÉREZ HIDALGO *et al*., 2016).

The known distribution of *Pachypappa* species and their chorology suggested aphidologists that their presence in territories of the Iberian Peninsula was unlikely since they were assumed to be boreal species (PÉREZ HIDALGO and NIETO NAFRÍA, 2003a).

Pachypappa warshavensis is widely distributed in Europe on *Populus alba* and probably also in the Iberian Peninsula. For this reason, we consider appropriate and useful the contribution that citizen science can give (WHEELER and VALDECASAS, 2010). Allowing naturalists to search and document the presence of organisms and interacting with experts to obtain information without excessive effort, may thus establish a beneficial symbiosis for all players in this game of knowledge enrichment (HAND, 2010; GURA, 2013).

Pachypappa species are recorded as important pests for both deciduous (as *Populus*) or evergreen (as *Picea*) forest trees (BARJADZE and JAPOSHVILI 2007). In particular, spruces can suffer from aphids attacks: for example, damages to spruce seedlings, some very severe, have been documented in nurseries in Canada (LAVALLÉE, 1987). Targeted plants appear severely affected, with gradually yellowing of needles and sometimes even needles falling. The genus can even affect the Norway spruce, grown for Christmas market (ALFORD, 1991). In Europe, *Pachypappa vesicalis* is considered as pest (WATT *et al*., 1997) and its presence is well established in spruce plantations in Britain, on trees of all ages (CARTER and DANIELSSON, 1991). *P. warshavensis* is recorded as pest of aspen with *P. marsupialis* in Georgian urban habitats (BARJADZE and JAPOSHVILI, 2007), but the commercial interest in *Populus* spp. is not relevant.

At the moment the genus *Pachypappa* is under control and causes very little damage (SUTHERLAND and VAN EERDEN, 1980; WONG, 1982; EL-SAYED, 2019) but we lack information about this aphid group as disease vector for plants and at the same time we know little about its natural predators and pathogens that can be used in its biological control. Experiments were carried out only for another species of Pemphigini, for example, *Pemphigus betae* Doane, and never with *Pachypappa* (LAVALLÉE, 1987; SWAN and PAPP, 1972; HARPER, 1961). More investigations are required to understand the possible risks for the agroforest business.

ACKNOWLEDGEMENTS

The authors are very grateful to the reviewers of the manuscript, to Barbara Osiadacz (Poznań University of Life Sciences, Poznań, Poland) and Andrey Stekolshchikov (Zoological Institute of Russian Academy of Science, Saint Petersburg, Russia) for facilitating the publications of N.V. Nasonov, A. Mordvilko and H. Szelegiewicz about *Pachypappa warshavensis* and to Francesca Maria Gatti (Civica Scuola Interpreti e Traduttori Altiero Spinelli, Milan, Italy) for the translation of articles in Russian. The investigation was conducted in the context of Project CGL2015-68188-P, funded by "Ministerio de Economia, Industria y Competitividad" of Spain (MIMECO).

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BARK BEETLES (COLEOPTERA CURCULIONIDAE SCOLYTINAE) ASSOCIATED WITH *PINUS PINEA* IN LEBANON: NEW RECORDS WITH REMARKS ON THEIR ECOLOGY, DISTRIBUTION AND POTENTIAL THREAT FOR FOREST STANDS

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El Khoury Y., Binazzi F., Nemer N., Noujeim E., Tarasco E., Roversi P.F., Pennachio F. – Bark beetles (Coleoptera Curculionidae Scolytinae) associated with *Pinus pinea* in Lebanon: new records with remarks on their ecology, distribution and potential threat for forest stands.

Five species of Scolytinae new to Lebanon were recorded on *Pinus pinea*: *Hylurgus ligniperda* (Fabricius), *Hylurgus micklitzi* Wachtl, *Hylastes angustatus* (Herbst), *Hylastes attenuates* Erichson, *Carphoborus pini* Eichhoff. Their ecology, distribution and potential phytosanitary risk are discussed in the context of stone pine conservation.

KEY WORDS: Scolytinae, *Pinus pinea*, new records, Lebanon.

INTRODUCTION

Lebanon is situated along the northeastern border of the Mediterranean Sea and it is part of the Middle East and Southwest Asia. The country is divided from South to North by the African-Syrian Rift Valley, which defines the following geographical regions: the coastal Plain, the Lebanon Mountain Range, the Bekaa Valley and the Anti-Lebanon Mountain Range (BEAUMONT *et al.*, 1976). Lebanon has a Mediterranean climate typically characterized by abundant rainfalls during the winter and marked drought conditions in the summer period. The high-altitude regions of the Lebanese Mountain Range are characterized by heavy rainfalls, contrary to the eastern part of the country where a more arid environment is dominant (BIEL, 1944).

These peculiar geomorphological and climatic conditions are ideal for the growth of the stone pine *Pinus pinea* L., a widespread conifer covering more than 12,000 ha in this country (FAO, 2005, 2010; ABOU-FAKHR HAMMAD, 2014).

From an economic and cultural perspective, the most important and emblematic *P. pinea* areas are those situated on Mount Lebanon in the districts: El Metn, Baabda and Aley (7,200 ha). In these sites, the majority of stands belong to private owners and are often located within small properties intensely managed with traditional practices (e.g. pruning and ground clearing) in order to increase cone productivity (NEMER, 2015 FAO). Stone pine is also sparsely spread in the district Chouf, though the second main area is located in the neighboring district Jezzine, South Governorate (about 2,000 ha), where the majority of stands are state-owned properties (ABOU DAHER, 2015; NEMER, 2015). Moreover, other stands are situated in the South, in the inner district Hasbaiya or in the North at Beit Menzer/Hadath El Jebbeh in the district of Bcharré. Furthermore, single trees are present almost everywhere along the coast, including the district of Akkar in the northern part of the country.

Pinus pinea is important for Lebanese rural economy as it is particularly praised by local communities for its nut production and use as fuel wood. In addition, its action against soil erosion in sandy slopes has been well documented in recent literature (NEMER, 2015 FAO). Nonetheless, in the last years, this conifer has increasingly suffered from Dry Cone Syndrome (DCS), a relatively recent disease responsible for the decline of pine nut production in the entire Mediterranean basin. The most relevant damages related to DCS are either a substantial conelet loss in the tree crown before ripening or a marked decline in pine nut yield per harvested cone weight (NEMER, 2015 FAO). This disease, which is still under investigation, could be the ultimate consequence of the combined action of multiple factors resulting in severe damages to stone pine forest vitality. Biotic agents such as pests and diseases, climatic factors, or traditional management of stands such as heavy pruning and clearing practices might play a crucial role in the genesis of this phenomenon.

In the light of the ecological complexity of stone pine ecosystems and because of their delicate equilibrium, there has been a growing emphasis, in the last years, on insects synergistically interacting with this plant (NEMER, 2015

– Received 19 June 2019 Accepted 10 October 2019

FAO). Among them, the most relevant in terms of threat to *P. pinea* nuts production is certainly *Leptoglossus occidentalis* Heidemann, a well-known coreid responsible for severe losses to pine nut production (Strong *et al.,* 2001; Strong, 2006; Roversi, 2009; Santini, 2010; Nemer *et al.,* 2019). Biological control agents against this pest are currently under investigation, as an alternative to chemical control (Masner, 1983; Bates and Borden, 2004; Roversi *et al.*, 2011; Sabbatini Peverieri *et al.*, 2012, 2013; Binazzi *et al.*, 2013; Paoli *et al.*, 2013; El Khoury *et al.*, 2018); nonetheless, their efficacy in the field has not been definitively proven and nut production remains endangered in most of the stone pine range. Other potential pests already reported as associated with *P. pinea* in Lebanon are *Tomicus destruens* (Wollaston) and *Orthotomicus erosus* (Wollaston).

Tomicus destruens is frequently attracted to pine forests heavily stressed by drought, fire, or root diseases, and in some Mediterranean countries, it has been often reported as a major cause of pine mortality (Lieutier *et al.*, 2016). Conversely, *O. erosus*, though being generally a secondary species breeding in logs or trees already colonized by more aggressive beetles, may sometimes attack plants weakened by stress factors such as fire or drought (Robertson, 2008). Moreover, this beetle has been reported as a potential vector of several *Bursaphelenchus* species such as *B. fuchsi* (Braasch and Philis, 2002), *B. fungivorus* (Arias *et al.*, 2005), *B. teratospicularis* (Penas *et al.,* 2006), *B. sexdentati* (Carletti *et al.*, 2008) and *B. minutus* (Torrini *et al.*, 2017).

Bark beetles represent the most economically relevant group of forest pests (Ciesla, 1993). However, despite the increasing attention to their activity, there is still limited knowledge on their interaction with stone pine in Lebanon. The focus of our paper was thus on new records of Scolytinae associated to *P. pinea* with remarks on their ecology, distribution and potential threat for forest stands.

MATERIALS AND METHODS

From August 2014 to October 2018, insects were collected in pure and mixed formations of *P. pinea*. Specimens were sampled from variable-size stands located in diverse habitats. Pine stumps, logs, branches and waste material were manually debarked and inspected for insects. The collected material was then identified based on the keys of Balachowsky (1949), Pfeffer (1995), Schedl (1981), Wood (1986), Wood and Bright (1992). The Scolytinae collections from the Research Centre for Plant Protection and Certification (CREA-DC, Florence, Italy) and the personal collection of Dr. Fabrizio Pennacchio were also used for comparisons. Information concerning current distribution of bark beetles and their association with host plants were mainly obtained from Wood and Bright (1992), Bright and Skidmore (1997), Bright and Skidmore (2002) Knížek (2011), Bright (2014), Lieutier *et al.*, (2016) and Alonso-Zarazaga *et al.,* (2017).

RESULTS

During the present study, five species of Scolytinae new to Lebanon were recorded on *P. pinea*: *Hylurgus ligniperda* (Fabricius), *Hylurgus micklitzi* Wachtl, *Hylastes angustatus* (Herbst), *Hylastes attenuates* Erichson, *Carphoborus pini* Eichhoff.

Hylurgus ligniperda Fabricius, 1787

Examined material: El Metn district, Bikfaya, N 33°92'30.28", E 35°67'94.72", 821 m a.s.l. breeding on *P. pinea*, Nabil Nemer leg., n 6 specimens, June 2018; Baabda district, Qsaybeh, N 33°86'74.31", E 35°65'47.38", 627 m a.s.l. breeding in logs of *P. pinea*, Yara El Khoury leg., n 5 specimens, October 2018.

The chorotype of *H. ligniperda* is European. This species had been previously reported in Africa, from Algeria, Azores Islands, Canary Islands, Madeira Island, Morocco, St. Helena Island (introduced), South Africa (introduced), Swaziland (introduced), and Tunisia, (Schedl, 1959; Wood and Bright, 1992; Bright and Skidmore 1997; CABI/EPPO 2010; CABI, 2019); in Asia from Turkey, China (Manchuria), India (likely introduced), Sri Lanka (likely introduced), Japan (introduced), (Serez, 1987; CABI/EPPO, 2010; Sarikaya and Avci, 2011; CABI, 2019); in Europe from Austria, Belarus, Belgium, Bosnia Hercegovina, Bulgaria, Cyprus, Croatia, former Czechoslovakia (Cech Republic and Slovakia) Denmark, Estonia, Finland, French mainland with Corsica, Germany, Greek mainland, Hungary, Italian mainland with Sicily and Sardinia, Latvia, Lithuania, Macedonia, Moldova, Montenegro, Netherlands, Norwegian mainland, Poland, Portugal mainland, Russian Federation (Caucasus, Central European Russia, Crimea, Daghestan and North European Russia) Serbia, Slovenia, Spanish mainland with Balearic islands, Sweden, Switzerland, UK, Ukraine (Schedl, 1967; Leker *et al.,* 1977; Rudnev and Vasechko, 1988; Burakowski *et al.,* 1992; Wood and Bright, 1992; Pfeffer, 1995; Bright and Skidmore 1997; Pileckis and Monsevicius, 1997; Bright and Skidmore 2002; Colonnelli, 2003; CABI/EPPO 2010; Knížek, 2011, 2013; Bright, 2014; Lieutier *et al.,* 2016; CABI 2019); in North and South America (where it is introduced) from United States, Argentina, Brazil, Chile, Paraguay, Uruguay (Shedl, 1959; Wood and Bright, 1992; Bright and Skidmore 1997, 2002; Wood, 2007; Tiranti, 2010; Bright, 2014; Kirkendall, 2018); in Oceania (where it is introduced) from Australia and New Zealand (Hosking, 1979; Wood and Bright, 1992; CABI/EPPO, 2010; Alonso-Zarazaga *et al.,* 2017; CABI, 2018).

Hylurgus ligniperda had been previously recorded on the following host plants: *Pinus brutia, P. canariensis, P. contorta*, *P. elliottii, P. halepensis, P. kochiana, P. montezumae*, *P. nigra, P. nigra austriaca, P. nigra nigra, P. nigra pallasiana, P. patula, P. pinaster*, *P. pinea, P. ponderosa, P. radiata, P. sylvestris, P. sylvestris var. hamata* and *P. strobus*; other records were also reported from *Cedrus; Larix*; *Picea; Pseudotsuga* (Wood and Bright, 1992; Bright and Skidmore 1997; Wood, 2007; Bright, 2014; Kirkendall, 2018; CABI, 2018).

Hylurgus micklitzi Wachtl, 1881

Examined material: El Metn district, Broumana, N 33° 88'19.29", E 35°63'94.55", 682 m a.s.l. breeding on *P. pinea*, Nabil Nemer leg., n 12 specimens, May 2018; El Metn district, Bikfaya, N 33°92'30.28", E 35°67'94.72", 821 m a.s.l. breeding on *P. pinea*, Nabil Nemer leg., n 7 specimens, June 2018; El Baabda district, Qsaybeh, N 33°86'74.31", E 35°65'47.38", 627 m a.s.l. breeding on *P. pinea*, Yara El Khoury leg., n 8 specimens, September and October 2018.

The chorotype of *H. micklitzi* is Mediterranean. This species had been already reported in Africa, from Algeria, Egypt, Libya, Morocco, Tunisia; in Asia, from Palestine and Turkey; in Europe, from Spanish mainland, French mainland, Germany, Greek mainland, Malta, Cyprus, Hungary, Italian mainland with Sicily and Sardinia, former W USSR (Russian Federation including Caucasus and Krasnodar) and former Yugoslavia (currently Croatia) (Pfeffer,1995; Shedl, 1967; Wood and Bright, 1992; Bright and Skidmore, 2002; Colonnelli, 2003; Bright, 2014; Knížek, 2013). *Hylurgus micklitzi* had been previously recorded on the following host plants**:** *Pinus halepensis*, *P. pinaster, P. pinea* and *P. nigra pallasiana balcanica* (Wood and Bright, 1992; Bright and Skidmore, 1997; Alonso-Zarazaga *et al.,* 2017).

Hylastes angustatus Herbst, 1793

Examined material: Baadba district, Arsoun, N 33°85'09.31", E 35°70'63.92", 954 m a.s.l. breeding on *P. pinea*, Yara El Khoury leg., n 7 specimens, July 2015, October 2015, and September 2018.

The chorotype of *H. angustatus* is European. This species had been previously reported in Africa from South Africa (where it is introduced); in Asia, from Turkey and Iran; in Europe, from Austria, Belarus, Bulgaria, Croatia, former Czechoslovakia (currently Czech Republic, Slovakia), Danish mainland, England, Estonia, European Turkey, Finland, French mainland, Germany, Greek mainland, Hungary, Italian mainland with Sicily and Sardinia, Latvia, Lithuania, Macedonia, Moldova, Norwegian mainland, Poland, Portuguese mainland, Spanish mainland, Sweden, Belgium, Switzerland, former USSR (currently Central European Russia, Dagestan, Northwest European Russia), UK, Ukraine, former Yugoslavia (currently Croatia, Kossovo, Macedonia, Montenegro Serbia and Slovenia) (Schedl, 1967; Lekander *et al.,* 1977; Wood and Bright, 1992; Bright and Skidmore, 1997; 2002; Colonnelli, 2003; Knížek, 2013; Bright, 2014; Lieutier *et al.,* 2016; Alonso-Zarazaga *et al.,* 2017).

Hylastes angustatus had been previously recorded on the following host plants: *Pinus elliottii, P. kochiana, P. laricio, P. montezumae, P. mugo* (sub. *P. montana*), *P. nigra var. austriaca, P. nigra nigra, P. nigra pallasiana, P. pinaster, P. pinea, P. pumila; P. sylvestris; Larix decidua, L. europea; Picea abies* and *P. orientalis* (Schedl, 1981; Wood and Bright, 1992; Bright and Skidmore, 1997; Bright, 2014; Lieutier *et al.,* 2016).

Hylastes attenuatus Erichson, 1836

Examined material: El Metn district, Qsaybeh, N 33°92'30.28", E 35°67'94.72", 627 m a.s.l. breeding on *P. pinea*, Yara El Khoury leg., n 3 specimens, October 2018.

The chorotype of *H. attenuatus* is European. This species had been previously reported in Africa from Azores Islands and Madeira island; in Asia from China "Manchuria", Iran, Japan, Korea, Taiwan (probably introduced) and North European territories (Russian Federation); in Europe from Austria, Belarus, Belgium, former Czechoslovakia (Czech Republic and Slovakia), Cyprus, England, Estonia, Finland, French mainland and Corsica, Germany, Greek mainland, Hungary, Italian mainland and Sicily, Latvia, Lithuania, Norwegian mainland, Poland, Portugese mainland, Spanish mainland, Sweden, Switzerland, Turkey, Ukraine, former Yugoslavia (Bosnia Herzegovina, Croatia, Kossovo, Macedonia, Montenegro, Serbia, Slovenia), former USSR (Daghestan, Central European Russia and Northwest European Russia) (Schedl, 1967; Lekander *et al.,* 1977; Wood and Bright, 1992; Pfeffer, 1995; Bright and Skidmore, 1997; 2002; Colonnelli, 2003; Mandelhstam *et al.,* 2006; Knížek, 2013; Bright, 2014; Lieutier *et al.,* 2016; Alonso-Zarazaga *et al.,* 2017).

Hylastes attenuatus had been previously recorded on the following host plants: *Pinus densiflora, P. halepensis, P. kochiana, P. nigra nigra, P. nigra austriaca, P. nigra laricio, P. pentaphylla, P. pinaster, P. pinea, P. radiata* and *P. sylvestris*; *Picea excelsa* (Wood and Bright, 1992; Bright and Skidmore, 1997; Bright, 2014).

Carphoborus pini Eichhoff, 1881

Examined material: Baadba district, Arsoun N 33°85'09.31", E 35°70'63.92", 954 m a.s.l. breeding on *P. pinea*, Yara El Khoury leg., n 1 specimens, September 2018.

The chorotype of *C. pini* is Mediterranean. This species had been previously reported in Africa from Algeria and Tunisia; in Asia from Cyprus and Turkey, and Iran; in Europe from French mainland with Corsica, Greek mainland, Hungary, Italian mainland with Sicily, Sardinia and Tuscany Islands, Spain, former Yugoslavia (current Bosnia Herzegovina and Croatia) (Wood and Bright, 1992; Pfeffer, 1995; Bright and Skidmore, 1997; Colonnelli, 2003; Bright, 2014; Lieutier *et al.,* 2016; Alonso-Zarazaga *et al.,* 2017).

Carphoborus pini had been previously recorded on the following host plants: *Pinus brutia, P. halepensis, P. nigra, P. pinaster* and *P. pinea* (Schedl, 1967*;* Wood and Bright, 1992; Bright and Skidmore, 1997).

DISCUSSION

The extensive investigation of mixed and pure stands of *P. pinea* allowed the identification of five species new to Lebanon, all belonging to three genera: *Hylurgus, Hylastes* and *Carphoborus.* In the genus *Hylurgus*, two species were recorded in the survey: *H. ligniperda* and *H. micklitzi.*

Hylurgus ligniperda, though native to central and southern Europe, Algeria, Asia Minor, Crimea (Ukraine) and Caucasus, successfully established in Japan, Australia, New Zealand, Brazil, Uruguay, Chile and South Africa (Bain, 1977; Neumann, 1987; Ciesla, 1988; Tribe, 1991; Pfeffer, 1995; Pawson *et al.,* 2014; Romo *et al.,* 2015). The patterns of spread clearly show that this beetle has currently a sub-cosmoplitan distribution; in fact, it has not only colonized all the available natural populations of pines but has also followed their extensive artificial introductions around the world.

In Europe, this species is widespread in all Mediterranean forests and its infestations generally affect only heavily weakened pines (Lieutier *et al.,* 2016). The lower part of trunks is the most subjected to the attack even though root collars and emerging roots of recently dead, stressed, unhealthy or fallen trees can be equally attractive (Brown and Laurie, 1968; Gil and Pajares, 1986; CABI, 2019).

Moreover, fresh stumps or bark portions of log piles in direct contact with the soil can be also colonized while slash and logging debris represent suitable breeding sites (Ciesla, 1988, 1993; CABI, 2019). Even though regarded as a minor pest with little economic importance, infestations have been observed in declining or dying stands weakened by diseases, nutrient deficiencies, mechanical injury or previous insect attacks (Viedma 1964; Lieutier *et al.,* 2016; CABI, 2018). In these conditions infestations are often heavy (Brown and Laurie, 1968) and areas involved in reforestation are particularly at risk (Browne, 1986; Robertson *et al*., 2008). In southeastern France, Fabre and Carle (1975) reported it as a secondary pest of *Pinus pinaster* severely weakened by the scale insect *Matsucoccus feytaudi* Duc**.** while Ciesla (1988), in Chile, observed secondary attacks in the root zone of pines infested by the pathogen *Verticicladiella* sp.

As many other scolytinae, *H. ligniperda*, in some circumstances, may be a significant pest not only directly, for its activity on host plants, but also indirectly as vector of nematodes carried under the elytrae (Robertson *et al*., 2008). Associations have been observed with *Bursaphelenchus sexdentati* in Greece and Portugal (Skarmoutsos and Skarmoutsos, 1999; Penas *et al.,* 2006)**,** *B. tusciae* in Portugal and Tunisia (Penas *et al*., 2006; Mejri *et al.,* 2016), *B. hellenicus* in Italy and Portugal (Penas *et al*., 2006; Carletti *et al*., 2008; d'Errico *et al*., 2015). Furthermore, several associations with fungi have been also reported. In particular, there is evidence that many *Grossmania* and *Leptographium* species causing root diseases are vectored by *H. ligniperda.* Though normally regarded as weak pathogens, these fungi may significantly contribute to the decline of already stressed conifers when their action is combined with bark beetle attacks. This phenomenon might be amplified by fungal cross contamination between insects overwintering in galleries (CABI 2019) (Zhou *et al*., 2001, 2004; Kirisits, 2004; Reay *et al*., 2006; Kim, 2010; Kim *et al*., 2011; Linnakoski, 2011; Jankowiak and Bilański, 2013; Davydenko *et al*., 2014; CABI, 2019). *H. ligniperda* is also known to be associated with many *Ophiostoma* species, often responsible for reduced growth, chlorosis, crown thinning and ultimately plant death (Kirisits, 2004; Kim, 2010; Davydenko *et al*., 2014). In South Africa this beetle has been sometimes reported to introduce blue stain fungi into the wood and to vector the root pathogens *Leptographium* spp. (Wingfield *et al.,* 1985; 1988; Tribe, 1991). In the same country, Zhou XuDong *et al.* (2001) investigated Ophiostomatoid fungi associated with *H. ligniperda* in artificial stands of *Pinus patula* and *Pinus elliottii* observing that the most commonly recorded species were *Leptographium serpens, L. lundbergii, Ophiostoma ips* and *O. serpens* (Zhou XuDong *et al*., 2001; CABI, 2019). More recently, in Ukraine, Davydenko *et al*., (2014) examined fungal communities associated with *H. ligniperda* colonizing *Pinus sylvestris*. Among the isolated taxa, those belonging to the genus *Ophiostoma* were the most frequent and included five species: *O. piceae*, *O. bicolor*, *O. ips*, *O. canum* and *O. rectangulosporium*, all of which are reported in literature as weak tree pathogens.

The second *Hylurgus* recorded in the survey is *H. micklitzi.* There is still controversy on the taxonomic status of this beetle: many Authors still consider this species as a valid taxon (Balachowsky, 1949; Pfeffer, 1995; Schedl, 1981; Wood and Bright, 1992; Wood, 2007), though others recently suggested that it could be simply a southern form of *H. ligniperda* differing from northern populations for its smaller size (Lieutier *et al*., 2016). This scolytid, whose behavior and biology are very similar to those of *H. ligniperda,* has been often reported as a secondary species attacking plants already colonized by more aggressive pests such as *T. destruens* (Lieutier *et al*., 2016). Nonetheless, there is very limited knowledge on its potential associations with pathogens such as fungi or nematodes and on its ecological role in forest stands.

In the current survey, two species of *Hylastes* were also recorded: *H. angustatus* and *H. attenuatus*. The genus *Hylastes* comprises bark beetles colonizing base of trunks, root collars or emerging roots of coniferous trees such as pine, spruce and occasionally larch. Moreover, stumps or humid parts of logs in direct contact with the soil can be equally attractive. Once new adults emerge after the winter, they disperse searching for conifer roots of young plants (3-10 years) where they feed in order to reach sexual maturation. This maturation process can be long (often up to several weeks) and represents the most critical phase of the life cycle in terms of potential phytosanitary risk.

*Hylastes angustatus***,** is native to Europe, Mediterranean Basin and Southern Russia where it feeds on conifer cambium and inner bark (Schedl, 1959; Schwenke, 1974; Tribe, 1990; 1992). In its native range, it is generally regarded as a secondary species colonizing mainly stressed or declining trees, though damages to pine bark and crown have been often reported in reforestation areas, where sexually immature adults may feed on young seedlings. In South Africa, where it was introduced through trade of plant material since 1930, it breeds almost exclusively on roots and stumps of dead or ailing conifers (Tribe, 1990). Nonetheless, in artificial monocultural pine plantations, newly emerged adults have been sometimes reported to be more aggressive infesting even healthy trees (Tribe, 1990; 1992). More recently, some authors reported, in the forest-steppe zone of eastern Ukraine, a consistent increase in diversity and population density of bark beetles; *H. angustatus* together with scolytids belonging to the genera *Orthotomicus, Hylurgus* and *Hylastes* became more frequent damaging forest stands, young plantations and timber of *Pinus sylvestris* (Meshkova and Sokolova, 2007).

The second species, *H. attenuates*, is very similar to *H. angustatus* in terms of biology and ecology, though it differs from the congeneric species for its smaller size and dark brown colour. It is mainly associated with pines, though records were also reported from *Picea abies*. This species is widespread in Europe and Asia where it is reported as an unaggressive beetle colonizing mainly roots of stumps and fallen trees.

Carphoborus pini is native to South-Western Mediterranean regions where it is associated with *Pinus* spp. (Pfeffer, 1995). Despite being able to withstand bark drying and phloem degradation, it is only a secondary species breeding primarily in small pine branches and tiny twigs even smaller than 1 cm. It poses no threat to forest stands as plants selected for colonization are only those already declining for adverse environmental or climatic factors such as drought, fire, winter winds or previous attacks by more virulent species (Lieutier *et al*., 2016).

Successful colonization of bark beetles depends on the interaction of two essential elements: tree resistance and insect density. If breeding material increases, population may shift from a latent phase (i.e. low population levels) to an outbreak phase (i.e. high population levels) (Berryman, 1982). The threshold between the two conditions may vary according to three factors: tree species, plant susceptibility and bark beetle aggressiveness. Moreover, in the initial phase of the attack, insects may adopt two strategies for neutralizing host resistance: exhausting tree defenses or avoiding tree defenses (LIEUTIER *et al*., 2016). The first one is the most common and anything stimulating energy expenditure in plants, may facilitate this type of approach (LIEUTIER *et al*., 2004; LIEUTIER *et al.,* 2009). Therefore, aggregation pheromones stimulating mass attacks as well as associations with pathogens such as fungi (e.g. some Ophiostomatoid fungi) may benefit beetles in their acticity. When populations of bark beetles reach the critical attack density threshold, tree death is often the ultimate and unavoidable consequence of this plant/insect interaction (BERRYMAN, 1972; 1976; RAFFA and BERRYMAN 1983; LIEUTIER *et al*., 2002; LIEUTIER *et al.,* 2009; 2016).

In Lebanon, *P. pinea* grows mainly in homogeneous, pure, and even-aged stands originating from extensive plantations established from the mid of the 18th century, during the late Ottoman Empire, to the French Mandate period before 1943. As a result, current stands are often highly fragmented and form unbalanced ecosystems consisting of mature single-layer stands with limited or no understory (FAO, 2010; NEMER, 2015). These formations are thus intrinsically vulnerable as they are approaching their final stage of over-maturity and their biological maximum age (PIQUÉ and MUTKE, 2015). Only in few circumstances, such as in more fertile sites and intensely pruned stands with incomplete tree crown cover (e.g. Falaise de Jezzine), a second pine layer may develop. In the latter context, *P. pinea* is often part of a typical mixed formation characterized by *Pinus brutia, Quercus infectoria* (on sandy soils), *Q. calliprinos* (on calcareous soils), *Q. cerris*, *Juniperus excelsa* and other shrubs (NEMER, 2015). However, even in these conducive conditions, natural regeneration cannot often develop under the old stands, and no significant regrowth has been observed. Forest ecosystems are hence extremely simplified, lacking adequate age class equilibrium and constant renovation over time (NEMER, 2015). It is worth noting that regular heavy pruning (each 5-10 years) of *P. pinea* is a widespread practice aimed at facilitating cone harvesting and improving yield. However negative side effects for pine ecosystems have been often reported, particularly when this practice is associated with clearing of understory vegetation performed to accelerate cone cropping. Moreover, since burning is less expensive than manual cutting, light controlled fires often represent a negative standard management practice resulting sometimes in accidentally provoked fires that lead to additional degradation of forest stands. Clearing and burning are particularly deleterious activities in terms of soil conservation as they rapidly deplete forest nutrients and natural organic matter in the highly permeable steep slopes characterized by poor sandy soils. Moreover, prolonged extraction of pruned branch biomass as well as excessive cone yields are equally hazardous procedures that may further endanger the fragile pine ecosystems. In Lebanon, these management practices have been intensively performed for many decades, though little is known on their impact on soil nutrient status as well as on pine resistence/resilience in a context of evolving climate change. In addition, other abiotic factors such as fluctuating precipitations or biotic agents such as pests or diseases might also have a long-term negative impact on pine conservation (NEMER, 2015).

In their native range, the five species of scolytinae newly recorded for Lebanon are minor pests deserving little attention in terms of phytosanitary risk. However, predicting their potential behaviour in the next future appears complex. In fact, not only Lebanese pine stands appear extremely vulnerable and progressively weakened by a wide range of factors, but climatic simulations predict that global change may affect Mediterranean ecosystems more than temperate and boreal ones. That would make their flora and fauna susceptible to temperature increase that might directly influence insect life cycles accelerating larval development and hence the occurrence of earlier spring flights (NEMER, 2015; LIEUTIER *et al*., 2016). That would cause, in turn, an increased voltinism and a potentially higher annual progeny production. Plant critical attack density thresholds might be reached more easily leading to recurrent and extended outbreaks enadangering local tree species. Moreover, temperature could severely influence the interactions between bark beetles and their associated pathogens even though long-term effets on plants remain substantially uknown. Changes in environmental conditions and interactions between organisms might also turn naïve species into actual pests, while an increment in storm frequency and intensity could produce more food sources and breeding sites for bark beetles (LIEUTIER *et al*., 2016).

Among the newly recorded taxa, *Carphoborus pini* is only a secondary species representing no threat for forest stands. Conversely, there is evidence that *Hylurgus* and *Hylastes* might turn, in some cases, into more relevant pests as their recent patterns of spread have clearly shown. In particular, trade in plant material should be carefully evaluated as, in the last decades, *H. ligniperda* and, to a lesser extent, *H. angustatus*, showed a strong ability to spread and establish in a wide variety of environments (CABI, 2019). Furthermore, the already established species *Tomicus destruens* and *Orthotomicus erosus* are known to cause economic damages as they experience rapid population shifts due to global warming (HORN *et al*., 2012). In a climate change scenario, associations of the recorded species with pathogens and phytoparasites such as fungi and nematodes represent a further potential threat, which should be carefully monitored over time. Additional research is thus needed to fully assess the long-term impact of these species on stone pine in Lebanon.

ACKNOWLEDGMENTS

This research was supported by grants from the National Council for Scientific Research (CNRS) in Lebanon and from the University of Bari Aldo Moro in Italy. Dr. Noujeim also acknowledge "L'OREAL-UNESCO foundation" for granting her a regional fellowship "L'OREAL-UNESCO for women in science, Levant and Egypt" on this project.

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REDIA, 102, 2019: 129-140 http://dx.doi.org/10.19263/REDIA-102.19.19

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LADYBIRD COMMUNITIES STRUCTURE IN THREE ITALIAN VINEYARDS

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Canovai R., Loni A. – Ladybird communities structure in three central Italian vineyards.

The knowledge of arthropods communities, their ecological structure and relationships are crucial for a correct management of agroecosystems, particularly in Integrated Pest Management (IPM) and Organic Management. The coccinellid community structure of three differently managed vineyards, two organic and one conventional, has been studied for two consecutive years. Ladybirds were sampled by using Malaise traps. Specimens collected were classified at species level. The Ladybirds communities structure has been analyzed at compositional and functional levels. The results obtained showed the presence of a high number of species all over the three vineyards (40 species in 2012 and 38 in 2013). Aphidophagous represented the main trophic guild. The communities of the three vineyards resulted well separated by the compositional and functional biodiversity analysis. The vineyards with herbaceous cover plants sustained a significant higher population of ladybirds than vineyards with a reduced herbaceous cover. Chemicals treatments produced a significant decreasing of coccinellid populations in the conventional vineyard. The study showed that a rich and diversified coccinellids population enhances the resilience of the agroecosystem vineyard, where the lost or reduction of a species was compensated with the arrival or a growth of other species.

KEY WORDS: Coccinellidae; trophic guilds; resilience; biodiversity; Malaise.

INTRODUCTION

The intensification of agricultural practices produces loss of habitats, landscape simplification and biodiversity reduction. An extended knowledge of the arthropod communities and their ecological structure in the agroecosystems is crucial to reduce the use of chemicals with the perspective to adopt strategies based on Integrated Pest Management (IPM) or Organic Management. Vineyard is one of the most economically important agroecosystems in the Mediterranean basin and represents a permanent crop that allows the development of well- established insect communities.

Previous studies on the vineyard arthropod fauna were mainly focused on the pest component, with about 150 species classified as noxious (BENTLEY *et al.*, 2005), and on their specific predators or parasitoids (ALTIERI *et al.*, 1993; MENALLED *et al.*, 1999), but scarce are the information regarding the general biodiversity (VINCENT *et al.*, 2012). Research carried out in the last decade, focused on the vineyard arthropodofauna in Canada, produced data about cicadellids (BOSTONIAN *et al.*, 2003), carabids (GOULET *et al.*, 2004), spiders (BOLDUC *et al.*, 2005), curculionids (BOUCHARD *et al.*, 2005), chrysomelids (LESAGE *et al.*, 2008) and coccinellids (LUCAS *et al.*, 2007). The results of all these papers converged in outlining the potentiality of the ecological structure of the vineyard in hosting a high level of biodiversity (VINCENT *et al.*, 2012). Ladybirds (Coleoptera: Coccinellidae) include major predators of harmful arthropods in agriculture and play an important role in reducing populations of many small insect pests such as scale insects, mealy bugs, aphids, psyllids, whiteflies, jassids and mites (CODERRE, 1988; WISE *et al.*, 1995; SHANTIBALA *et al.*, 1997; JOSHI *et al.*, 2012; ALI *et al.*, 2014).

Coccinellids occur in any terrestrial ecosystem and agroecosystem, resulting very able to colonize various habitats, but showing strong sensitiveness to pesticide use in agricultural practice (ZAHOOR *et al.*, 2003).

Many studies underlined as such group of predators would take advantage by the presence of a rich, wild vegetation surrounding the crops. It can offer shelters, alternative food resources as well as the possibility to perform spillover from the crops to the surrounding areas in case of human perturbations or treatments (ROOT, 1975; LANDIS, 1994; ALTIERI, 1999; NICHOLS *et al.*, 2000, 2001, 2008; THOMSON & HOFFMAN, 2009). For these reasons, coccinellids represent good bioindicators, producing useful information on the habitats where they live (ANDERSEN, 1999; IPERTI, 1999; HEMCHANDRA *et al.*, 2010).

More than 90% of coccinellids are predators (IPERTI, 1999), feeding on a wide range of insect orders (OBRYCKI *et al.*, 2009; EVANS, 2009; HODEK & HONĚK, 2009) as well as on mites (BIDDINGER *et al.*, 2009). By considering the totality of the world coccinellid population, their main food resource are the coccid scale insects and mealybugs, preyed by 36% of all the coccinellid species, followed by aphids (20%); on the contrary, in the regions characterized by cold-temperate climes, the rate completely changes and aphids represent the main resource, exploited by 68% of coccinellids species (HODEK & HONĚK, 2009). Most of vineyards fall in these climatic areas and host populations of both these pests.

The knowledge of the coccinellidofauna of the Italian vineyards is scarce and only in the recent years contributions to this topic were produced (CANOVAI *et al.*, 2014; LONI *et al.*, 2014; SOMMAGGIO & BURGIO, 2014; BURGIO *et al.*, 2016; LAMI *et al.*, 2016).

In this paper we investigated for two consecutive years the coccinellid communities in three vineyards of Tuscany. The aim of this research was to evaluate how different management systems can influence the population of coccinellids occurring in such agroecosystem. In particular,

– Received 30 May 2019 Accepted 11 October 2019

we focused on the effect of vegetation structure of the three vineyards and on the coccinellids compositional and functional biodiversity associated with it by analyzing: a) the structure of the community at the species level; b) the potentiality to discriminate different vineyards on the base of the community structure of the coccinellids; c) the species richness of each environment; d) the vegetal habitat variables that contributed in determining the community structure of the coccinellids.

MATERIALS AND METHODS

Experimental area

Surveys were carried out in 2012 and 2013 in three vineyards (labelled as Md, O and C) in the Province of Pisa, District of Crespina. The three vineyards extended for 40, 30 and 2 hectars respectively and developed between 15 and 50 meters above sea level. All the experimental units were included in an area of 3 kilometers of diameter consenting to reduce variation of topographical and weather conditions, though their borderlines remain away to each other of at least 1 kilometer. The sites of the observations have these coordinates: Md (43°59'43"N, 10°57'25"E), O (43°58'49"N, 10°53'75" E) and C (43°59'65" N, 10°53'72' E). All the vineyards resulted homogeneous as regards the average age of the plants (ranging between 8 and 10 years) and adopted their specific management systems by at least 5 years. That allowed operating in an ecological structure with an established insect community, well integrated with the study environment. The three vineyards adopted different management strategies to control the main pest *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera, Tortricidae). Vineyard Md is an organic vineyard adopting the mating disruption technique (MD), O is an organic farm not using any chemicals and C is conventional, where Chlorpyrifos was sprayed once or twice a year. As regards the soil management, during the spring and summer periods, Md adopted an alternate tillage within the rows, maintaining an herbs cover on the no tillage row, whereas O and C were tilled within all the rows to reduce water traspiration from the soil. Rows with herbs cover of Md vineyard, were periodically cutted to make easier the access onto the vineyard.

Insect sampling methods

Insects were sampled by using two Malaise traps in each vineyard. They were set up approximately in the center and near the edge of the studied plots, maintaining a distance of about 60 meters between both traps. Such positioning allowed obtaining a more complete sampling of the entomofauna graviting in the experimental area (Fraser *et al.*, 2007, 2008; Loni & Lucchi, 2014). Malaise continuously worked from the half of May to the beginning of October, both in 2012 and 2013 (17/05-20/09/2012 and 16/05-8/10/2013). Trap containers have been changed bi-weekly.

Local Vegetation Assessment

To describe the vegetation and the habitat sources we delimited a circular transect (50-meter ray) around each trap and scored all the trees (woody species higher than 3 meters) and the number of the wild, woody shrub species. During the same dates of the samples collection, we estimated the surface covered by herbs by throwing randomly a square grid of 20 cm of side and by evaluating the herbs covering inside it. We performed thirty throws in each trap site, visually choosing the zones with macroscopically different herbs occurrence. We extended the average cover percentage to the entire experimental area, obtaining the herbs cover relative to each date. Trees, shrubs and herbs represent the main structural vegetation components, adopted as habitat indicators in determining arthropods abundance and diversity (Fraser *et al.*, 2007).

Coccinellids determination

We separated coccinellids from the other specimens and stored them under alchool solution (70% volume). Classification at the species level was carried out, basing on morphological features and, if needed, by the genitalia analysis, according to the descriptions of Porta (1929), Fürsch *et al.*, (1967), Gourreau (1974), Canepari (1983 and 2000), Canepari & Tedeschi (1977) and Canepari *et al.* (1985). Information regarding the food preferences of coccinellids were given by Saharoui (1994), Iperti (1999), Gerson *et al.* (1975), Iablokof-Khnzorian (1982), Majerus (1994), Klausnitzer & Klausnitzer (1997), Saharoui & Gourreau (1998), Kontodimas *et al.* (2007), Hodek & Honěk (2009). The community structure of coccinellids of the three vineyards was analyzed on the base of their species compositional biodiversity and also by grouping them on the base of the trophic guild they belong (Saharoui,1994 modified). Six trophic guilds were selected: predators of aphids (Aphidophagous = Ap), of coccids (Coccidophagous = Co), of mites (Acarophagous = Ac), of aleyrodids (Aleurodophagous = Al), mycophagous coccinellids (Mycophagous = My) and others (O), the last one considering all the species that can prey either on aphids or coccids as well as species whose diet is not well defined. The nomenclature adopted is that proposed by Canepari (2013). The coccinellid classifications usually adopted, were proposed by Sasaj (1968) and Kovar (1996). Ślipiński (2007) found these classifications artificial and phylogenetically unacceptable and proposed the splitting of Coccinellidae into two subfamilies: Microweiseinae and Coccinellinae. Afterwards Ślipiński & Tomaszewska (2010) and Seago *et al.* (2011) formalized the separation of Coccinellidae into the two subfamilies, with Coccinellinae containing most of the tribes. We followed the taxonomy proposed by Ślipiński (2007) and Seago *et al.* (2011).

Statistical analysis

Total abundance of ladybird populations in both the years, among the three vineyards, has been confronted by Chi square test, adopting a $p< 0,001$ value, by using an interactive calculation tool for chi-square tests of goodness of fit and independence (Preacher, 2001).

We organized all the sampled specimens in two raw data matrices. In the first, we arranged an array of rows containing all the species and columns with all the Malaise trap samples. In the second one, we grouped the species on the base of their trophic guilds. A $\log(X+1)$ transformation of the raw data was applied to avoid the right-skewed distribution of the species. On the transformed data matrix, we calculated the Bray-Curtis similarity coefficient to obtain a similarity matrix reporting the similarity percentage values among all the couples of samples. We performed an ordination technique as the non-metric multidimensional scaling (NMDS), producing a configuration in two dimensions of the coccinellid species samples.

To visualize differences or similarities among the coccinellid populations we labelled the samples on the base of the factors "Site" and "Period" of collection. Each year we tested the null hypothesis that no differences would exist among the coccinellids population, showing the same level of similarity across different vineyards. We performed a permutational multivariate analysis of variance (Permanova) adopting a two-way crossed design with two fixed factors: "Vineyard" with three levels (Md, O, and C) and "Period" again with three levels. The term "Period", considered three levels by grouping three consecutive sample dates, obtaining the "initial" the "medium" and the "late" period. In this way, we reduced the probability to find some significant differences across the high number of dates, just as result of a stochastic event. We considered the period as a fixed factor, because we expected a change in community due to the different period of sampling, further, Malaise catches continuously covered all the experimental period and the "Period" factor does not represent a subsample of a longer range of time. Actually, we were more interested in considering how much of the variation could be assigned to the period and if this factor had a significant interaction with the vineyard factor. In 2012, we performed nine sampling dates. In 2013 we collected samples of ten different dates, so that the Permanova analysis, performed adopting the term "period", produced an unbalanced design with the levels 1 and 2, each one grouping three dates and the level 3 grouping the last four dates. We considered such data adopting sums of square called Types III, where every term in the model is fitted only after taking into account all other terms in the full model. We verified the influence of the dispersion of the data on the Permanova results by testing the homogeneity of their distribution (Permdisp analysis). We choose a p value < 0.01 for both the analysis. We compared the estimates of species richness among data sets from the different vineyards by using the two non-parametric estimators Chao1 and Chao 2. Chao 1 is considered an abundance estimator, whose calculations are firstly based on the occurrence of "singletons" and "doubletons" species, represented just from one or two specimens across all the samples. The Chao 2 is an incidence estimator and takes into account the rare species observed only in one or two of all the samples. Calculation of the two estimators was performed by running the data of the samples in random order (permute option) for 9,999 times. To relate the coccinellid communities structure to the habitat variable(s), we tested the null hypothesis that such variable(s) were not associated to the coccinellids abundance by using a distance-based linear model analysis, adopting a step-wise selection procedure and an adjusted R2 criteria of selection. P values for testing the null hypothesis were obtained using a permutation method. We adopted a P value < 0.01. All these analyses were performed by using the Primer Program v6, 2nd edition (Clarke & Warwick, 2001; Clarke & Gorley, 2006) and the Permanova+ for Primer (Anderson *et al.*, 2008).

RESULTS

Total captures

A total of 4,923 coccinellids were captured during the two years, 4,057 in 2012 and 866 in 2013. In 2012 we identified 40 species and 38 in 2013. All these species belong to six tribes of Coccinellinae subfamily: Coccinellini, Coccidulini, Platynaspini, Chilocorini, Diomini and Hyperaspini, according to Seago *et al.* (2011). Thirty species were shared between the years; eight species were collected only in 2012 and two only in 2013. Each year, vineyard Md gave more than half of specimens collected, followed by vineyard O and C respectively. Total abundance differences among the three vineyards resulted statistically significant (Table 1). Despite the different number of specimens collected from each vineyard, their percentages on the total captures, remained very similar in both the years. A complete list of the captured species in each year is reported in the appendix material at the end of this paper (Tables Appendix 1 and 2).

Tribes

Coccidulini and Coccinellini were the most represented tribes, followed by Platynaspini, Chilocorini, Diomini and Hyperaspini. In 2012 and 2013 the number of Coccidulini was markedly higher in Md, followed by O and C, while populations of Coccinellini showed a lower fluctuation of abundance across the three vineyards (Table 1).

Trophic guilds

Composition of the trophic guilds resulted quite similar in both the years. The main group was the aphidophagous covering 80% of the captures, followed by coccidophagous, mycophagous and acarophagous. Predators of aleyrodids showed a very low presence. This community structure has been maintained across the vineyards Md and C, whereas in vineyard O mycophagous was the second abundant group. Only in the vineyard C, acarophagous were higher than 5% of population (Table 2).

Main species

Hippodamia (*Hippodamia*) *variegata* Goeze, *Scymnus* (*Scymnus*) *frontalis* (Fabricius) and *Scymnus* (*Scymnus*) *interruptus* (Goeze) were the most represented species among aphidophagous across the two years.

Nephus (Bipunctatus) bisignatus (Boheman), predator of *Planococcus citri* (Risso) (Kontodimas *et al.*, 2007), *Scymnus* (*Mimopullus*) *flagellisiphonatus* (Fursch) and *Chilocorus bipustulatus* (Linnaeus) resulted the most represented species preying on Coccoidea.

Tytthaspis sedecimpunctata (Linnaeus) and *Psyllobora vigintiduopunctata* (Linnaeus), feeding on fungi of the family *Erysiphaceae,* were the only mycophagous species (Iablokoff-Khnzorian, 1982).

Stethorus pusillus (Herbst) and *Stethorus gilvifrons* (Mulsant) were the only acarophagous species, predators of Tetranychidae mites (Biddinger *et al.*, 2009).

Only few specimens of *Clitostethus arcuatus* (Rossi), predators of Aleyrodids, were collected (Tables Appendix 1 and 2).

Statistical multivariate analysis

Species and Guild matrices 2012

The graphical representation of NMDS, based on the species matrix, showed two separated main groups of samples at the similarity level of 52 %. The first, on the up-left corner of the graphic, containing almost all the samples of the vineyards Md and O together, and the second group, represented by the left-below group, containing most of vineyard C samples (Fig. I, 1). The same analysis, based on the matrix of the trophic guilds, showed the same groups of samples, but at a similarity level of 72 % (Fig. I, 2).

The Permanova performed on the species matrix, confirmed the separation of the groups, highlighted by the graphical NMDS. Differences with statistical significance resulted for the vineyard factor groups and for the period factor ones without significant interaction (Table 3). The analysis of the dispersion of the samples for the period factor, produced significant differences, resulting in a strong

Table 1 – Abundance of the different tribes in the three vineyards in 2012 and 2013.

2012	Coccidul.	Coccinel.	Chilocor.	Diomini	Platynasp.	Hyperasp.	Tot
Md	1,445	694	17	4	131	0	2,291 a (56.47) (56.47%)
O	441	482	4	8	33	0	968 b (23.86%)
C	208	518	2	2	61	7	798 c (19.67%)
Tot	2,094	1,694	23	14	225	7	4,057
%	51.61	41.75	0.57	0.35	5.55	0.17	100.00
N. species	23	11	1	1	1	1	38
2013							
Md	390	81	23	1	25	0	520 a (60.05%)
O	113	76	4	1	19	0	213 b (24.59%)
C	45	76	2	0	10	0	133 c (15.36%)
Tot	548	233	29	2	54	0	866
%	63.28	26.90	3.35	0.23	6.24	0	100.00
N. species	19	10	1	1	1	0	32

Different letters on the totals, represent significant differences (2012: Md vs O X^2: 537.1, p <0.001; Md vs C X^2: 721.6, p <0.001; O vs C X^2: 16.4, p <0.001, df 1; 2013: Md vs O X^2: 128.6, p <0.001; Md vs C X^2: 213.1, p <0.001; O vs C X^2: 48.1, p <0.001, df 1).

Table 2 – Abundance of the different guilds in the three vineyards in 2012 and 2013.

Vineyard 2012	Ap	Co	My	Ac	Al	O	Tot
Md	2,087	133	36	28	1	6	2,291
O	483	188	265	29	2	1	968
C	676	24	28	59	2	9	798
Tot	3,246	345	329	116	5	16	4,057
N. species	21	9	2	2	1	3	38
Vineyard 2013							
Md	414	80	16	9	1	0	520
O	157	22	29	4	1	0	213
C	105	13	6	7	0	2	133
Tot	676	115	51	20	2	2	866
N. species	19	6	2	2	1	2	32

Ap = Aphidophagous; Co = Coccidophagous; My = Mycophagous; Ac = Acarophagous; Al = Aleurodophagous; O = Others (generic predators)

influence on the Permanova results (Table 4). Permanova results for the guilds-based matrix produced the same separation of the sample groups (Table 3), without suffering influence by the dispersion of the samples (Table 4).

SPECIES AND GUILDS MATRICES 2013

The NMDS representation based on species matrix showed two main groups containing most samples at the similarity level of 36 %. The first group in the center of the graphic, with almost all samples of the Md and O vineyards, separated from a cluster with four sample of C vineyard on the right part (Fig. II, 1). NMDS based on Guilds matrix produced the same groups of samples at the similarity level of 65 %. The first on the left part of the graph grouping most samples of vineyards Md and O, while the group in the right part of the pictures contains most samples of the vineyard C (Fig. II, 2).

The Permanova performed on the species matrix analysis found a statistical significance for the separation of the groups both for the vineyard than the period factor without significant interaction (Table 5), not suffering a significant difference due to the dispersion of the samples (Table 6). Same results resulted by Permanova based on the Guilds matrix (Tables 5 and 6).

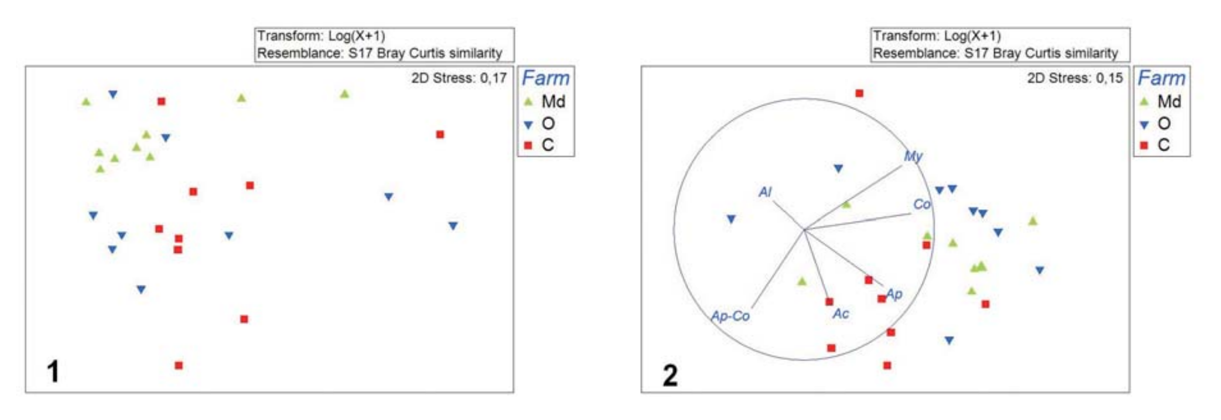

Fig. I – NMDS 2012 graphical representations. 1: graphic based on the Species composition matrix, 2: graphic based on the Guild groups matrix. The same symbols represent samples of the same vineyard. Vectors overlaid in Fig. I 2, represent the Spearman correlation among the guild groups and the positioning of samples.

Table 3 – Permanova analysis two fixed factors, Vineyard and Period for species and guilds matrices.

Species matrix	df	P	Perms.	Guilds matrix	df	P	Perms.
Vineyard	2	0.0001	9,935	Vineyard	2	0.001	9,945
Period	2	0.0008	9,938	Period	2	0.004	9,955
Vi x Pe	4	0.69	9,906	Vi x Pe	4	0.3	9,923
Residual	18			Residual	18		
Total	26			Total	26		

Table 4 –Distance-based test for homogeneity of multivariate dispersions. Vineyard and Period for species and guilds matrices.

Species matrix Vineyard factor	Period factor	Guilds matrix Vineyard factor	Period factor
F: 1.54, df1:2, df2: 24	F: 7.65, df1:2 df2: 24	F: 0.25, df1:2, df2: 24	F: 2.08, df1:2, df2: 24
P(perm): 0.4	P(perm): 0.003	P(perm): 0.83	P(perm): 0.18

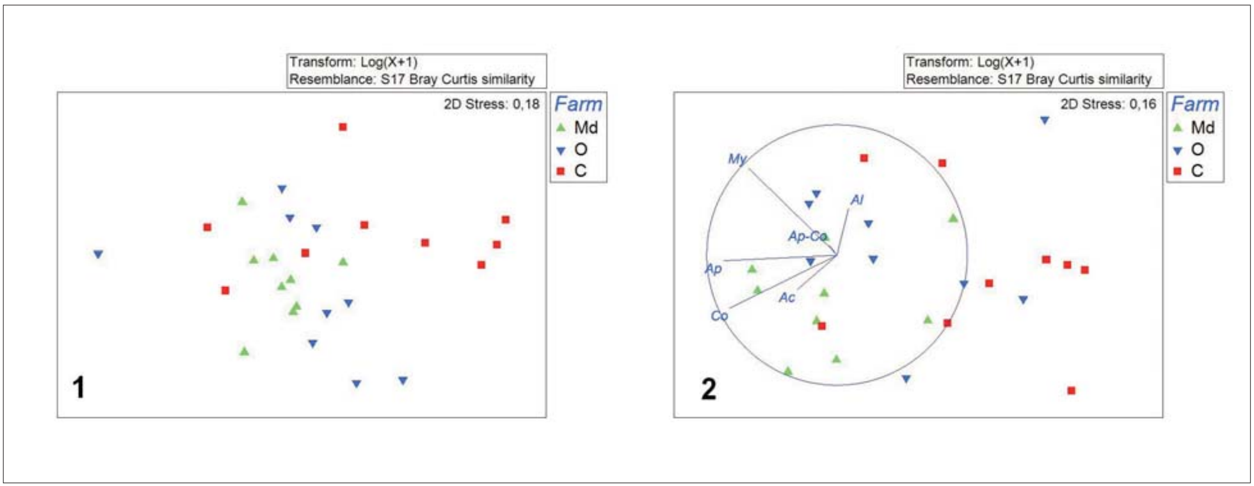

Fig. II – NMDS graphical representations. 1: graphic based on the Species composition matrix, 2: graphic based on the Guild groups matrix. The same symbols represent samples of the same vineyard. Vectors overlaid in Fig. II 2 represent the Spearman correlation among the guild groups and the positioning of samples.

Table 5 – Permanova analysis two fixed factors, Vineyard and Period for species and guilds matrices.

Species matrix	df	P	Perms.	Guilds matrix	df	P	Perms.
Vineyard	2	0.0001	9,921	Vineyard	2	0.0007	9,954
Period	2	0.0001	9,927	Period	2	0.0003	9,954
Vi x Pe	4	0.0481	9,886	Vi x Pe	4	0.89	9,944
Residual	18			Residual	18		
Total	26			Total	26		

Table 6 – Distance-based test for homogeneity of multivariate dispersions. Vineyard and Period for species and guilds matrices.

Species matrix Vineyard factor	Period factor	Guilds matrix Vineyard factor	Period factor
F: 6.5, df1: 2, df2: 27	F: 2.34 , df1: 2, df2: 24	F: 1.56 df1: 2, df2: 27	F: 0,41 df1:2; df2:24
P(perm): 0.016	P(perm): 0.15	P(perm): 0.29	P(perm): 0.7

SPECIES RICHNESS ESTIMATORS

In 2012 non-parametric estimators Chao1 and Chao 2 showed an increasing tendency in vineyard Md, suggesting that more species (7 - 9) could be found over the number of the observed species. In vineyard O they stabilize, suggesting that from five to ten additional species still remain undetected. In vineyard C both estimators showed a reduction trend suggesting that no more species have to be found (Fig. III).

In 2013 estimators Chao1 and Chao 2, very quickly increased and stabilize in vineyards Md and O, suggesting that from 2 to 5 further species could be found respect to the Sobs. Again, both estimators showed a reduction trend in vineyard C, suggesting that no additional species have to be found (Fig. IV).

DISTANCE BASED LINEAR MODEL

In 2012 the marginal test shows that only the variable herbs had a significant relationship with the derived multivariate data cloud of the species and alone explains more than 12% of the variability in data cloud. No significant relationships emerged by considering the other habitat variables. Very similar consideration resulted by analyzing the results of 2013. Again, the variable herbs produced a significant relationship with the species data cloud, explaining more 18% of all the data cloud variability (Table 7).

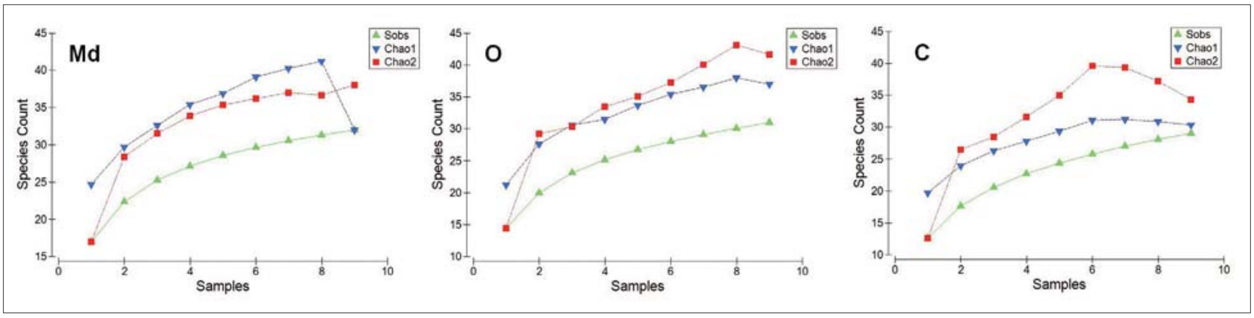

Fig III – Functions describing the non-parametric species richness estimators: Chao 1: blue triangles dotted line, Chao 2: squares dotted line, Species observed (Sobs): green triangles dotted line.

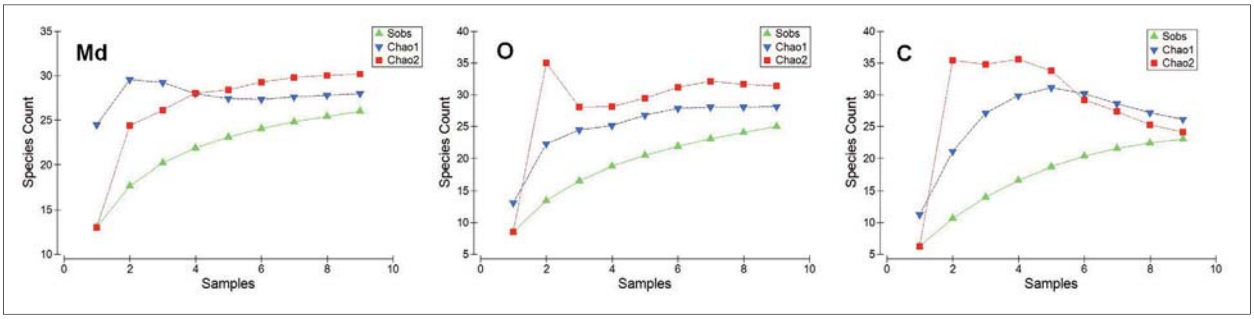

Fig IV – Functions describing the non-parametric species richness estimators: Chao 1: blue triangles dotted line, Chao 2: squares dotted line, Species observed (Sobs): green triangles dotted line

Table 7 – Distance-based linear model for habitat variables using step-wise selection procedure. Boldface values are statistical significant.

	2012				2013			
Variable	SS(trace)	Pseudo-F	P	Prop.	SS(trace)	Pseudo-F	P	Prop.
Vineyard	2,540.3	1.86	0.07	6.91E-02	4,041.4	1.59	0.13	6.00E-02
Trees	2,103.4	1.52	0.14	5.72E-02	6,483	2.67	0.012	9.6E-02
Bushes	2,495.5	1.82	0.087	6.79E-02	4,045.6	1.56	0.12	6.01E-02
Herbs	4,533.7	3.52	**0.002**	0,12	11,835	5.3	**0.0002**	0.18

DISCUSSION

The fluctuation of insect populations through time and space is a common phenomenon, influenced by many ecological factors (Loni & Lucchi, 2014) and well explains the drastic fall of the total captures in 2013. Nevertheless, the percentage contribution to the total captures of each vineyard remained the same. These data describe an ecological structure of populations well established and integrated with the habitat hosting them. The high number of species observed (40), covering about a third of all the species described in Italy (Canepari, 2013), represents a very interesting result by considering the reduced surface of sampled area. Moreover it is interesting to underline the presence of the exotic species *Harmonia. axyridis* (Pallas), introduced in Italy in greenhouses for biocontrol between 1995 and 1999 and recorded in natural environments since 2006 (Burgio *et al.*, 2008) and nowadays well spread all over Italian territory (Menichetti *et al.*, 2015). *H. axyridis* is a generic predator whose feeding action involves the aphid *Daktulosphaira (=Viteus) vitifoliae* (Fitch) (Kogel *et al.*, 2013). This exotic species need to be monitored because of its capacity to taint the resulting wine with alkaloids flavor (Ejbick, 2003; Koch, 2003; Pickering *et al.*, 2004; Galvan *et al.*, 2007; Pfeiffer *et al.*, 2012). The three vineyards showed a decreasing abundance of ladybirds population, passing from Md to O and C respectively. This is consistent with the three different habitat structures and the management systems adopted. The extended herbaceous cover of MD vineyard supported the significatively higher ladybirds number collected, in comparison with O and even more with C vineyard, both tilled into the rows. This result is in agreement with what described by other Authors about the importance of herbs cover for predators (Root, 1975; Landis, 1994; Altieri, 1999; Nichols *et al.*, 2000; 2001; 2008; Thomson & Hoffman, 2009). The smallest population of C vineyard also evidences the coccinellids sensitiveness to the use of chemicals in agreement with Yardim & Edwards (1998), Obrycki & Kring (1998), Rodriguez *et al.* (2003), Santos *et al.* (2007; 2010).

Coccidulini and Coccinellini always resulted the most represented tribes in agreement with Italian coccinellids fauna (Audisio *et al.*, 1995). The Coccidulini number decreased passing from Md, to O and C vineyards respectively, in line with the general trend of the entire ladybirds population. Coccinellini abundance showed a low abundance fluctuation all over the three vineyards, without to be affected by the different habitat structures and management systems as possible consequence of their lower susceptibility to chemicals, due to their higher average body sizes. Indeed it is documented that the toxicity of an insecticide is related to the size of the organism exposed (Obrychi & Kring, 1998; Gilbert, 2004; Kraiss & Cullen, 2008; Zhu, 2008).

Aphidophagous ladybirds were always the prevalent guild in each vineyard in agreement with Hodek & Honěk (2009) about the distribution of coccinellids trophic guilds in temperate regions. Predators of aleyrodids were very scarce and this could be due to the reduced occurrence of these phytophagous into the vineyards (Pajovic *et al.*, 2012). Also acarophagous ladybird populations, were quite reduced and, only in the conventional vineyard (C), this component of predators was well represented. Their higher presence in this vineyard could represent the ecological answer to a major concentration of phytophagous mites, due to the mites stimulant-action, induced by the adoption of chemical insecticides (Van de Vrie *et al.*, 1972; Girolami, 1981; Gruys, 1982; Rossi & Conti, 1997).

The analysis of the coccinellids community structure allowed to discriminate very well the conventional C vineyard by the two organic Md and O that shared a more similar coccinellid community structure. The higher level of similarity of the samples composition, resulted by the analysis of the trophic guilds of the species, suggested that the changes of the community structure, in terms of species composition, did not affect the functional structure of the population in agreement with a functional study approach of biodiversity (Laureto *et al.*, 2015). That is consistent with a good resilience of the ecosystem, where the lost or reduction of one species can be compensated by the arrival or the growth population of other species that have similar functions (Naeem, 1998; Schmid *et al.*, 2009; Mori *et al.*, 2013).

According to the two non-parametric species estimators, a richer community of coccinellids is expected in the two organic vineyards (MD and O), supporting that the organic management offers more suitable conditions to host a rich biodiversity.

CONCLUSIONS

Herbs cover resulted a crucial resource in sustaining an abundant coccinellids population as reported by other authors (Altieri, 1999; Nichols *et al.*, 2000; 2001; 2008). Coccinellids resulted a good bioindicators group that resulted sensitive to the use of chemicals. The analysis of their community structure, based on the compositional and functional biodiversity, allows to discriminate different environments and to obtain useful information about the resilience of an agroecosystem.

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Table Appendix 1-2012 – Ladybird species collected in vineyards studied and their guild .

Tribe Chilocorini	Md	O	C	Tr. guild
Chilocorus bipustulatus (Linnaeus)	17	4	2	Co
Tribe Coccidulini				
Stethorus gilvifrons (Mulsant)	17	12	28	Mi
Stethorus pusillus (Herbst)	11	17	31	Mi
Scymnus (*Mimopullus*) *flagellisiphonatus* (Fursch)	28	102	5	Co
Scymnus (*Neopullus*) *haemorrhoidalis* Herbst	36	14	2	Ap
Scymnus (*Neopullus*) *limbatus* Stephens	5	1	2	O
Scymnus (*Pullus*) *auritus* Thunberg	13	43	9	Ap
Scymnus (*Pullus*) *fraxini* Mulsant *	-	-	1	Co
Scymnus (*Pullus*) *subvillosus* (Goeze)	7	2	3	Ap
Scymnus (*Pullus*) *suturalis* Thunberg	1	-	-	O
Scymnus (*Scymnus*) *apetzi* Mulsant	27	4	8	Ap
Scymnus (*Scymnus*) *apetzoides* Capra e Fursch	3	2	-	Ap
Scymnus (*Scymnus*) *femoralis* (Gyllenhal)	7	17	-	Ap
Scymnus (*Scymnus*) *frontalis* (Fabricius)	653	70	50	Ap
Scymnus (*Scymnus*) *interruptus* (Goeze)	514	66	27	Ap
Scymnus (*Scymnus*) *marginalis* (Rossi)	3	3	3	Ap
Scymnus (*Scymnus*) *rubromaculatus* (Goeze)	13	4	1	Ap
Scymnus (*Scymnus*) *rufipes* (Fabricius)	22	8	22	Ap
Nephus (*Nephus*) *quadrimaculatus* (Herbst) *	-	1	-	Co
Nephus (*Bipunctatus*) *bipunctatus* (Kugelann)	-	1	1	Co
Nephus (*Bipunctatus*) *bisignatus* (Boheman)	79	71	13	Co
Scymniscus anomus (Mulsant) *	1	-	-	Co
Clitostethus arcuatus (Rossi)	1	2	2	Al
Rhyzobius lophanthae (Blaisdell)	4	1	-	Co
Tribe Coccinellini				
Hippodamia (*Hippodamia*) *variegata* Goeze	445	82	381	Ap
Hippodamia (*Hemisphaerica*) *tredecimpunctata* Linnaeus	1	-	1	Ap
Adalia (*Adalia*) *decempunctata* (Linnaeus) *	-	1	-	Ap
Tytthaspis sedecimpunctata (Linnaeus)	5	245	22	My
Coccinella (*Coccinella*) *septempunctata* Linnaeus	9	24	43	Ap
Coccinula quatuordecimpustulata (Linnaeus)	5	20	2	Ap
Oenopia conglobata (Linnaeus)	1	-	-	Ap
Harmonia axyridis (Pallas)	-	1	3	Ap
Harmonia quadripunctata (Pontoppidan) *	1	-	-	Ap
Propylea quatuordecimpunctata (Linnaeus)	196	89	60	Ap
Psyllobora vigintiduopunctata (Linnaeus)	31	20	6	My
Tribe Diomini				
Diomus rubidus (Motschulsky)	4	8	2	Co
Tribe Hyperaspini				
Hyperaspis chevrolati Canepari *	-	-	7	O
Tribe Platynaspini				
Platynaspis luteorubra (Goeze)	131	33	61	Ap
Total	2291	968	798	-
%	56,47	23,86	19,67	-
N species	32	31	29	-

Mi = predators of mites, Ap = Aphidophagous, Co = predators of coccids, My = Mycophagous, Al = Predators of aleyrodids, O = others, Tr.guild = Trophic guild. * shows the species obtained only in 2012

Table Appendix 2-2013 – Ladybird species collected in vineyards studied and their feeding

Tribe Chilocorini	Md	O	C	Tr. guild
Chilocorus bipustulatus (Linnaeus)	23	4	2	Co
Tribe Coccidulini				
Stethorus gilvifrons (Mulsant)	3	2	3	Mi
Stethorus pusillus (Herbst)	6	2	4	Mi
Scymnus (*Mimopullus*) *flagellisiphonatus* (Fursch)	24	9	6	Co
Scymnus (*Neopullus*) *haemorrhoidalis* Herbst	53	-	3	Ap
Scymnus (*Neopullus*) *limbatus* Stephens	-	-	1	O
Scymnus (*Pullus*) *auritus* Thunberg	4	14	2	Ap
Scymnus (*Pullus*) *subvillosus* (Goeze)	3	5	3	Ap
Scymnus (*Pullus*) *suturalis* Thunberg	-	-	1	O
Scymnus (*Scymnus*) *apetzi* Mulsant	5	10	3	Ap
Scymnus (*Scymnus*) *apetzoides* Capra e Fursch	-	1	-	Ap
Scymnus (*Scymnus*) *femoralis* (Gyllenhal)	8	3	-	Ap
Scymnus (*Scymnus*) *frontalis* (Fabricius)	109	52	9	Ap
Scymnus (*Scymnus*) *interruptus* (Goeze)	134	2	2	Ap
Scymnus (*Scymnus*) *rubromaculatus* (Goeze)	2	4	-	Ap
Scymnus (*Scymnus*) *rufipes* (Fabricius)	6	-	3	Ap
Nephus (*Bipunctatus*) *bipunctatus* (Kugelann)	-	-	1	Co
Nephus (*Bipunctatus*) *bisignatus* (Boheman)	30	6	4	Co
Clitostethus arcuatus (Rossi)	1	1	-	Al
Rhyzobius lophanthae (Blaisdell)	2	2	-	Co
Tribe Coccinellini				
Anisosticta novemdecimpunctata (Linnaeus) **	-	-	1	Ap
Hippodamia (*Hippodamia*) *variegata* Goeze	12	4	45	Ap
Tytthaspis sedecimpunctata (Linnaeus)	1	22	-	My
Coccinella (*Coccinella*) *septempunctata* Linnaeus	3	10	3	Ap
Coccinula quatuordecimpustulata (Linnaeus)	2	8	2	Ap
Oenopia conglobata (Linnaeus)	1	3	-	Ap
Oenopia lyncea (Olivier) **	1	-	-	Ap
Harmonia axyridis Pallas	2	4	1	Ap
Propylea quatuordecimpunctata (Linnaeus)	44	18	18	Ap
Psyllobora vigintiduopunctata (Linnaeus)	15	7	6	My
Tribe Diomini				
Diomus rubidus (Motschulsky)	1	1	-	Co
Tribe Platynaspini				
Platynaspis luteorubra (Goeze)	25	19	10	Ap
Total	520	213	133	-
%	60,05	24,59	15,36	-
N species	27	25	23	-

Mi = predators of mites, Ap = Aphidophagous, Co = predators of coccids, My = Mycophagous, Al = Predators of aleyrodids, O = others, Tr. Guilds = Trophic guilds. ** shows the species obtained only in the 2013

REDIA, 102, 2019: 141-148 http://dx.doi.org/10.19263/REDIA-102.19.20

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COMMUNITIES OF PLANT PARASITIC AND FREE-LIVING NEMATODES IN ITALIAN HOP CROPS

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Landi S., d'Errico G., Papini R., Gargani E., Simoncini S., Amoriello T., Ciccoritti R., Carbone K. – Communities of plant parasitic and free-living nematodes in Italian hop crops.

The cultivation of hop has had a notable expansion due to growing interest of craft beer in Italy in the past few years. Thus, surveys were conducted to evaluate nematodes associated with the Italian hop cultivations. The aims of this work were to determine the occurrence of plant parasite and virus-vector nematodes and to evaluate the ecosystem services associated with the control of plant parasitic nematodes. Six different sites located in North and Central Italy were monitored to assess the structure of nematode communities. Many plant parasitic nematodes widely distributed in Italy were found in association with hop plants such as *Mesocriconema xenoplax* and *Heterodera* spp. The *Xiphinema* genus was the most abundant and ubiquitous plant parasitic nematode. The most frequent species were *X. pacthaicum* followed by *X. diversicaudatum,* the Arabis mosaic virus (ArMV)-vector. Predators were not able to regulate the ecosystem pest control service. The agronomic strategies that adopted less intensive tillage practices, such as minimum tillage or cover grass, showed the best regulation.

KEY WORDS: ecology, free-living nematodes, hop crop, North and Central Italy, plant parasitic nematodes.

INTRODUCTION

Over the last few decades, Italy has been assisting to the rise and the establishment of a new type of business in the brewing industry: the craft beer industry, which gained great success and credibility, becoming one of the most significant phenomenon of the Italian agri-food sector. The direct, indirect and induced contribution of the craft brewing sector to the Italian economy is becoming considerable, with approximately 600 microbreweries operating in the country, for an average annual beer production of 445,000 hectoliters, accounting for about 3 % of the total volume of beer produced. Besides, Italy imports almost all the brewing raw materials, especially hop cones, the inflorescences of the female plant of *Humulus lupulus* L., which play a pivotal role as flavouring/bittering and preservative agent (CARBONE *et al.*, 2017). In order to address the growing internal demand, several farmers have begun cultivating hops in different Italian regions. Currently, the hop cultivating area in Italy amounts to approximately 56 hectares with an average surface of 6351 m^2 (www.luppolo.crea.gov.it).

As with many crops the occurrence of infection by pathogens and the establishment of infestations by harmful organisms such as arthropods and nematodes represent a limiting factor for hop crop cultivations and cause quantitative and qualitative yield losses. This risk is seriously aggravated by the presence of several weeds that may serve as pest or pathogen sources of inoculum (D'ERRICO *et al.*, 2014). Given the recent interest in hop crop in Italy, surveys have been conducted to evaluate the phytosanitary status of the Italian hop crops (GARGANI *et al.,* 2017) and nematode populations associated with this agroecosystem. The first records on the occurrence of nematodes in hop fields mainly showed the presence of *Heterodera humuli* Filipjev in Germany (VOIGT, 1894) and in England (PERCIVAL, 1895). Then, this species has been reported from USA, Canada, South Africa, New Zealand and in many European countries (DANILOVA YU, 1996). *H. humuli* is the obligate parasite hop cyst and represents the most common nematode species associated with hop. At present, this species has not been found in Italy (GARGANI *et al.,* 2017). In general, plant parasitic nematodes associated with hop production are considered of minor importance (HAY, 2010), except for virus-vector species of the genus *Xiphinema* (VALDEZ *et al.,* 1974). By contrast, few studies have been carried out to investigate the whole nematode community and its multiple roles within the soil food web (LIŠKOVÁ and RENČO, 2007). Several management factors still need to be addressed, such as the use of different agronomic practices, aimed to preserve biodiversity and ecosystem services with particular regard to regulative services for pest control (LANDI *et al.,* 2018a). In fact, ecosystems are strongly affected by crop management strategies, soil chemical properties and soil microbial, nematode, microarthropod and plant communities (MOCALI *et al.,* 2015a; 2015b; LAZZARO *et al.,* 2018).

The objectives of the present work were to i) determine the structure of nematode communities in Central and North Italy, the main Italian production areas, focusing on the occurrence of plant parasitic and virus-vector nematodes; ii) evaluate main ecosystem services associated with nematodes such as the control of plant parasitic nematodes.

– Received 17 March 2019 Accepted 14 October 2019

MATERIALS AND METHODS

Field sites and soil sampling design

Six fields of approximately half hectare each, distributed in North and Central Italy, were surveyed (Fig. I). The characteristics of sites are reported in Table 1. Soil samples were collected from three different points of each field during summers 2017 and 2018. To determine soil texture, soil pH and Total Organic Carbon (TOC), samples were taken at 0-20 cm depth. To characterize soil nematode community, a further set of soil samples was collected close to previous ones. The sampling was carried out using a hand auger (5 cm inside diameter) from the 20 cm deep top layer of bulk soil. For each soil sample, six cores were randomly sampled and then mixed to form one composite sample. Each sample was then placed in a plastic bag, labelled and stored in a cold chamber at 4°C.

Fig. I – Monitored hop growing sites located in Northern and Central Italy.

Soil physical and chemical analysis

The soil samples were air dried at room temperature (~20°C) and sieved through a 2 mm mesh for pH and texture, then through a 0.5 mm mesh for TOC. The texture was determined by modified pipette methods (Indorante *et al.,* 1990). The soil pH was measured potentiometrically in a 1:2.5 soil-water suspension. TOC was determined by hot oxidation with potassium dichromate and sulphuric acid according to Springer and Klee (1954).

Soil nematode community analysis

Nematodes were isolated from 100 ml of each soil sample using the cotton-wood filter extraction method. Nematodes were extracted for 48 h at room temperature, approximately 20°C. Each nematode suspension was sieved through a 25 μm mesh and the nematodes were counted under a stereomicroscope (50x magnification). Nematodes were mounted on temporary slides and identified at higher magnification to species or genus level using keys from Mai *et al.* (1962), Bongers (1988), Cordero *et al.* (2012), Marinari-Palmisano and Vinciguerra (2014), Crozzoli, 2014. Taxonomic families were assigned to a trophic grouping based on Yeates *et al.* (1993) and Okada *et al.* (2005). Nematode communities were characterised using: i) absolute abundance of individuals; ii) richness determined by counting the number of taxa; iii) the Maturity (MI) and Plant Parasitic (PPI) indices according to Bongers (1990); iv) diversity-weighted abundance (θ) calculated using biomass values (Ferris, 2010) as reported by Ferris and Tuomisto (2015) in order to evaluate the ecosystem services efficiency. In addition, plant parasitic nematode communities were characterised using the relative abundance and the distribution frequency.

Statistical analysis

One-way ANOVA was performed to assess the differences of sites on nematode taxa abundance, indicators of nematode community structure and θ index. When the *F*-test was significant at $P < 0.05$, treatment means were compared using the Student-Newman-Keuls test using CoStat statistical software package (http://www.Cohort.com/costat.html). In addition, Canonical Correspondence Analysis (CCA) was carried out in order to link plant parasitic nematode communities (abundance of plant parasitic nematode taxa) and soil phys-

Table 1 – Characteristics of climate and management in the six selected sites.

Site[a]	Italian Province	Köppen climate types	Previous crops	Year of hops implanting	Hops varieties	Agronomic management
LAZ-1	Latina	Csa[b]	Vineyard	2014	Cascade, Chinook, Columbus, Tettnanger	Conventional tillage
LAZ-2	Roma	Csa	Uncultivated	2016	Columbus, Yeoman, Cascade	Minimum tillage
LAZ-3	Rieti	Cfa[c]	Crop rotation: wheat/corn/ vegetables	2014	Hallertauer Magnum, Spalt Spalter, Hallertau Mittelfrüh, Northern Brewer, Perle, Saazer	Green cover
TOS-1	Pisa	Csa	Mother plant for vineyard	2013	Northern Brewer, Fuggle, Cascade, Perle, Centennial	Conventional tillage
EMI-2	Reggio Emilia	Cfa	Vineyard	2016	Cascade	Conventional tillage
EMI-3	Ravenna	Cfa	Nectarines	2016	Cascade, Nugget, Centennial, Sorachi Ace, Mounth Hood, Crystal, Chinook, Fuggle	Conventional tillage

[a] The geographical coordinates are not available due to the lack of the farm owners' authorisation.
[b] Hot-summer Mediterranean.

ical and chemical variables (texture, soil pH and TOC) by the Past analysis package (Hammer *et al.,* 2001) (http://folk.uio.no/ohammer/past). Vectors represented the environmental axes; the length of vectors indicates the importance of the environmental variable with other variables; vectors in the same direction are positively correlated, while vectors pointing in opposite directions are negatively related. The statistical significance of the relationship between community and environmental variables was assessed by permutation test of both the first ordination axis and the combination of both the first and second axes.

RESULTS

Soil physical and chemical properties

Soil textures of sampled sites were classified according to the USDA Soil Taxonomy. EMI-2 and EMI-3, both in Emilia Romagna Region, were classified as clay loam and silty clay. The site located in Tuscany, TOS-1, was classified as sandy clay loam. Finally, in Latium Region, collected soils were classified as clay (LAZ-1 and LAZ-3) and clay loam (LAZ-2). Soil pH values ranged from 6.7 to 8.2 in Emilia Romagna and Latium, respectively, while pH was 5.1 in Tuscany. The highest TOC value was found in LAZ-2, followed by LAZ-3 and the lowest in TOS-1 (Table 2).

Soil nematode community structures

Twenty-seven genera, belonging to twenty plant parasitic and free-living nematode families, were identified in soil samples collected in the six sites (Table 3). In general, the nematode population was low, only the Rhabditidae family was prominent among sites. Moreover, the abundance of Aphelenchidae and Dorilaymidae families were higher in LAZ-3 than in other sites. Conversely, the taxa richness was high in all sites except for TOS-1. Thirteen different genera of plant parasitic nematodes belonging to ten families were identified (Table 4). In general, the plant parasitic relative communities showed the absence of dominant species. *Xiphinema pacthaicum* was the most abundant species followed by *Meloidogyne incognita*, *Mesocriconema xenoplax*, and *Helicotylenchus digonicus*. *Xiphinema pacthaicum* was also the most widely distributed species among four sites, followed by *M. xenoplax* found in three sites. Finally, the species *Pratylenchus penetrans*, *Ditylenchus* sp., *Rotylen chus robustus*, *M. incognita*, and *X. diversicaudatum* were reported by less than 20% of sites. Other plant parasitic species occurred rarely.

Soil nematode indicators

MI values ranged from 1.3 to 2 indicating the presence of generalist and opportunistic species (Table 3). Conversely, the PPI reached higher values (from 2.6 to 3.4) associated with the presence of families of persistent plant parasitic nematodes like Longidoridae. The MI and PPI were significantly higher in LAZ-3 than in other sites. Average values of diversity-weighted abundance (θ) are reported in Fig. II. The nematode assemblage was arranged in three functional classes: i) detritivores (bacterial and fungal feeders); ii) plant parasitic nematodes; iii) predators (including omnivores). The biomass of plant parasitic nematodes was significantly higher in EMI-2, LAZ-1, LAZ-2 and LAZ-3 than EMI-3 and TOS-1. By contrast, no significant differences were found in the other functional classes. In general, the detritivores channel was greater than other functional classes in all sites, while predator channel was low in all sites. Therefore, the regulation functions of opportunistic and plant parasitic nematodes by predation were insufficient: the predators/preys ratio ranged from 1:6.23 in LAZ-3 to 1:17.47 in EMI-3.

Relationship among soil variables and plant parasitic nematode community structure

In the CCA conducted between plant parasitic nematode taxa abundance (expressed as genus) and soil variables (texture, soil pH and TOC), the total variance explained by the first two axes was 56.7% (30.7 % axis 1 and 26% axis 2). The ordination diagram in the plane defined by the first and second CCA axes were statistically significant for $P<0.04$ and $P<0.001$, respectively (Fig. III). Sand (0.72), TOC (-0.68), soil pH (-0.67) clay (-0.55) and silt (-0.55) are the soil variables best correlated with axis 1, whereas silt (0.52), soil pH (0.51) and sand (-0.47) are best correlated with axis 2. CCA confirmed a clear separation of sites according to soil parameters; the main parameter was represented by the texture. The community variables were considered significant if their values exceed 1. Thus, significant community variables were associated to genera: *Tylenchorhynchus* and *Ditylenchus* (dominated by sand content and inversely related to TOC), *Heterodera* (its dominant drivers were silt content and soil pH), *Paratylenchus* genus (inversely related to soil pH). By contrast, *Xiphinema* and *Mesocriconema* genera were the least influenced by the environmental gradient established within the study areas.

DISCUSSION

The selected sites were representative of different climatic areas of Italy, soil types and agronomic practices allowing a broad evaluation of soil nematode communities associated to hop crops. In fact, the monitored area corresponded to 5% of the whole extent covered by hop crops in Italy and interested two climatic zones (Csa, Hot-summer Mediterranean; Cfa, Humid subtropical). The soil textures belonged to the main soil types (silty, sandy and clay soils) and a broad vari-

Table 2 – Soil physical and chemical properties of the topsoil (0-20 cm) in the six sites.

	Sand	Silt	Clay	Soil texture classification	Soil pH	TOC (g kg^{-1})
LAZ-1	25.0±1.4	24.6±0.4	50.4±1.5	Clay	6.7±0.2	1.3±0.1
LAZ-2	30.8±3.0	36.7±4.4	32.5±1.4	Clay loam	7.3±0.1	1.9±0.05
LAZ-3	10.0±1.4	30.0±3.8	60.0±3.8	Clay	7.8±0.	1.5±0.03
TOS-1	73.3±1.7	4.2±4.2	22.5±2.5	Sandy clay loam	5.1±0.1	0.7±0.1
EMI-2	15.0±1.4	50.8±0.8	34.2±2.2	Silty clay loam	7.8±0.1	1.3±0.04
EMI-3	1.7±1.7	42.5±1.4	55.8±0.8	Silty clay	8.2±0.02	1.2±0.1

Table 3 – Abundance of nematode taxa (number of nematodes 100 ml^{-1} soil). Samples were collected from the six sites during 2017 and 2018 (n=6). Standard errors are reported. Values in the row followed by different letters are significantly different at $P < 0.05$. The significant p-values are reported in bold.

	LAZ-1	LAZ-2	LAZ-3	TOS-1	EMI-2	EMI-3	P-values
Rhabditidae	45.3±4.1	49.0±7.9	33.7±7.9	36.3±12.8	44.7±3.5	48.7±6.6	0.64
Monhysteridae	0 b	0 b	0 b	0 b	0 b	1.3±1.3 a	**0.02**
Cephalobidae	4.0±1.5	2.0±1.0	7.0±2.3	26.3±14.4	7.0±0.6	2.3±0.7	0.11
Plectidae	0 b	1.3±0.7 a	0 b	0 b	0 b	0 b	**0.02**
Alaimidae	4.7±1.2 a	0 b	0 b	0 b	0 b	0 b	**0.0001**
Aphelenchidae	0 b	6.0±1.5	8.3±1.9 a	4.3±0.7	2.0±1.5	3.0±1.0	**0.006**
Discolaimidae	0 b	3.0±1.0 a	0 b	0 b	0 b	1.0±1.0 b	**0.004**
Dorylaimidae	5.0±2.6 b	6.0±1.5 b	13.0±1.5 a	2.7±1.8 b	10.0±1.0	2.7±1.8 b	**0.004**
Seinuridae	1.0±0.6	0.7±0.7	0	0.7±0.74	0.3±0.3	1.0±1.0	0.74
Mononchidae	1.7±1.7	0	1.7±0.9	0 a	1.7±0.3	0.3±0.3	0.38
Tylenchidae	0 b	12.7±4.9 a	8.3±2.3	7.0±1.2	8.7±2.6	2.0±0.6	**0.04**
Paratylenchidae	0.7±0.7	0	0	0	0	0	0.46
Anguinidae	0	0.7±0.7	0.7±0.7	2.0±1.0	2.3±1.5	0	0.26
Pratylenchidae	2.7±1.3	0	0.7±0.7	5.3±2.7	3.0±0	0	0.07
Hoplolaimidae	7.0±4.0	1.3±1.3	3.0±3.0	0	7.7±2.9	3.3±0.9	0.25
Criconematidae	2.7±1.8	0	1.3±0.9	0	4.0±2.1	1.3±0.7	0.21
Heteroderidae	0 b	1.7±0.9 b	0 b	0 b	1.0±1.0 b	6.0±3.2 a	**0.05**
Meloidogynidae	6.3±6.3	7.8±7.8	0	0	0	0	0.20
Dolichodoridae	2.3±0.3 a	0 b	0 b	0 b	0 b	0 b	**0.00001**
Longidoridae	6.0±1.2	7.0±0.6 a	6.7±1.3 a	0 b	6.3±1.8 a	0 b	**0.0005**
Total abundance	**89.3±2.2**	**99.0±10.7**	**85.3±13.1**	**84.7±9.5**	**98.7±3.5**	**73.0±6.4**	0.32
Taxa richness	**9.3±0.3 a**	**9.0±0.6 a**	**9.3±0.3 a**	**6.7±0.7 b**	**11.0±0.6 a**	**9.0±0.6 a**	**0.003**
MI	*1.6±0.0*	*1.6±0.1*	*2.0±0.1 a*	*1.6±0.2*	*1.6±0.1*	*1.3±0.1 b*	***0.05***
PPI	*3.2±0.2*	*3.1±0.3*	*3.4±0.2 a*	*2.6±0.2 b*	*3.2±0.2*	*2.8±0.1*	***0.05***

ability was also revealed in soil pH values, from acid to sub alkaline to alkaline. Being recent hop plants (established from 2013 to 2016 in all sites), soil organic carbon content was affected both by previous managements and current agronomic practices. LAZ-2 and LAZ-3 showed the highest organic matter content close to 2%, which is considered as the critical threshold for soil quality in temperate region (Loveland and Legendre, 1988). In these sites, conservative agronomic techniques have been applied both before and after hop planting. LAZ-2 was uncultivated since 2016 and then the hop cultivation was managed by minimum tillage, LAZ-3 was managed by crop rotation since 2014 and then by green cover. By contrast, the traditional tillage has caused low content of organic matter. In particular, the sandy soil with acid pH showed the lowest values.

Soil nematode structure

A great variability in soil nematode community was found among different climatic areas, soil types and agronomic managements, according to Neher *et al.* (1995).

Many plant parasitic nematodes widely distributed in Italy were also found associated with hop plants such as the ring nematode *M. xenoplax* common on fruit trees in Europe and reported associated with hop only in South Africa (Nyczepir and Halbrendt, 1993; Crozzoli, 2014; Gargani *et al.,* 2017). This species is known to occur, also, in grape-growing areas in Italy (Malossini *et al.,* 2008; Malossini *et al.,* 2011) and it often causes significant economic losses in the grape wine areas of the world (Téliz *et al.,* 2007). The ring nematode is often occurring in association with *Xiphinema* spp. (Ambrogioni and d'Errico, 1980). *H. humuli*, the most common species infesting hop in Europe, was not found during this survey, but other species belonging to *Heterodera* genera were found: *H. carotae*, *H. schachtii* and *H. cruciferae* were detected in LAZ-2, EMI-2 and EMI-3, respectively. No dominant plant parasitic nematode species have been found in any site, however the species belonging to *Xiphinema* genus were widely distributed in the study areas and some of them could transmit plant virus. The most frequent species were *X. pacthaicum* followed by *X. diversicaudatum*. The Arabis mosaic virus (ArMV), the most common nepovirus identified in hop and transmitted by *X. diversicaudatum* has not been found yet in Italy (Gargani *et al.,* 2017), but the presence of this virus-vector nematodes *X. diversicaudatum* is of particular concern. Multiple plant parasitic nematode association and/or their interaction with virus or fungi may also constitute a problem because this can cause synergistic damages to plants (Ragozzino and d'Errico, 2011; Anwar and McKenry, 2012).

Several genera of plant parasitic nematodes were affected by soil parameters. CCA suggested that edaphic variables soil texture, pH and organic carbon, are the primary determinants that influence the structure and diversity of plant parasitic nematode community. The texture influenced three genera: *Heterodera* was favoured by silt content, while genera *Ditylenchus* and *Tylenchorhynchus* increased with high sand content. As reported by Pedersen *et al.* (2010), *Heterodera* genus was also influenced by soil pH. In fact, the population density was associated with high soil pH. In accordance with Barker and Koening (1998), Bird (2000) and Landi *et al.* (2016), the increase of organic matter content was negatively correlated with plant parasitic nematodes

Table 4 –Specific structure of plant parasitic nematode communities in hop sites.

Nematode species	Mean value X±SE	Relative abundance %	Distribution frequency %
Tylenchidae			
Tylenchorhynchus sp.	0.4±0.4	2.3	5.6
Paratylenchidae			
Paratylenchus sp.	0.1±0.1	0.6	5.6
Anguinidae			
Ditylenchus dipsaci (Kühn) Filipjev	0.4±0.3	2.3	11.1
Ditylenchus sp.	0.6±0.3	3.2	22.2
Pratylenchidae			
Pratylenchus penetrans (Cobb) Filipjev and Schuurmans Stekhoven	0.8±0.3	4.5	27.8
Pratylenchus vulnus Allen and Jensen	0.2±0.2	1.3	5.6
Pratylenchus sp.	1.0±0.6	5.8	16.7
Hoplolaimidae			
Helicotylenchus digonicus Perry in Perry, Darling and Thorne	1.4±0.6	8.4	27.8
Helicotylenchus sp.	0.3±0.2	1.9	11.1
Rotylenchus robustus (de Man) Filipjev	0.7±0.3	3.9	22.2
Rotylenchus sp.	1.3±0.8	7.4	16.7
Criconematidae			
Mesocriconema xenoplax (Raski) Loof and De Grisse	1.4±0.5	8.4	44.4
Criconema sp.	0.1±0.1	0.6	5.6
Meloidogynae			
Meloidogyne incognita (Kofoid and White) Chitwood	2.3±1.2	13.5	22.2
Heteroderidae			
Heterodera carotae Jones	0.3±0.2	1.6	11.1
Heterodera cruciferae Jones	1.0±0.7	5.8	11.1
Heterodera schachtii Schmidt	0.2±0.2	1	5.6
Dolichodoridae			
Merlinius brevidens (Allen) Siddiqi	0.4±0.2	2.3	16.7
Longidoridae			
Longidorus macrosoma Hooper	0.2±0.1	1.3	16.7
Xiphinema diversicaudatum (Micoletzky) Thorne	0.9±0.4	5.2	22.2
Xiphinema italiae Meyl	0.4±0.2	2.3	16.7
Xiphinema pacthaicum (Tulaganov) Kirjanova	2.8±0.6	16.4	66.7

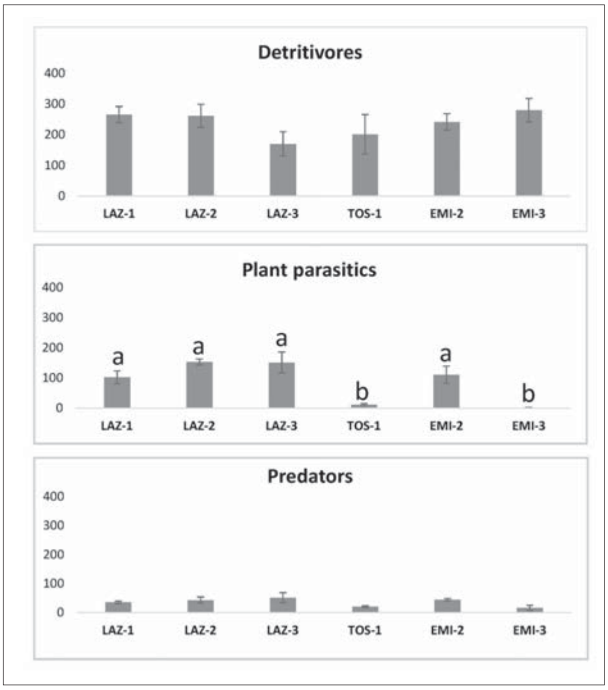

Fig. II – Diversity-weighted abundance (θ) expressed as biomass for functional classes of soil nematode assemblages in six sites located in three Italian regions: Lazio (LAZ-1, LAZ-2 and LAZ-3), Tuscany (TOS-1) and Emilia Romagna (EMI-1 and EMI-2).

belonging to the genera *Ditylenchus* and *Tylenchorhynchus*. By contrast, in these study areas the genera *Mesocriconema* (harmful for vineyards) and *Xiphinema* (vector of plant virus) resulted ubiquitous. As reported by HARRISON and WINSLOW (1961) and BOAG *et al.* (1991) *Xiphinema* genus could be expected to require well-aerated soils, whereas they are also found in soil with medium or heavy texture. Moreover, the increment of native soil organic matter did not suppress larger nematodes. At this regard, PONCHILLIA (1972) found that only large additions of organic matter as muck have a deleterious effect on *Xiphinema* species.

The nematode indicators showed that the nematode community composition varied as a result of the previous and current agricultural practices. In fact, the LAZ-3 site, characterized by previous crop rotation and the current cover grass management, showed the highest values in MI and PPI indices. The lack of soil tillage may have reduced the negative impact on the nematode populations, as previously reported (THOMAS, 1978; WARDE, 1995; LANDI *et al.,* 2018a; 2018b). In contrast, the lowest MI value was found where the agronomic technique included annual deep ploughing. In the context of ecosystem services, nematode community plays an important role in pest control (regulating services) and in nutrient recycling (supporting service). Nematode predators provide to regulate plant parasitic nematodes, while detritivores and predators provide nutrient recycling service. The use of diversity-weighted abundance expressed as biomass showed that the predators are unable to regulate

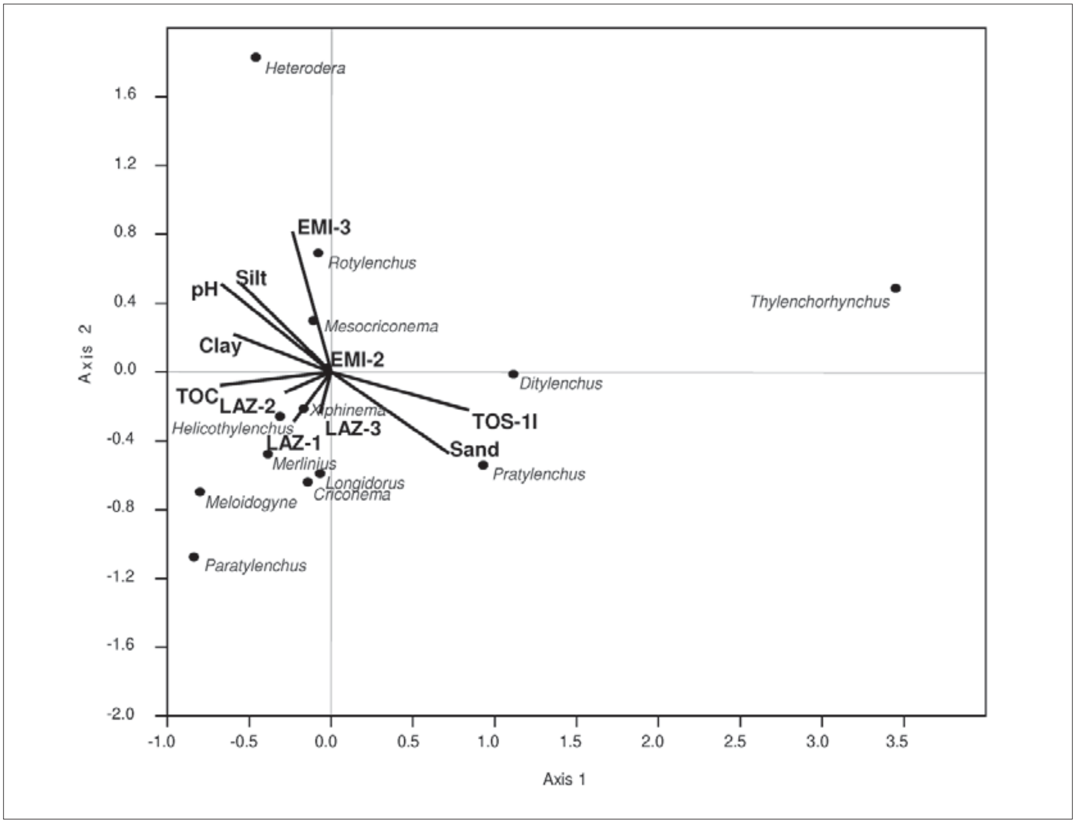

Fig. III – Scatter plot of CCA ordination showing relationships between soil properties and plant parasite nematode taxa abundance. Percentage of variance explained was 30.7% for axis 1 ($P < 0.04$) and 26% for axis 2 ($P < 0.001$).

the ecosystem services of plant parasitic nematodes and carbon mineralization. However, the differences among sites were relevant: the site previously managed using crop rotation and then by grass cover showed the best regulation.

Conclusion

By evaluating six different sites located in Northern and Central Italy, we are able to assess the structure of nematode communities. Overall, with regard to plant parasitic nematodes, the virus-vector *Xiphinema* genus is the most abundant and widely distributed and for this reason periodic soil monitoring is necessary to evaluate the phytosanitary status. Moreover, the predators were not able to regulate the ecosystem services and in particular the pest control service. At this purpose, agronomic practices that reduce tillage such as minimum tillage or, better, cover grass are recommended.

ACKNOWLEDGMENTS

Financial support for this work was provided by the Ministero delle Politiche Agricole Alimentari e Forestali (MiPAAF) under the Project LUPPOLO.IT “Miglioramento competitivo e qualitativo del luppolo da birra”.

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REDIA, 102, 2019: 149-152 http://dx.doi.org/10.19263/REDIA-102.19.21

DARIA PATRIZIA LOCATELLI [a] - FATIMA BEATRIZ PEREZ GARCIA [a] - LIDIA LIMONTA [a]

THE EFFECTS OF COMPETITION BETWEEN LARVAE OF STORED-PRODUCT MOTHS

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Locatelli D.P., Perez Garcia F.B., Limonta L. – The effects of competition between larvae of stored-product moths.

The competition between *Idaea inquinata*, *Corcyra cephalonica*, and *Plodia interpunctella* on an artificial diet was investigated. The experiments were carried out with eggs laid within 24 hours. In the first experiment, 20 eggs of one species were placed in a ventilated Petri dish with 10 g of diet and 20 eggs of one of the other species; in the second experiment, 20 eggs of one species and, after 7 days, 20 eggs of one of the other species were added. Experiments were carried out at 27±1 °C, 70±5% R.H. Each experiment was replicated 5 times and the number of days to complete the development and the number of emerged adults were recorded.

The number of *P. interpunctella* adults was not affected by the presence of *C. cephalonica* or *I. inquinata*. A delay in the development was only observed when *P. interpunctella* eggs were added to the medium already colonized by the other species. *I. inquinata* was the less competitive, as the number of adults decreased and the days to complete the cycle increased. The development of *I. inquinata* was prevented if *P. interpunctella* eggs were started at the same time. Larvae of this last species cannibalized eggs of the other two species.

I. inquinata was the least competitive due to the longer cycle, the lower mobility and the aggressiveness of the larvae of the other two species that cannibalized its eggs.

KEY-WORDS: *Plodia interpunctella*, *Corcyra cephalonica*, *Idaea inquinata*, Pyralidae, Geometridae.

INTRODUCTION

Plodia interpunctella (Hübner) and *Corcyra cephalonica* (Stainton) (Lepidoptera Pyralidae) are cosmopolitan and polyphagous species. *P. interpunctella* is less demanding in relation to temperatures, as the lower threshold of egg development is 15 °C (ARBOGAST, 2007), and of pupae 13.8 °C (JOHNSON *et al*., 1995); larvae develop between 18 and 32 °C, the optimum temperature is 28 °C (SAVOV, 1973). *C. cephalonica* requires higher temperatures, the optimum temperature is at 30-32 °C, and the minimum temperature for reproduction is 20 °C (COX *et al*., 1981). *Idaea inquinata* (Scopoli) (Lepidoptera Geometridae) is present all over Europe (SKINNER, 1984; NAVES, 1995; FLAMIGNI & BASTIA, 1998; GIANTI, 2001), develops on withered plants, hay and officinal plants (dried spices, herbs, and medicinal plants), but it can also consume cereal products (CANDURA, 1931a, b; TEMPEL, 1941; KRATOCHVIL, 1948; LOCATELLI *et al.,* 2005)**.** The trade in dried aromatic plants or vegetable packaging can favor the spread of this species in food plants and warehouses (NAVES, 1995). The optimum temperature, in a laboratory experiment on an artificial diet, is 26 °C (LIMONTA & LOCATELLI, 2013). Larvae are grey-green colored and adults are brown-yellow colored, so an infestation can be underestimated as this species is mimetic with the stored products.

These three species can develop on the same food products, and they could compete in warehouses. In the literature, papers deal with competition in natural habitats (DUDLEY *et al*., 1990; FABRE *et al*., 2004), or competition among phytophagous species (KARBAN, 1986; KAPLAN & DENNO, 2007; DEVESCOVI *et al*., 2015; EATOUGH JONES *et al*., 2015; SIGMON, 2015; NTIRI *et al*., 2016; TUEHELER *et al*., 2016). Also, competition in stored-product insects has been studied (SMITH, 1986; GIGA & CANHAO, 1993; OLLSON *et al.* 2006; CUI *et al*., 2006; SAKKA & ATHANASSIOU, 2018). In particular, ALLOTEY & KUMAR (1985) published results on competition between *C. cephalonica* and *Cadra cautella* (Walker) on cocoa beans, and *C. cephalonica* was the predominant species. In another paper *P. interpunctella* and *C. cautella* were reared on peanuts and the predominant species was *C. cautella*, as it can develop on rearing media with a high content in fat; however, with an artificial diet as a rearing medium, *P. interpunctella* was more competitive (ALLOTEY & GOSWAMI, 2002).

In this paper, we consider the intraspecific competition between *P. interpunctella* and *C. cephalonica,* two widespread species, and *I. inquinata* that can develop on the same stored foods. Experiments were carried out on an artificial diet that contains a balanced amount of nutrients, as insects perform better on this than on natural diets (SHOONHOVEN *et al*., 2005).

The research aims to verify the hierarchy between species that can coexist on the same commodity in order to guide the choice and timing of control measures.

MATERIALS AND METHODS

REARING

Laboratory cultures of *Plodia interpunctella* (Hübner), *Corcyra cephalonica* (Stainton) and *Idaea inquinata* (Scopoli), maintained in a rearing room at 27±1 °C, 70±5% R.H. and L16:D8 photoperiod, were used for the experiments. The ingredients of the diet were 62 g bran, 8 g cornflour, 7 g wheat flour, 4 g wheat germ, 3 g dried yeast, 9 g glycerol, and 7 g honey (LIMONTA & LOCATELLI, 2013). Previous development tests proved this diet suitable for all the species studied.

– Received 21 March 2019 Accepted 17 October 2019

EXPERIMENTS

All the experiments were carried out with eggs laid within 24 hours. The eggs were obtained by placing newly emerged adults in a glass jar closed with tulle, turned upside down on a ventilated Petri dish with filter paper.

For each species, groups of 20 eggs were placed in a Petri dish with 5 g of diet, an amount sufficient for the development of the specimen.

Two sets of experiments were carried out in order to observe the competition among the species. In the first set of experiments, 20 eggs of one species were placed in a ventilated Petri dish with 10 g of diet and 20 eggs of one of the other species, namely 20 eggs of *P. interpunctella* and 20 eggs of *C. cephalonica*; 20 eggs of *P. interpunctella* and 20 eggs of *I. inquinata*; 20 eggs of *C. cephalonica* and 20 eggs of *I. inquinata*.

In the second set of experiments, 20 eggs of one species were placed in a ventilated Petri dish with 10 g of diet and, after 7 days, 20 eggs of one of the other species were added; namely 20 eggs of *P. interpunctella* and after 7 days 20 eggs of *C. cephalonica*, 20 eggs of *P. interpunctella* and after 7 days 20 eggs of *I. inquinata*, 20 eggs of *C. cephalonica* and after 7 days 20 eggs of *P. interpunctella*, 20 eggs of *C. cephalonica* and after 7 days 20 eggs of *I. inquinata*, 20 eggs of *I. inquinata* and after 7 days 20 eggs of *P. interpunctella*, 20 eggs of *I. inquinata* and after 7 days 20 eggs of *C. cephalonica*.

Each experiment was replicated 5 times and the number of days to complete the postembryonic development, in the tables indicated as development, and the number of adults emerged was recorded daily.

STATISTICAL ANALYSIS

Data, when normally distributed, were submitted to One-way ANOVA and LSD test (α=0.05), otherwise, the Kruskal-Wallis test and Multiple comparisons of groups were performed (IBM SPSS Statistics 24).

RESULTS

The number of *Plodia interpunctella*, *Corcyra cephalonica,* and *Idaea inquinata* adults, developed from 20 eggs of each species reared alone on the artificial diet, was 18.8±0.58, 17.4±0.75, and 16.2±1.28 respectively, and not significantly different (One-way ANOVA: $F_{2,\ 12}$= 2.292 P=0.144). The development time of the three species was significantly different (χ^2= 266,666 P<0.001), it was 82.8±2.16 days in *I. inquinata*, 37.9±0.31 days in *C. cephalonica*, and 22.3±0.25 days in *P. interpunctella*.

As shown in Table 1, the number of *P. interpunctella* adults was never influenced by the presence of *C. cephalonica*. On the contrary, when *P. interpunctella* eggs were started seven days before, the number of *C. cephalonica* that developed into adults decreased. Both species developed in a significantly shorter time when reared alone.

In the experiments with *I. inquinata*, the number of *P. interpunctella* adults was not significantly different (Tab. 2), only the development into an adult took longer. When the two species were started at the same time, no *I. inquinata* adults were observed. The number of adults and the development period of this species were negatively influenced by the presence of *P. interpunctella*, as the number of emerged adults was significantly lower and the development time was significantly higher.

C. cephalonica was not influenced by the presence of *I. inquinata* as regards the number of emerged adults, while the days required to develop into adults significantly increased (Tab. 3). In the presence of *C. cephalonica*, the number of *I. inquinata* adults decreased and the development days increased. However, the development days of *I. inquinata* significantly increased and decreased when *C. cephalonica* eggs were added 7 days before and after, respectively.

Table 1 – Number of emerged adults (±S.E.) and development days (±S.E.) of *Plodia interpunctella* (Hübner) (P.i.) reared alone, with *Corcyra cephalonica* (Stainton) (C.c.), and with the other species added 7 days after or before the experiment started.

Experiment	*Plodia interpunctella*		*Corcyra cephalonica*	
	Adults (±S.E.)	Development (±S.E.)	Adults (±S.E.)	Development (±S.E.)
One species	18.8±0.58	22.3±0.25c	17.4±0.75a	37.9±0.31c
P.i. + C.c.	17.8±1.02	27.9±0.17b	18.6±0.98a	40.6±0.22b
P.i. + after 7 dd C.c.	19.8±0.20	30.3±0.49a	12.2±1.88b	56.5±2.69a
C.c. + after 7 dd P.i.	16.4±2.71	30.2±0.43a	20.0±0.00a	40.0±0.39b

One-way ANOVA: *Plodia interpunctella* adults $F_{3,\ 16}$= 0.960 P=0.436; Development time χ^2 = 197.331 P<0.001. *Corcyra cephalonica* adults $F_{3,\ 16}$= 54.733 P<0.001; Development time χ^2 = 87.282 P<0.001. Values followed by different letters are significantly different (LSD test and Kruskal-Wallis test).

Table 2 – Number of emerged adults (±S.E.) and development days (±S.E.) of *Plodia interpunctella* (Hübner) (P.i.) reared alone, with *Idaea inquinata* (Scopoli) (I.i.), and with the other species added 7 days after or before the experiment started.

Experiment	*Plodia interpunctella*		*Corcyra cephalonica*	
	Adults (±S.E.)	Development (±S.E.)	Adults (±S.E.)	Development (±S.E.)
One species	18.8±0.58	22.3±0.25c	17.4±0.75a	37.9±0.31c
P.i. + C.c.	17.8±1.02	27.9±0.17b	18.6±0.98a	40.6±0.22b
P.i. + after 7 dd C.c.	19.8±0.20	30.3±0.49a	12.2±1.88b	56.5±2.69a
C.c. + after 7 dd P.i.	16.4±2.71	30.2±0.43a	20.0±0.00a	40.0±0.39b

One-way ANOVA: *Plodia interpunctella* adults $F_{3,\ 16}$= 0.960 P=0.436; Development time χ^2 = 197.331 P<0.001. *Corcyra cephalonica* adults $F_{3,\ 16}$= 54.733 P<0.001; Development time χ^2 = 87.282 P<0.001. Values followed by different letters are significantly different (LSD test and Kruskal-Wallis test).

Table 3 – Number of emerged adults (±S.E.) and development days (±S.E.) of *Corcyra cephalonica* (Stainton) (C.c.) reared alone, with *Idaea inquinata* (Scopoli) (I.i.), and with the other species added 7 days after or before the experiment started.

Test	*Corcyra cephalonica*		*Idaea inquinata*	
	Adults (±S.E.)	Development (±S.E.)	Adults (±S.E.)	Development (±S.E.)
One species	17.4±0.75	37.9±0.31c	16.2±1.28a	82.8±2.16c
C.c.+I.i.	16.4±0.87	49.4±0.35b	9.2±2.27b	124.3±4.38a
C.c.+after 7 dd I.i.	15.0±0.89	51.6±0.84a	4.8±2.22bc	99.8±4.25b
I.i.+ after7 dd C.c.	15.6±0.98	48.9±0.49b	2.8±0.49c	69.1±1.50d

One-way ANOVA: *Corcyra cephalonica* adults $F_{3,16}$= 1.403 P=0.278; Development time χ^2=182.664 P<0.001. *Idaea inquinata* adults $F_{3,16}$= 176.183 P<0.001; Development time χ^2=74.447 P<0.001. Values followed by different letters are significantly different (LSD test and Kruskal-Wallis test).

CONCLUSIONS

Among the species considered in this study, *Plodia interpunctella* was the most competitive. In fact, the number of individuals of this species that became adults was not influenced by the presence of *Corcyra cephalonica* or *Idaea inquinata*. Only a delay in the development was observed when *P. interpunctella* eggs were added to the medium already colonized by the other species. Also in this case, however, the development required fewer days than for the other two species. The short life cycle could be an explanation for the competitiveness of this species, as observed in soybeans in Hemiptera Heteroptera *Euschistus heros* (F.), that presents a higher number of generations per year and was more competitive than *Piezodorus guildinii* (Westwood) (Tuehler *et al.*, 2016).

C. cephalonica was not influenced by the presence of *P. interpunctella*, whether the eggs of the other species were started at the same time or added seven days after. However, the number of *C. cephalonica* adults decreased and the days required to complete the development increased if the eggs of *P. interpunctella* were put in the rearing medium seven days before. In this case, the lower number of *C. cephalonica* adults can be explained by *P. interpunctella* larvae that cannibalize eggs (White & Huffaker, 1969, in Allotey & Goswami, 1992). In another study, it was observed that larvae of *T. castaneum* (Coleoptera) cannibalized eggs of *C. cephalonica* (Nagalakshmi & Balaji, 1999). The number of *C. cephalonica* adults was not affected by *I. inquinata*. In this case, *C. cephalonica* was the most competitive species, as was observed also in experiments with *Cadra cautella* (Allotey, 1986).

I. inquinata is the least competitive species, as it was affected by the presence of both *C. cephalonica* and *P. interpunctella*. In fact, the number of adults decreased and the number of days to adult emergence increased. Actually, *I. inquinata* development was prevented when eggs of *P. interpunctella* were started contemporaneously. In this case, it was observed that larvae of *P. interpunctella* cannibalized eggs of *I. inquinata*, even if the food was abundant.

I. inquinata development is slow and this characteristic makes it less competitive (Allotey, 1986). Usually, a higher number of generations per year is considered a favored biology (Tuehler *et al.*, 2016). Krijger *et al.* (2001) observed in *Drosophila* "species with a longer development time suffer more from interspecific competition".

I. inquinata is less competitive compared to the other pest species also due to the adults' lower mobility (Authors' observation). In addition, *C. cephalonica* and *P. interpunctella* larvae weave a silk thread that incorporates food and makes food accession for *I. inquinata* larvae more difficult.

ACKNOWLEDGMENTS

We would like to thank Dr. Lesley Currah for editing the manuscript, and the anonymous reviewers for their suggestions that improved the manuscript.

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REDIA, 102, 2019: 153-162 http://dx.doi.org/10.19263/REDIA-102.19.22

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FAUNISTIC AND ECOLOGICAL STUDIES ON APIDAE (HYMENOPTERA APOIDEA) IN NATURAL AND CULTIVATED ECOSYSTEMS IN SICILY

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Mazzeo G., Longo S., Seminara A.R., Bella S. – Faunistic and ecological studies on Apidae (Hymenoptera Apoidea) in natural and cultivated ecosystems in Sicily.

The authors give the results from more than a decade of studies on Apidae (Hymenoptera, Apoidea) in eastern Sicily, carried out in natural and cultivated ecosystems at different altitudes in the Mediterranean Basal (0-1450 m a.s.l.), and Mountain-Mediterranean plans (1450-2100 m a.s.l.). Seven species are reported for the Mountain-Mediterranean plan at the highest altitude (Piano Provenzana, 1845 m, rich in endemic plants from Etna and Sicily, such as *Astragalus*, *Rubus* and *Senecio*): *Anthophora balearica* (Friese), *A. retusa* (L.), *Apis mellifera* L., *Ceratina chalybea* Chevrier, *Epeolus julliani* Peréz, *Eucera aterrima* Friese and *Xylocopa violacea* (L.). Thirty-one bee species were recorded in the Mediterranean Basal plan, with *Apis mellifera* and *Xylocopa violacea* ubiquitous and abundant in all sites surveyed. Overall, 35 species of Apidae belonging to 11 genera are reported here: *Amegilla* (2 species), *Anthophora* (6 species), *Apis* (1 species), *Ceratina* (7 species), *Epeolus* (1 species), *Eucera* (10 species), *Habropoda* (1 species), *Melecta* (1 species), *Nomada* (3 species), *Tetraloniella* (1 species), and *Xylocopa* (2 species). Data on the surveyed species are discussed in relation to the altitudinal level, the plants visited, and the ecological quality of the sites where the specimens were found.

KEY WORDS: Apidae; Biodiversity; Plants; Ecosystems; Sicily; Altitudinal plans.

INTRODUCTION

The evaluation of the biodiversity and quality of natural environments and agro-ecosystems is based on the knowledge of the organisms living there, particularly on the study of key species used as bioindicators. Insects are considered good bioindicators, particularly solitary and social bees, due to their relationships to the plants they visit for taking nectar and pollen (PORRINI *et al.*, 2002; CELLI and MACCAGNANI, 2003).

Bees contribute significantly to the pollination of crops and wild plants, and are of great importance for the protection of natural environments (LONGO *et al.*, 2002; BELLA, 2014; MARGIOTTA *et al.*, 2017). In Europe, this group is threatened by habitat loss from agricultural intensification, urban development, and a changing climate. Knowledge about bees and their ecological role is particularly poor in southern European countries. It is therefore essential to analyse recent data about the distribution, population trends, and ecology of the species living in a certain territory that is considered critical for the conservation of bee diversity and for the benefits associated with pollination (NIETO *et al.*, 2014; BALZAN *et al.*, 2016).

In order to know the ecological status of natural and anthropic ecosystems in Sicily, observations were conducted for a period of 11 years on anthophilous insects detected on flowers in different ecosystems. Some of the results regarding crops and some groups of anthophilous insects have already been published, and the data showed that the majority of the insects belonged to Hymenoptera and to the family Apidae (QUARANTA *et al.*, 2004; MAZZEO *et al.*, 2004, 2006, 2007a,b,c; SEMINARA *et al.*, 2009). This paper, is focused on Apidae (Hymenoptera, Apoidea) species recorded in 11 years of surveys, from sea level to the high slopes of the volcano Etna (eastern Sicily). Twelve species (13 taxa) of *Bombus* Latreille recorded in the same period of investigation, were treated in detail in MAZZEO *et al.* (2015) and were thus excluded from the discussion.

In this context, this paper represents a first step to the study of ecological traits of bees in a very particular area, the Mount Etna, by providing a list of species, with data on visited plants, altitudinal quotes and features of the areas where the specimens were found.

MATERIALS AND METHODS

THE INVESTIGATED TERRITORY

The surveys were carried out from 1997 to 2007 in nine sites representative of the main environments of eastern Sicily, from sea level to 1900 m a.s.l. on Mount Etna, in Catania province. The sites differed in climate and vegetational aspects. Overall, the climate is Mediterranean, with variations in slope, altitude, and exposure. In the basal plane on the coast, the climate is thermo-Mediterranean (xerothermic-hot), with average temperatures ≥16 °C; the following survey sites were located here: Catania (Torre Allegra, and Spina Santa), Giarre (Contrada Nespole), Motta Sant'Anastasìa, Paternò and Ramacca. In the hilly zone, the climate is meso-mediterranean with average temperatures between 12 and 16 °C; the following sites were here: Bronte, and Milia (Monte San Leo, Belpasso). In the higher sites of

– Received 5 July 2019 Accepted 29 October 2019

the high mountain ranges, the climate is oro-mediterranean with average temperatures ≤8 °C (SIAS, 2014; MAZZEO *et al*., 2015; POLI MARCHESE and PATTI, 2000); the site of Piano Provenzana (Monte Tanaurpi, Linguaglossa) was here. The vegetational aspects characterising these sites are influenced both by human activity, especially in the mid-baseline plane, and by the activity of the volcano in the central summit. The bioclimatic zones ("altitudinal plans" and "horizons") constituted by plant groupings at various altitudes, include a medium-baseline plan (0-1450 m a.s.l.) that is characterised by cropland (mainly citrus orchards, olive groves, vineyards, and orchards), oak and pine forests, a mountain plan (1450-2100 m a.s.l.) that comprise pine and beech forests and pioneer plants in upper altitudinal limit of the arboreal vegetation, a high plan (2100-2950 up to the volcanic desert at 3330 m a.s.l.) where a characteristic group of species is present. In this area, the predominant plants are *Astragalus siculus* Biv., capable of colonising the slopes of loose volcanic sand and providing shelter to other species, and some endemic to the volcano, such as *Senecio aetnensis* Jan ex DC., *Viola aetnensis* Parl., *Anthemis aetnensis* Schouw, *Rumex aetnensis* (C. Presl), *Galium aetnicum* Biv., *Sclerantus aetnensis* Strobl, and *Saponaria sicula* Rafin. (POLI MARCHESE, 1991).

SAMPLING SITES

The nine surveyed areas were divided into the following three types based on floristic complexity and the degrees of human and agronomic aspects: agro-ecosystems [*AE*]; natural ecosystems [*NE*]; and semi-natural ecosystems [*SN*]. The sites are described in Table 1.

SAMPLING METHODS

The protocol of the surveys was developed within the Italian project A.M.A. (Ape, Miele, Ambiente) (QUARANTA *et al*., 2004) with some modifications. Anthophilous insects were recorded in each site along a transect of 400-600 m, representative of the spontaneous flora that were preliminarily defined in all surveyed areas. The wild plants were collected, prepared and identified in case of doubt by the specialist. The observations were conducted on wild plants once a month, while the cultivated plants were observed three times during the flowering period. The distribution, locality and date of capture, number and sex of examined specimens, number of observed specimens in brackets, and the plants visited are reported for each species. Each specimen was prepared dry and specifically identified after the observation of male sexual structures in cases of doubt.

The classification used in this paper followed MICHENER (2007) for supra-specific taxa, and their nomenclature was according to STOCH (2003) and POLASZEK (2013). Updated distributional data were taken from the online check-list by COMBA (2015), KUHLMANN *et al*. (2015) and RASMONT and HAUBRUGE (2018). The species were assigned to chorological categories according to LA GRECA (1964).

The collected material is preserved in the collection of the Department of Agriculture, Food and Environment, sect. Applied Entomology, University of Catania.

Acronyms used in the text for the collectors and determiners as follows: AS (Alice Seminara); SB (Salvatore Bella); VL (Vincenzo Lizzio); MQ (Marino Quaranta); MS (Maximilian Schwarz); VN (Vittorio Nobile).

RESULTS

FAUNISTIC DIVERSITY

Over the course of 11 years more than 14600 specimens of Apidae were collected or observed, belonging to 35 species.

Table 1 – Sites where the surveys were carried out in 1997-2007.

Sites	**m a.s.l.**	**Ecological types**	**Geographic coordinates**	**Description**	
				General info	**Crops and plants**
Torre Allegra	5	[*SN*]	37°24′N 15°04′E	Within the nature reserve "Oasi del Simeto", behind the coastline	Sulla and wheat, surrounded by large wetlands with characteristic wild plants
Spina Santa	18	[*AE*]	37°24′N 14°57′E	Large cultivated areas	Cereals, sulla, and vegetables, with natural flora present in the residual zones
Motta Sant'Anastasia	70	[*AE*]	37°30′N 14°53′E	Large cultivated areas	Citrus and little natural vegetation
Giarre	81	[*AE*]	37°44′N 15°10′E	Large cultivated areas	Avocado, citrus, mango, fejoia, loquat, and other fruits, with native flora in marginal zones
Ramacca	200	[*AE*]	37°23′N 14°41′E	Agro-ecosystem	Cereals and oilseeds; marginal areas with native flora
Paternò	256	[*AE*]	37°33′N 14°54′E	Large cultivated areas	Citrus groves in intensive culture with residual natural vegetation
Bronte	625	[*AE*]	37°48′N 14°47′E	Large cultivated areas	Peach and pear, surrounded by oak forests
Milia	1046	[*SN*]	37°39′N 14°59′E	Within Etna Park	Pome and stone-fruit orchards with an abundance of wild flowers
Piano Provenzana	1845	[NE]	37°47′N 15°02′E	Within Etna Park (site Natura 2000 ITA 070009) at the altitudinal limit of pioneer vegetation	Rich in plants of high ecological value, with the presence of many endemic species

The species belonged to eleven genera: *Amegilla* (2 species), *Anthophora* (6 species), *Apis* (1 species), *Ceratina* (7 species), *Epeolus* (1 species), *Eucera* (10 species), *Habropoda* (1 species), *Melecta* (1 species), *Nomada* (3 species), *Tetraloniella* (1 species), and *Xylocopa* (2 species). Overall, the Apidae genera were represented as follows: *Amegilla* (12 specimens; 0.08% of the total number), *Anthophora* (94 specimens; 0.65%), *Apis* (13.666 specimens; 94.57%) *Ceratina* (124 specimens; 0.86%), *Epeolus* (1 specimen; 0.01%), *Eucera* (421 specimens; 2.91%), *Habropoda* (13 specimens; 0.09%), *Melecta* (1 specimen; 0.01%), *Nomada* (18 specimens; 0.12%), *Tetraloniella* (3 specimens; 0.02%), and *Xylocopa* (98 specimens; 0.68%). *Apis mellifera* Linnaeus, was the most numerous and common species, recorded in all environments investigated, due to the widespread practice of beekeeping.

Table 2 shows the recorded entities with the list of plants visited in relation to the altitudinal plans.

FOOD SOURCES

The surveyed Apidae were observed on 68 species of plants, comprising a total of 63 plant genera within 27 plant families (Tables 2, 3, and Fig. I). Particularly interesting was the site of Piano Provenzana, where there were species

Table 2 – Taxa and related number of specimens of the bees (Apidae family, bumblebees excluded) recorded on Mount Etna in the period 1997-2007 on cultivated and wild plants at different altitudinal plans.

Bees		Plants		Altitude	No. specimens
Genus	**Species/subspecies**	**Family**	**Species**		
Amegilla	*albigena*	Verbenaceae	*Vitex agnus-castus*	0-500	5
	quadrifasciata	Asteraceae, Brassicaceae	*Helianthus annuus, Moricandia arvensis*	0-500	4
		Boraginaceae, Scrophulariaceae, Asteraceae	*Echium vulgare ssp. pustulatum, Linaria purpurea,Onopordum illyricum*	1000-1450	3
Anthophora	*aestivalis*	Asteraceae	*Centaurea napifolia*	0-500	1
	balearica	Brassicaceae	*Erysimum aetnense*	1450-2100	1
	balneorum	Boraginaceae	*Echium vulgare* ssp. *pustulatum*	1000-1450	1
	crinipes	Asteraceae	*Onopordum illyricum*	1000-1450	10
	plumipes squalens	Boraginaceae	*Echium vulgare* ssp. *pustulatum*	0-500	67
		Boraginaceae, Rosaceae	*Anchusa hybrida, Pyrus communis*	1000-1450	8
	retusa	Brassicaceae	*Raphanus raphanistrum*	500-1000	2
		Rosaceae	*Rubus aetnicus*	1450-2100	4
Apis	*mellifera*	Apiaceae, Asteraceae, Boraginaceae, Brassicaceae, Convolvulaceae, Cucurbitaceae, Dipsacaceae, Euphorbiaceae, Fabaceae, Mirtaceae, Oxalidaceae, Resedaceae, Rosaceae, Rutaceae, Tamaricaceae, Verbenaceae	*Ammi visnaga, Anthemis arvensis, Borago officinalis, Brassica nigra, Calendula arvensis, Centaurea napifolia, Cerinthe major, Chrozophora tinctoria, Citrullus lanatus, Citrus sinensis, Calystegia sepium, Diplotaxis erucoides, Diplotaxis tenuifolia, Dittrichia viscosa, Echium vulgare* ssp. *pustulatum, Eriobotrya japonica, Eucalyptus camaldulensis, Galactites tomentosa, Hedysarum coronarium, Helianthus annuus, Heliotropium europaeum, Knautia integrifolia, Melilotus officinalis, Oxalis pes-caprae, Reseda alba, Rubus ulmifolius, Scabiosa maritima, Sonchus oleraceus, Tamarix gallica, Vitex agnus-castus*	0-500	10.737
		Asteraceae, Brassicaceae, Onagraceae, Rosaceae	*Diplotaxis erucoides, Dittrichia viscosa, Epilobium hirsutum, Malus domestica, Prunus spinosa, Pulicaria dysenterica, Pyrus communis, Rubus ulmifolius*	500-1000	400
		Asteraceae, Fagaceae, Geraniaceae, Labiatae, Lamiaceae, Rosaceae, Scrophulariaceae	*Amygdalus communis, Calamintha foliosa, Carlina nebrodensis, Castanea sativa, Erodium acaule, Linaria purpurea, Malus domestica, Micromeria graeca, Onopordum illyricum, Pyrus communis, Salvia verbenaca, Satureja fruticosa, Senecio glaber, Rubus ulmifolius*	1000-1450	448
		Asteraceae, Berberidaceae, Fabaceae, Onagraceae, Rosaceae, Rubiaceae, Scrophulariaceae	*Astragalus siculus, Berberis aetnensis, Epilobium angustifolium, Galium aetnicum, Linaria purpurea, Rosa canina, Rubus aetnicus, Senecio aetnensis, Tanacetum siculum*	1450-2100	2.081
Ceratina	*chalcites*	Boraginaceae	*Echium vulgare* ssp. *pustulatum*	500-1000	2
		Asteraceae	*Onopordum illyricum*	1000-1450	1
	chalybea	Asteraceae	*Senecio aetnensis, Tanacetum siculum*	1450-2100	7

(continued)

Continued Table 2

Bees		Plants		Altitude	No. specimens
Genus	**Species/subspecies**	**Family**	**Species**		
Ceratina	*cucurbitina*	Boraginaceae, Verbenaceae	*Echium vulgare* ssp. *pustulatum, Vitex agnus-castus*	0-500	9
		Asteraceae, Boraginaceae, Brassicaceae, Rosaceae	*Diplotaxis erucoides, Echium vulgare ssp. pustulatum, Galactites tomentosa, Malus domestica*	500-1000	15
		Asteraceae, Boraginaceae, Rosaceae, Scrophulariaceae	*Bellis perennis, Echium vulgare* ssp. *pustulatum, Linaria purpurea, Malus domestica, Pyrus communis, Senecio glaber*	1000-1450	65
	cyanea	Asteraceae, Verbenaceae	*Dittrichia viscosa, Vitex agnus-castus*	0-500	15
	dallatorreana	Asteraceae, Boraginaceae, Verbenaceae	*Echium vulgare* ssp. *pustulatum, Helianthus annuus, Vitex agnus-castus*	0-500	7
	dentiventris	Boraginaceae	*Echium vulgare* ssp. *pustulatum*	500-1000	1
		Boraginaceae	*Heliotropium europaeum*	1000-1450	1
	nigrolabiata	Boraginaceae	*Echium vulgare* ssp. *pustulatum*	500-1000	1
Epeolus	*julliani*	Scrophulariaceae	*Linaria purpurea*	1450-2100	1
Eucera	*aterrima*	Fabaceae, Rosaceae	*Astragalus siculus, Rubus aetnicus*	1450-2100	7
	caspica	Boraginaceae, Fabaceae	*Anchusa hybrida, Vicia sativa*	1000-1450	3
	eucnemidea	Asteraceae, Boraginaceae, Fabaceae, Malvaceae	*Centaurea napifolia, Echium vulgare* ssp. *pustulatum, Galactites tomentosa, Glebionis coronaria, Hedysarum coronarium, Lavatera trimestris, Reichardia picroides*	0-500	364
		Asteraceae	*Galactites tomentosa*	500-1000	2
	nigrescens	Fabaceae	*Hedysarum coronarium*	0-500	1
		Boraginaceae, Fabaceae	*Borago officinalis, Vicia sativa*	500-1000	6
		Fabaceae	*Vicia sativa*	1000-1450	1
	notata	Boraginaceae	*Echium vulgare* ssp. *pustulatum*	0-500	3
		Asteraceae, Fabaceae	*Lathyrus grandiflorus, Onopordum illyricum*	1000-1450	5
	numida numida	Fabaceae	*Hedysarum coronarium*	0-500	2
	oraniensis	Asteraceae	*Galactites tomentosa*	0-500	9
		Rosaceae	*Amygdalus communis*	1000-1450	5
	proxima	Fabaceae	*Hedysarum coronarium*	0-500	3
	seminuda	Asteraceae, Rutaceae	*Citrus sinensis, Galactites tomentosa*	0-500	7
	vulpes	Asteraceae, Boraginaceae	*Centaurea napifolia, Echium vulgare* ssp. *pustulatum*	0-500	3
Habropoda	*tarsata*	Boraginaceae, Fabaceae, Rosaceae	*Anchusa hybrida, Lathyrus grandiflorus, Pyrus communis*	1000-1450	13
Melecta	*albifrons nigra*	Boraginaceae	*Echium vulgare* ssp. *pustulatum*	0-500	1
Nomada	*discrepans*	Asteraceae, Rosaceae	*Pulicaria dysenterica, Rubus ulmifolius*	0-500	9
	distinguenda	Asteraceae, Verbenaceae	*Pulicaria dysenterica, Vitex agnus-castus*	0-500	6
	succincta	Rosaceae	*Amygdalus communis, Pyrus communis*	1000-1450	3
Tetraloniella	*dentata fulviscopa*	Asteraceae, Lamiaceae	*Marrubium vulgare, Onopordum illyricum*	1000-1450	3
Xylocopa	*iris*	Verbenaceae	*Vitex agnus-castus*	0-500	14
		Lamiaceae, Rosaceae	*Amygdalus communis, Micromeria graeca*	1000-1450	2
	violacea	Boraginaceae, Rosaceae, Verbenaceae	*Borago officinalis, Eriobotrya japonica, Rubus ulmifolius, Vitex agnus-castus*	0-500	20
		Asteraceae, Boraginaceae, Rosaceae	*Borago officinalis, Dittrichia viscosa, Prunus persica, Pulicaria dysenterica*	500-1000	6
		Asphodelaceae, Asteraceae, Boraginaceae, Lamiaceae, Rosaceae	*Amygdalus communis, Anchusa hybrida, Asphodelus ramosus, Echium vulgare* ssp. *pustulatum, Marrubium vulgare, Pyrus communis, Senecio glaber*	1000-1450	41
		Caryophyllaceae, Fabaceae, Rosaceae	*Astragalus siculus, Rubus aetnicus, Saponaria sicula*	1450-2100	15

Table 3 – Species of Apidae and botanical families.

Plant's family / **Apidae species**	ASPHODELACEAE	APIACEAE	ASTERACEAE	BERBERIDACEAE	BORAGINACEAE	BRASSICACEAE	DIPSACACEAE	CARYOPHYLLACEAE	CONVOLVULACEAE	CUCURBITACEAE	EUPHORBIACEAE	FABACEAE	FAGACEAE	GERANIACEAE	LABIATAE	LAMIACEAE	MALVACEAE	MYRTACEAE	ONAGRACEAE	OXALIDACEAE	RESEDACEAE	ROSACEAE	RUBIACEAE	RUTACEAE	SCROPHULARIACEAE	TAMARICACEAE	VERBENACEAE
Amegilla albigena																											5
Amegilla quadrifasciata			3		1	2																			1		
Anthophora aestivalis			1																								
Anthophora balearica						1																					
Anthophora balneorum					1																						
Anthophora crinipes			10																								
Anthophora plumipes squalens					74																	1					
Anthophora retusa						2																4					
Apis mellifera		2	1550	70	878	127	21		60	21	2	2690	50	1	40	100		154	386	37	2	4836	8	2194	43	365	29
Ceratina chalcites			1		2																						
Ceratina chalybea			7																								
Ceratina cucurbitina			8		7	1																67			1		5
Ceratina cyanea			3																								12
Ceratina dallatorreana			2		1																						4
Ceratina dentiventris					2																						
Ceratina nigrolabiata					1																						
Epeolus julliani																									1		
Eucera aterrima												5										2					
Eucera caspica					1							2															
Eucera eucnemidea			99		16							1					248										
Eucera nigrescens					2							6															
Eucera notata			2		3							3															
Eucera numida numida												2															
Eucera oraniensis			9																			5					
Eucera proxima												3															
Eucera seminuda			6																					1			
Eucera vulpes			2		1																						
Habropoda tarsata					5							7										1					

(continued)

Continued Table 3

Plant's family / **Apidae species**	ASPHODELACEAE	APIACEAE	ASTERACEAE	BERBERIDACEAE	BORAGINACEAE	BRASSICACEAE	DIPSACACEAE	CARYOPHYLLACEAE	CONVOLVULACEAE	CUCURBITACEAE	EUPHORBIACEAE	FABACEAE	FAGACEAE	GERANIACEAE	LABIATAE	LAMIACEAE	MALVACEAE	MYRTACEAE	ONAGRACEAE	OXALIDACEAE	RESEDACEAE	ROSACEAE	RUBIACEAE	RUTACEAE	SCROPHULARIACEAE	TAMARICACEAE	VERBENACEAE
Melecta albifrons nigra					1																						
Nomada discrepans			6																			3					
Nomada distinguenda			3																								3
Nomada succincta																						3					
Tetraloniella dentata fulviscopa			2													1											
Xylocopa iris																1						1					14
Xylocopa violacea	1		3		4			6				7				4						45					12

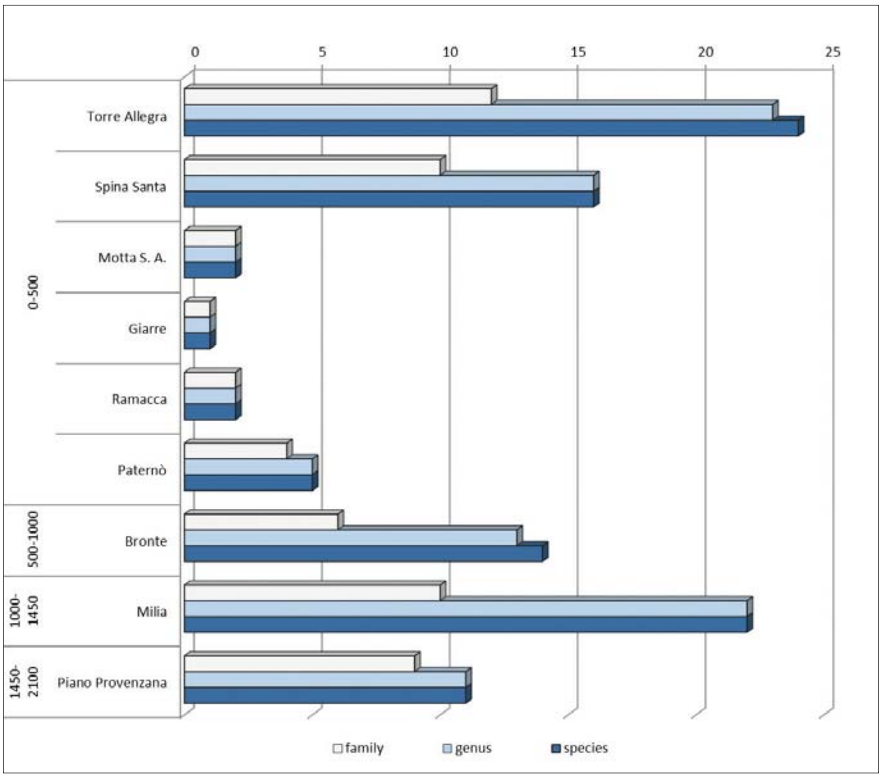

Fig. I – Number of families, genera, and species of plants visited by bees in the period 1997-2007 in relation to the altitudinal plans.

of considerable phytogeographic importance, such as *Astragalus siculus* (Fabaceae) and *Senecio aethnensis* (Asteraceae), characteristic of high altitudes of Etna, and *Erysimum aetnense* Jord. (Brassicaceae), all endemic to Sicily. In this environment, most of the bees were recorded on plants with the highest potential of honey, such as *Astragalus*, *Rubus* and *Senecio*. Here, despite the position inside a protected area, the total number of plants was low (Fig. I) due to the extreme climatic conditions of this high mountain environment. The largest number of plant species was found in the two semi-natural ecosystems of Torre Allegra and Milia and in the agro-ecosystem of Bronte (Fig. I). In this latter, this is explained with the presence of a large number of wild plants surrounding the agro-ecosystems. The herbaceous and woody cultivated plants visited by bees included 11 species within *Castanea*, *Citrus*, *Eucalyptus*, *Malus*, *Prunus*, and *Pyrus* (spring flowering); *Hedysarum* and *Helianthus* (summer flowering); and *Amygdalus* and *Eriobotrya* (winter flowering). Only five species of bees were found on almond trees (cultivated at an altitude of

1000 m) and loquat (81 m) in December-January and December-February, respectively (Table 2). In this adverse season, only the following species were noticed: *Apis mellifera*, *Eucera oraniensis* Lepeletier, *Nomada succincta* Panzer, *Xylocopa iris* (Christ) and *X. violacea* (L.).

Table 3 shows the number of species of bees observed on each botanical family of plants surveyed. Most of the species of the bees were found on Asteraceae (19 species), Rosaceae (11 species), and Fabaceae (10 species). As regards the food preferences, only a few bee species visited a large number of plants belonging to different families (polylectic bees, e.g. *Apis mellifera*, *Ceratina cucurbitina* and *Xylocopa violacea*), whereas the majority were attracted by only two or three botanical species (oligolectic bees) (Table 3).

Detected taxa

Family Apidae Latreille, 1802

Genus *Amegilla* Friese, 1897

Amegilla (*Zebramegilla*) *albigena* (Lepeletier, 1841)

Distribution: Central-Asiatic, European-Mediterranean.
Examined specimens: Torre Allegra, 28.VII.1998, 1 ♂, (3), 23.VII.1999, 1 ♂, SB leg., MQ det.

Amegilla (*Amegilla*) *quadrifasciata* (De Villers, 1789)

Distribution: Palaearctic.
Examined specimens: Ramacca, 23.VI.1999, 1 ♀, 02.VII.1999, 2 ♀♀, 12.VII.1999, 1 ♂, SB leg., MQ det.; Milia, 21.IX.2004, (1), 16.VII.2005, 1♀, (1), SB leg. and det.

Genus *Anthophora* Latreille, 1803

Anthophora (*Anthophora*) *crinipes* Smith, 1854 (= *salviae* Panzer, 1804)

Distribution: Asiatic-European.
Examined specimens: Milia, 07.V.2003, 1 ♂, (9), SB leg., MQ det.

Anthophora (*Anthophora*) *plumipes squalens* Dours, 1869

Distribution: South-central Europe, and Maghreb.
Examined specimens: Torre Allegra, 14.IV.1998, 5 ♀♀, 1 ♂, (12), 22.IV.1998, 5 ♀♀, (28), VL leg., 06.V.1998, 5 ♀♀, (10), 04.V.1999, 1 ♀, SB leg., MQ det.; Milia, 24.III.2000, 1 ♂, (6), 7.V.2003, 1 ♀, SB leg., MQ det.

Anthophora (*Paramegilla*) *balneorum* (Lepeletier, 1841)

Distribution: South-West Mediterranean.
Examined specimens: Milia, 30.V.2006, 1 ♂, SB leg., MQ det.

Anthophora (*Pyganthophora*) *aestivalis* (Panzer, 1801)

Distribution: Europe, Anatolia, and Caucasia.
Examined specimens: Torre Allegra, 26.V.1999, 1 ♀, SB leg., MQ det.

Anthophora (*Pyganthophora*) *balearica* (Friese, 1896)

Distribution: Spain and Baleari Islands, southern Italy (Apulia and Sicily), and Anatolia.
Examined specimens: Piano Provenzana, 19.VI.2007, 1 ♂, AS & SB leg., VN det.
Remarks: Maidl (1922) reported this species for Spain, Italy and Anatolia.

Anthophora (*Pyganthophora*) *retusa* (Linnaeus, 1758)

Distribution: Palaearctic.
Examined specimens: Bronte, 13.IV.2000, 1 ♂, (1), SB leg., MQ det.; Piano Provenzana, 27.VII.2007, 1 ♀, (3), AS & SB leg., VN det.

Genus *Apis* Linnaeus, 1758

Apis mellifera Linnaeus, 1758

Distribution: Cosmopolitan.
Examined specimens: abundant in all sites surveyed (Table 2).
Remark: in relation to the widespread presence in the island territory of beekeeping activity, the specimens observed were mainly related to the two subspecies present in Sicily (*Apis mellifera siciliana* Dalla Torre, 1896 and *A. m. ligustica* Spinola, 1806) and their hybrids.

Genus *Ceratina* Latreille, 1802

Ceratina (*Ceratina*) *cucurbitina* (Rossi, 1792)

Distribution: W-Palaearctic.
Examined specimens: Torre Allegra, 28.VIII.1997, 1 ♀, (2), VL leg., 04.V.1999, 1 ♀, (3), 28.VI.1999, 2 ♀♀, SB leg., MQ det.; Bronte, 30.VI.2000, 1 ♀, SB leg., MQ det., 27.IV.2005, 1 ♂, 2 ♀♀, (9), 29.V.2006, 2 ♀♀, SB leg., VN det.; Milia, 30.V.2000, 1 ♀, 02.V.2003, 3 ♂♂, (52), SB leg., MQ det., 23.IV.2005, 2 ♂♂, (4), 24.IX.2005, 1 ♀, 11.IV.2006, 1 ♂., 30.V.2006, 1 ♂., SB leg., VN det.

Ceratina (*Euceratina*) *chalcites* Germar, 1839

Distribution: Europe and northern Asia (excluding China).
Examined specimens: Bronte, 19.VII.2005, 1 ♀, (1), SB leg., VN det.; Milia, 23.VI.2006, 1 ♀, SB leg., VN det.

Ceratina (*Euceratina*) *chalybea* Chevrier, 1872

Distribution: W-Palaearctic.
Examined specimens: Piano Provenzana, 16.VI.2007, 1 ♂, (4), 16.VIII.2007, 1 ♂, 28.VIII.2007, 1 ♀, AS & SB leg., VN det.

Ceratina (*Euceratina*) *cyanea* (Kirby, 1802)

Distribution: European.
Examined specimens: Spina Santa, 27.X.1998, 1 ♂ (2), SB leg., MQ det.; Torre Allegra, 28.VI.1999, 1 ♂, 23.VII.1999, 2 ♂, (9), SB leg., MQ det.

Ceratina (*Euceratina*) *dallatorreana* Friese, 1896

Distribution: Holartic.
Examined specimens: Torre Allegra, 04.V.1999, 1 ♀, 28.VI.1999, 1 ♀, 1 ♂, (2), SB leg., VN det.; Ramacca, 02.VII.1999, 2 ♀♀, SB leg., MQ det.

Ceratina (*Euceratina*) *dentiventris* Gerstaecker, 1869

Distribution: Caucasia, southern Europe, Anatolia and the Maghreb.
Examined specimens: Bronte, 30.VI.2000, 1 ♂, SB leg., MQ det.; Milia, 20.VIII 2005, 1 ♂, SB leg., VN det.

Ceratina (*Euceratina*) *nigrolabiata* Friese, 1896

Distribution: Europe and northern Asia (excluding China).
Examined specimens: Bronte, 29.V.2006, 1 ♀, SB leg., MQ det.

Genus *Epeolus* Latreille, 1802

Epeolus julliani Peréz,1884

Distribution: Southern Europe and Anatolia.
Examined specimens: Piano Provenzana, 16.VIII.2007, 1 ♀, AS & SB leg., VN det.

Genus *Eucera* Scopoli, 1770

Eucera (*Atopeucera*) *seminuda* Brullé, 1832

Distribution: Medium-South Europe, Anatolia and Maghreb.
Examined specimens: Spina Santa, 25.IV.1998, 2 ♀♀, (4), SB leg., MQ det.; Motta Sant'Anastasia, 10.V.2000, 2 ♀♀, SB leg., MQ det.

Eucera **(*Eucera*)** ***proxima*** Morawitz, 1875

(= *graeca* Radoszkowski, 1875)

Distribution: South-central Europe, Anatolia, and eastern Mediterranean.
Examined specimens: Spina Santa, 16.IV.1998, 1 ♂, (2), SB leg., MQ det.

Eucera **(*Eucera*)** ***nigrescens*** Pérez, 1879

Distribution: Europe and Anatolia.
Examined specimens: Torre Allegra, 14.IV.1998, 1 ♀, SB leg., MQ det.; Bronte, 13.IV.2000, 1 ♂, 1 ♀, 26.IV.2000, 1 ♂, 1 ♀, (2), SB leg., MQ det.; Milia, 07.IV.2006, 1 ♂, SB leg., MQ det.

Eucera **(*Eucera*)** ***numida numida*** Lepeletier, 1841

Distribution: South-West-Europe and Maghreb.
Examined specimens: Torre Allegra, 06.V.1998, 2 ♀♀, SB leg., MQ det.

Eucera **(*Hetereucera*)** ***notata*** Lepeletier, 1841

Distribution: Mediterranean.
Examined specimens: Paternò, 27.IV.1998, 1 ♀, (2), SB leg., MQ det.; Milia, 29.III.2000, 1 ♂ (2), 27.VI.2000, (2), SB leg.. MQ det.

Eucera **(*Hetereucera*)** ***oraniensis*** Lepeletier, 1841

Distribution: South-West Europe, and northern African.
Examined specimens: Torre Allegra, 14.IV.1998, 1 ♀, (2), SB leg., MQ det.; Spina Santa, 23.III.1998, 1 ♀, (2), 16.IV.1998, 1 ♀, (2), SB leg., MQ det.; Milia, 26.III.2003, 1 ♂ (4), SB leg., MQ det.

Eucera **(*? Pareucera*)** ***aterrima*** Friese, 1896

Distribution: Mediterranean.
Examined specimens: Piano Provenzana, 02.VII.2006, 1 ♀, 16.VII.2006, 1 ♀, (1), 19.VI.2007, 1 ♀, 27.VII.2007, 1 ♀, (2), AS & SB leg., VN det..
Remark: in this context and until the males are identified, we prefer to believe that *Eucera aterrima* is a good species and not a subspecies of *E. nigrita* Friese 1896, as in RISCH (2001).

Eucera **(*Pareucera*)** ***caspica*** Morawitz, 1873

Distribution: Central-Asiatic, Europe, and Anatolia.
Examined specimens: Milia, 08.IV.2005, 1 ♂, 07.IV.2006, 1 ♀, (1), SB leg.,VN det.

Eucera **(*Pteneucera*)** ***eucnemidea*** Dours, 1873

Distribution: South-West Europe, and Maghreb.
Examined specimens: Torre Allegra, 06.V.1998, 10 ♂♂, (78), VL leg., MQ det.; Spina Santa, 16.IV.1998, 8 ♂, (34), 25.IV.1998, 15 ♂♂, (215), 28.V.1999, 1 ♂, (1), SB leg., MQ det.; Bronte, 31.V.2000, 1 ♂, (1), SB leg., MQ det.

Eucera **(*Rhyteucera*)** ***vulpes*** Brullé 1832

(= *parvula* Friese, 1896)

Distribution: South-Europe, and Anatolia.
Examined specimens: Torre Allegra, 04.V.1999, 2 ♀♀, (1), SB leg., MQ det.

Genus *Habropoda* Smith, 1854

Habropoda tarsata (Spinola, 1838)

Distribution: European extended to Anatolia.
Examined specimens: Milia, 17.IV.2000, 1 ♂ (4), 22.IV.2000, 1 ♂, (1), SB leg., MQ det., 07.V.2003, 1 ♀ (5), SB leg., VN det.

Genus *Melecta* Latreille, 1802

Melecta albifrons nigra Spinola, 1806

Distribution: South-West Europe.
Examined specimens: Torre Allegra, 14.IV.1998, 1 ♀, SB leg., MQ det.

Genus *Nomada* Scopoli, 1770

Nomada discrepans Schmiedeknecht, 1882

Distribution: South-Europe, Maghreb, and Macaronesia.
Examined specimens: Torre Allegra, 04.VIII.1997, 1 ♂, (2), 28.VIII.1997, 1 ♂, (5), VL leg., MQ det.

Nomada distinguenda Morawitz, 1874

Distribution: Palaearctic.
Examined specimens: Torre Allegra, 04.VIII.1997, 1 ♂, (2), 28.VIII.1997, 1 ♂, (2), VL leg., MS det.

Nomada succincta Panzer, 1798

Distribution: Asiatic-European.
Examined specimens: Milia, 29.III.2000, 1 ♀, 31.III.2006, 1 ♂, 04.IV.2006, 1 ♀, SB leg., MQ det.

Genus *Tetraloniella* Ashmead, 1899

Tetraloniella dentata fulviscopa Hedicke, 1933

Distribution: South-Europe, and Caucasia.
Examined specimens: Milia, 22.VI.2005, 1 ♂, 23.VI.2006, 2 ♂♂, SB leg., VN det.

Genus *Xylocopa* Latreille, 1802

Xylocopa **(*Copoxyla*)** ***iris*** (Christ, 1791)

Distribution: Mediterranean and Central-Asiatic.
Examined specimens: Torre Allegra, 04.VIII.1997, 1 ♀, (2), 27.IX.1997, 1 ♂, (2), VL leg., 28.VII.1998, 1 ♂, (1), 28.VI.1999, 1 ♂, 23.VII.1999, 1 ♂, 1 ♀, (3), SB leg., MQ det.; Milia, 13.III.2003, 1 ♂, VN det., 16.VII.2005, (1), SB leg. and det.

Xylocopa **(*Xylocopa*)** ***violacea*** (Linnaeus, 1758)

Distribution: Europe extended to the Maghreb and Anatolia.
Examined specimens: Torre Allegra, 28.VIII.1997, 3 ♂♂, 1 ♀, (8), 27.IX.1997, 1 ♂, (2), VL leg., MQ det.; Giarre, 14.XI.2000, (2); Paternò, 27.IV.1998, 1 ♂, (2), SB leg., MQ det.; Bronte, 07.IV.2005, (1), 27.IV.2005, (3), 26.IX.2005, (1), 28.IX.2006, (1); Milia, 24.III.2000, 2 ♂♂, (1), 20.IV.2000, 1 ♀, 5.III.2003, 1 ♂, (4), 13.III.2003, 1 ♂, (29), 19.III.2003, 1 ♂, (1), SB leg., MQ det.; Piano Provenzana, 19.VIII.2006, 1 ♂, (14), AS & SB leg., VN det.

DISCUSSION AND CONCLUSIONS

In the 11 years of investigation, more than 20000 specimens of Apoidea were collected and/or observed, more than 18000 of which belonged to the family Apidae, accounting for 88% of the total. Excluding bumblebees that accounted for 3426 specimens in 12 species (MAZZEO *et al.*, 2015), 14451 Apidae specimens in 11 genera and 35 species were detected. Overall, the Apidae family (*Bombus* genus included) was present with 48 taxa.

Apis mellifera, with 13666 specimens was the predominant species for number and range, having been found on Mount Etna at all altitudes. *Xylocopa violacea* was also very common and widely distributed. Only seven species were recorded for the site at the highest altitude, Piano Provenzana (1845 m a.s.l.): *Anthophora balearica*, *A. retusa*, *Apis mellifera*, *Ceratina chalybea*, *Epeolus julliani*, *Eucera aterrima* and *Xylocopa violacea*. The greatest number of species was found in the thermo-Mediterranean (0-500 m a.s.l.) and meso-Mediterranean (500-1000 m a.s.l.) horizons (Fig. II), where the

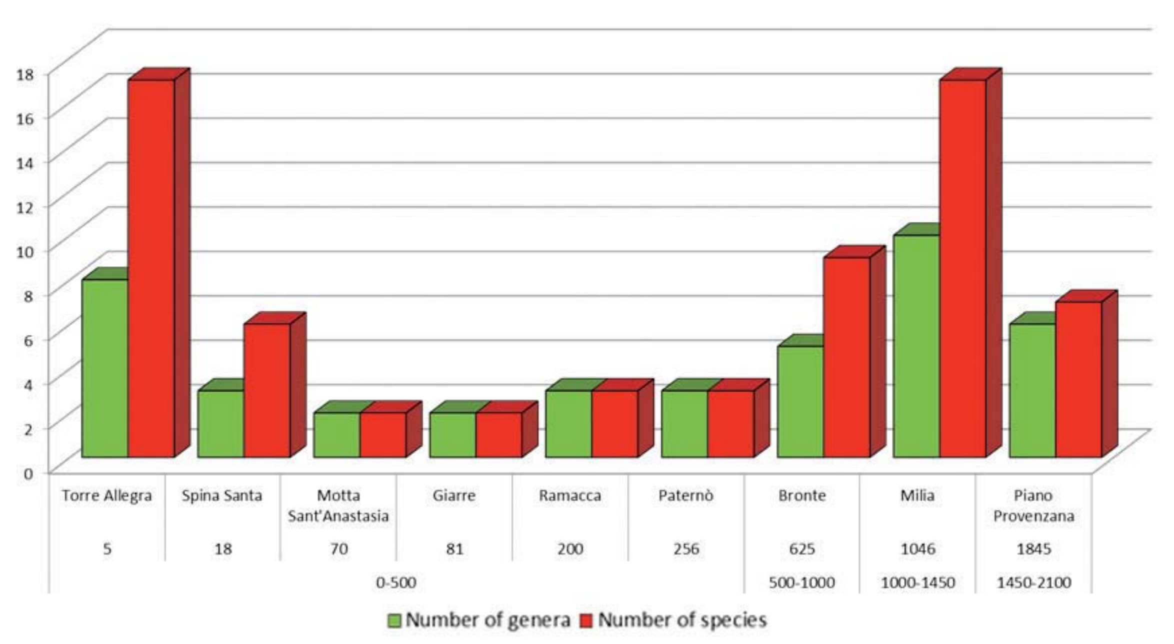

Fig. II – Number of Apidae genera and species (bumblebees excluded) per site recorded on cultivated and wild plants at different altitudinal plans.

presence of bees was abundant even in the coldest months of the year on winter-flowering crops, as in the case of almond (5 species) and loquat (2 species). In this horizons *Anthophora plumipes*, *Apis mellifera* and *Eucera eucnemidea*, were the most abundant species. In the mountain-mediterranean (1450-2100 m a.s.l.) plan and in the supra-mediterranean horizon (1000-1450 m a.s.l.), *Apis mellifera* and *Xylocopa violacea* were most abundant.

Anthophora aestivalis, *A. balearica*, *A. balneorum*, *Ceratina chalcites*, *C. dentiventris*, *C. nigrolabiata*, *Epeolus julliani*, *Eucera proxima*, *E. numida*, *E. parvula*, *Melecta albifrons nigra*, *Nomada succincta* and *Tetraloniella dentata* were very rare in the period of investigation, with only 1-3 specimens recorded for each species.

Most of the species were recorded inside the protected areas and in particular: *Amegilla albigena*, *Anthophora aestivalis*, *Apis mellifera*, *Ceratina cucurbitina, C. cyanea*, *C. dallatorreana*, *Eucera nigrescens*, *E. numida*, *E. oraniensis*, *E. eucnemidea*, *E. vulpes*, *Melecta nigra*, *Nomada discrepans*, *N. distinguenda*, *Xylocopa iris* and *X. violacea* from Torre Allegra; *Amegilla quadrifasciata*, *Anthophora crinipes*, *Anthophora plumipes squalens*, *Anthophora balneorum*, *Anthophora balearica*, *Anthophora retusa*, *Apis mellifera*, *Ceratina cucurbitina*, *C. chalcites*, *C. chalybea*, *C. dentiventris*, *Epeolus julliani*, *Eucera nigrescens*, *E. notata*, *E. oraniensis*, *E. aterrima*, *E. caspica*, *Habropoda tarsata*, *Nomada succincta*, *Tetraloniella dentata fulviscopa*, *Xylocopa iris*, and *X. violacea* from the Etna Park.

The qualitative and quantitative characterisations of the pollinators of a given area provide the “health status” of that territory and, through subsequent monitoring, show the environmental changes that have occurred and how much these have affected biodiversity.

The data from this study, together with those about the *Bombus* species in Mazzeo *et al.* (2015), analysed the biodiversity of the Apidae family in the cultivated, semi-natural, and natural ecosystems of the Etna territory, a wide area of eastern Sicily, placing it in relation to the different human activities, altitudinal plans, plants visited, and ecological qualities of the areas.

ACKNOWLEDGEMENTS

We express our gratitude to Dr Vittorio Nobile (Ragusa, Italy) and to Dr Marino Quaranta (CREA-AA, Bologna, Italy) for identifying most of the Apidae collected, and to Dr Giovanna Ferrauto and to prof. Gianpietro Giusso del Galdo (University of Catania, Italy) for the nomenclatural information on the reported plants, and to the anonymous reviewers for their constructive commentary. We are especially grateful to Etna Park and the Metropolitan City of Catania for authorising the surveys in the Regional protected areas.

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REDIA, 102, 2019: 163-170 http://dx.doi.org/10.19263/REDIA-102.19.23

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ISOLATION AND CHARACTERIZATION OF *METARHIZIUM* ISOLATES FROM THE SOIL OF AFGHANISTAN AND THEIR MYCOINSECTICIDE EFFECTS AGAINST SUBTERRANEAN TERMITE (*ANACANTHOTERMES VAGANS*)

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Fallahzadeh H., Karimi J., Moravvej G.H., Tarasco E. – Isolation and characterization of *Metarhizium* isolates from the soil of Afghanistan and their mycoinsecticide effects against subterranean termite (*Anacanthotermes vagans*).

The entomopathogenic fungi are widely used as a biocontrol agent against many insect pests. In the present study, strains of *Metarhizium* were isolated and identified from Badakhshan province of Afghanistan. The taxonomic identity of the isolates was confirmed by molecular data, using (ITS) rDNA gene sequence, and morphology: *Metarhizium robertsii* and *Metarhizium anisopliae.* The virulence and the mass conidial production of these strains were compared with two Iranian isolates of *Beauveria* (*B. bassiana* and *B. varroae*) using four different raw substrates (broken rice, broken wheat, green gram, and saw dust) by solid-state fermentation. The *M. robertsii* strain had the highest total conidial yields obtained after 18 days on broken rice and the least conidial growth was observed on saw dust. For *B. bassiana,* the highest conidial yields were obtained after 18 days on green gram, and also like to *M. robertsii,* the least conidial growth was observed on saw dust. The conidia suspension was applied against subterranean termite, *Anacanthotermes vagans,* at four different concentrations (1×10^5, 1×10^6, 1×10^7, 1×10^8 conidia/ml^{-1}). The highest mortality rate was obtained from 1×10^8 conidia/ml^{-1} for both *M. robertsii* and *B. bassiana*. In general, the indigenous isolate of *M. robertsii* was more virulent than *B. bassiana* while *B. varroae* had not effect on termites.

Key Words: Entomopathogenic fungi, Identification, Phylogenetical analysis, Termites, Substrate.

INTRODUCTION

Termites are medium-sized, soft-bodied, light-colored, polymorphic and cellulose-eating social insects living in large communities of several hundred to several million individuals. The fossil record indicates the long history of termite's evolution (Sindha *et al*., 2011; Azmi *et al*., 2016). Due to the feeding function, the worker casts cause widespread destruction resulting in major economic losses in tropical and subtropical areas by destroying agricultural crops, live trees, and wooden structures in the houses. About 300 species have been reported to cause significant damage to agriculture crops and have been recorded as pests (Abonyo *et al*., 2016; Jamil *et al*., 2017). Termites also feed and often destroy various other structures or materials that people use, *i.e.* wooden portions of buildings, furniture, books, utility poles, fence posts, many fabrics, and other useful materials. The termites also attack telephone pole, boats, and other finished goods (*e.g.* paper and fabric), valuable manuscripts and paintings (Cheraghi *et al*., 2012). Control of termites has depended on the use of insecticides. However, due to relatively high human toxicity and unacceptable environmental consequences, this situation has required research to find effective and safe alternatives for termite control (Ghaffari *et al*., 2017). It is therefore essential that alternative safe management strategies be developed to replace chemical agents and reduce risks of environmental contamination (Hussain *et al*., 2010; Brunner-Mendoza *et al*., 2019).

Biological control agents can be used as a component of integrated pest management. Several natural enemies, including entomopathogenic fungi (EPF), have been under consideration as potential biological control agents. Under natural conditions, fungal pathogens are frequent and often cause natural mortalities within the insect populations (Keppanan *et al*., 2018a).

Mitosporic fungi in the genera *Metarhizium* Sorokīn and *Beauveria* Vuillemin are widespread in soil and are known with virulence to most arthropods (Perez-Gonzalez *et al*., 2014). These genera have shown great potential for management of various insect pests (Singha *et al*., 2010). *Metarhizium anisopliae* (Metch.) Sorokin is being developed as insecticide for use against locusts and grasshoppers in Australia and Africa (Dong *et al*., 2007). Development of similar biopesticides is being undertaken in many countries. In this paper, isolation and characterization of two *Metarhizium* strains obtained from soils of Afghanistan were carried out and the pathogenicity of the Afghan strain *Metarhizium robertsii* Bischoff *et al*., compared with Iranian isolates of *Beauveria bassiana* (Bals.-Criv.) Vuill. and *Beauveria varroae* S.A. Rehner & Humber, on subterranean termite (*Anacanthotermes vagans* Hagen) was surveyed.

MATERIALS AND METHODS

Galleria melonella L. (Lepidoptera: Pyralidae) was reared in laboratory. Adult wax moths and larvae were kept

– Received 7 June 2019 Accepted 11 November 2019

in 3L plastic bottles and stored at room temperature in an incubator (28 ± 1 °C). The cultures were maintained by a continuous supply of nutrient media made with wax, flour, bran, yeast, glycerin and honey (Lephoto, 2013).

Collection of soil samples and Baiting Technique

One hundred and 58 samples of soil from Badakhshan province with a geographic of longitude: 71° 00' 0.00" E, and latitude 38° 00' 0.00" N, located in the northeast of Afghanistan, were collected. The soil samples, collected from depth of 25-30 cm were transferred to Biocontrol and Insect Pathology Lab., Ferdowsi University of Mashhad. The samples were kept in separate plastic containers and stored at 22 ± 3 °C during transport to the laboratory. Prior baiting, water was added to give a moisture content not greater than 10% moisture and the samples were then stored at room temperature overnight.

Extraction of the fungi from the soil samples has done using *Galleria* larvae as bait insect. About 6 insect larvae were placed on top of each sample. Each container was turned over and stored in an incubator set at 28 ±1 °C (Keppanan *et al*., 2018b). Observations were done daily to monitor the infection and dead larvae (Sepulveda *et al*., 2016).

All the dead larvae were collected after 96 hours. The dead larvae were removed and surface sterilized with 1% sodium hypochlorite for 1 min, then washed three times in sterile distilled water, placed on damp filter paper within a sealed medium petri dish (9 cm diameter) and incubated at 28±1 °C for 6 days. After incubation period, fungi were isolated from the dead larvae showing external mycelia growth on potato dextrose agar (PDA) with antibiotics (tetracycline at 40 µm/ml) for suppression of bacterial growth. After growth of mycelia on PDA, they transferred to the liquid potato dextrose (PD) and were incubated for 10 days at 25 ± 1 °C on a shaker at 250 rpm (Sayed *et al*., 2018).

DNA Extraction and Molecular Characterization

Fungal genomic DNA was extracted from the hyphae using a partially chemical lysis method. Approximately 30 mg of crushed mycelium was used for DNA extraction, and the rest of the sample was stored at -20 °C until needed. The DNA extraction was done using DNeasy Blood & Tissue Kits. The extracted DNA was stored at -20 °C until use as a template for PCR (Fernandes *et al*., 2009).

The PCR mixture was carried out in a reaction volume of 25 µl, containing 2.7 µl of 10×PCR buffer, 14.4 µl of H2O, 1 µl of Mgcl2 (25mM), 0.5 ul of dNTPs (10 Mm), 0.3 µl *Taq* polymerase (5 units/ µl), 1 µl of forward primer (10 pmol/ µl), 1 µl of reverse primer (10 pmol/ µl), and 4 µl of template DNA (Darsouei *et al*., 2015). The ITS gene was amplified using the ITS4 and ITS5 primers (White *et al*., 1990). The condition for PCR was as follows: denaturation at 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 1min, annealing at 52 °C for 1min, and extension at 72 °C for 5min. A final extension was performed at 72 °C for 10 min. The PCR products were electrophoresed on 1% agarose gels and subsequently, the gels were stained using Green viewer (SYBR) (Karimi *et al*., 2018). The PCR products were sequenced by Bioneer Company of South Korea. The sequences chromatograms were checked using BioEdit software. Forward the reverse sequence was assembled in BioEdit software.

For phylogenetic analysis of the recovered population, the DNA sequences were compared with those of other available in the GeneBank using the BLAST homology search program. The ITS rDNA sequences of some valid strains of *Metarhizium* isolates available in the GeneBank were retrieved and aligned using MEGA7 (Kumar *et al*., 2018). The ITS sequence of *Armillaria gallica* Marxm. & Romagn (KP288483.1) was used as outgroup. The MEGA7 program (Kumar *et al.,* 2016) was used to calculate nucleotide distances. Phylogenetic analyses were performed using the maximum likelihood with 10000 relocations of bootstrap.

Morphology and Mass Production of Conidia

The morphology of the conidia taken from the cadaver of *Galleria* larvae was studied by light microscopy. Culture of the fungus was grown on potato dextrose agar in the dark for 10, 15 and 20 days. The morphology of the conidial clumps taken from the potato dextrose agar medium was studied by light microscopy. The colonies were evaluated according to the technique described by Humber & Steinkraus (2005). Slides were prepared with lactophenol cotton blue, and micromorphologies observed at 100× magnification. Thirty conidia were measured by eyepiece measuring system as presented in the table1.

Four different solid culture which including broken rice, broken wheat, green gram and sawdust were used for evaluating the mass production of *M. robertsii* and Iranian strain of *B. bassiana* at 28 ±1 °C. One hundred and 50 g of each sample was washed well. The broken rice and broken wheat were soaked in water for two hours and green gram and saw dust for seven hours prior to starting the experiments (Migiro *et al*., 2010). The excess water was drained by decanting and shade drying for 30 minutes to further remove the excess moisture. Three replications were maintained for each sample. The samples were packed in individual 1000 ml bottle for everyone separately.

The bottles were plugged with cotton wool and autoclaved for 1 h. After cooling, 3 ml of the spore suspension of the fungal pathogen was inoculated into each bottle, separately. All these procedures were done under laminar air flow chamber (Prasad & Pal, 2014). The bottles were incubated in the incubator at 28 ±1 °C, separately for 18 days. To avoid clumping, after 9 days of inoculation, the bottles were shaken vigorously to separate the media and break the mycelial material. After 18 days of incubation, 450 ml sterilized water containing Tween 80 (0.05%) was added to each bottle and then shake for 10 min (Agale *et al*., 2018). The suspension was filtered through double-layered muslin cloth. Counting of spores was made after serial dilution of the suspension using double ruled Neubauer hemocytometer for determining the number of conidia in 1g of each sample.

Evaluation of fungal Pathogenicity against Termites in Laboratory Bioassays

The insecticidal activity of three entomopathogenic fungi, *Metarhizium robertsii* (Afghan isolate), *Beauveria bassiana*

Table 1 – Conidia morphometrics of *Metarhizium robertsii* and *M. anisopliae* strains collected in the current work compared with relative isolates of *Metarhizium.*

Species name	isolate	measurement (µm)
M. robertsii	current work	7.6 (7.0 - 8.9) × 3.6 (3.4 - 3.8)
M. robertsii	NR_132011.1	7.9 (7.5 - 9.0) × 3.7(3.5- 4.0)
M. guizhouense	Hkd25-2	11.6 (9.3 - 13.6) × 4.2 (3.4 - 4.7)
M. anisopliae	Current work	7.0 (6.4 - 7.8) × 3.6 (3.2 – 3.8)
M. anisopliae	Kgs15-1	9.0 (7.5 - 10.1) × 3.4 (2.8-3.8)
M. anisopliae	Sag6-1	9.3 (8.0 - 10.3) × 3.7 (3.4× 4.2)
M. majus	Kkj2-1	9.4 (8.4 - 10.1) × 3.5 (3.3 – 4.0)

(FUM102) and *Beauveria varroae* (FUM121) (Iranian isolates) were determined on *Anacanthotermes vagans*. The *Beauveria* strains came from Ferdowsi University of Mashhad Insect Pathogens Collection. The fungal strains were tested at a series concentration of 1×10^5, 1×10^6, 1×10^7 and 1×10^8 conidia/ml^{-1}. Six hundred µm suspension of each fungal concentration was poured by a pipet into sterile 9 cm petri dish and allowed to dry partially. Termites were allowed to walk on the partially dried fungal suspension for 1 min. One milliliter 0.05 Tweem-80 solution was added on the filter paper in Petri dishes as the control (KEPPANAN *et al*., 2018b). Three replicates were performed for each concentration of conidial suspensions with ten individual termites which maintained. Mortality was observed at different day's intervals (every 24 h) for 10 days. The infected insect cadavers were transferred aseptically to another sterile Petri dish. At each observation, dead termites were removed, and surface sterilized with 1% sodium hypochlorite for 1 min, then washed three times in sterile distilled water and placed in plastic petri dish (90 mm) with wet filter paper and maintained at 28 ±1 °C in growth chamber for mummification and sporulation to confirm the pathogenicity.

EVALUATION OF FUNGAL PATHOGENICITY AGAINST TERMITES IN SEMI-FIELDS CONDITIONS

For semi-field experimental setup, plastic dishes were prepared at 14cm on the level of the balcony. The soil with saw dust and added moisture was placed in container. The open shape plastic dishes were laid out on the soil and saw dust to prevent termite's escape and ant attack and also protect the termites from direct sunlight. In this test, two fungal strains, *Metarhizium robetsii* (Afghan isolate) and *Beauveria bassiana* (Iranian isolate) were used. The fungal concentrations were 1×10^5, 1×10^6, 1×10^7 and 1×10^8 conidia/ml^{-1}. One ml suspension of *M. robetsii* and *B. bassiana* was sprayed by small sprayer into each plastic container and put 10 termites in each plastic container. Three replicates were performed on each concentration of conidial suspensions with ten individual termites which maintained and mortality was recorded at different day's intervals (every 24 h) for 12 days. Sterile distilled water was sprayed at the inner side of the container every day to maintain the humidity. One milliliter 0.05% Tween-80 solution was sprayed on the plastic container as the control. Similar to lab assay, the infected insect cadavers were transferred aseptically to another sterile petri dish. At each observation, dead termites were removed, counted and placed in a plastic petri dish (90 cm) lined with wet filter paper and maintained at 28 ±1 °C in a growth chamber for mummification and sporulation.

DATA ANALYSIS

Mortality data were corrected according to Abbott's formula (ABBOTT, 1925). Differences between the fungal isolates and control group, with respect to mortality, was determined by analysis of variance (ANOVA). And subsequently by LSD multiple comparison test. All analyses were performed using program SAS (GUPTA *et al*., 2016).

RESULT

Two species of *Metarhizium* were identified from the collected soil samples. The general morphology of the fungal mycelia was similar to *Metarhizium* genus. The infected *Galleria* larvae turned from grayish green and grey to brown or black in color and the cadavers were abundantly covered with white mycelia, grayish green and green conidial spores. The first isolate had ovoid conidia formed in a chain, which is characteristic in *M. robertsii.* The conidia average length was 7.6 µm and the average conidia width was 3.6 µm. The second isolate had cylindrical conidia formed in a chain, which is characteristic in *M. anisopliae*. The conidia average length was 7 µm and the average conidia width was 3.6 µm as indicated in the table1. Preliminary confirmation of microscopic study revealed that the morphological appearance of the isolated fungi such as conidial shape and size of the spores were identical to those of *M. robertsii* and *M. anisopliae*. To confirm the identity of the *M. robertsii* and *M. anisopliae*, the partial sequence (550 fragments) of the ITS4-ITS5 gene was sequenced and its identify compared with representative valid sequences of *Metarhizium* isolates (Fig. I). According to phylogenetic analysis, the new *Metarhizium* isolates made a single clade along with other isolates of *M. robertsii* and *M. anisopliae* with high bootstrap. The Afghan strains of *Metarhizum* had the lowest genetic distance with those of other isolates of *M. robertsii* and *M. anisopliae.* The partial DNA sequence was deposited in the genebank.

EFFECT OF MEDIA ON FUNGAL GROWTH

In the present study, several naturally available substrates of solid media were tested for mass production of *B. bassiana* and *M. robertsii.* Those isolates were grown under standard conditions on different solid substrates and harvested after 18 days. There were significant difference between substrates (F= 2061.04, df= (1, 20), $P<0.0001$), fungal species (F= 4706.74, df= (4, 20), $P<0.0001$) and the interaction between them (F= 655.93, df= (4, 20), $P<0.0001$). For *M. robertsii*, the broken rice substrate with 1.4×10^8 conidia/g showed maximum conidial production and the saw dust substrate with 1.8×10^7 conidia/g showed minimum conidial production (Fig. II).

For *B. bassiana* after 18 days, the maximum conidial production was obtained on the green gram and broken wheat at 1.4×10^8 and 1×10^8 conidia/g, respectively, and the minimum conidial production with 4×10^7 conidia/g was observed on saw dust (Fig. II).

PATHOGENICITY OF *M. ROBERTSII*, *B. BASSIANA* AND *B. VARROAE* UNDER LABORATORY CONDITIONS

Four different conidial concentrations (1×10^5, 1×10^6, 1×10^7 and 1×10^8 conidia/ml^{-1}) of *M. robertsii*, *B. bassiana*, and *B. varroae* were administrated for insecticidal activity against *A. vagans* in laboratory conditions. After application of each conidial concentration they cause few mortality so during the 24h, although termites were very weak and hardly moving. It is certain that *M. robertsii* was virulent and pathogenic to the termite *A. vagans;* the termite mortality increased with the indigenous newly identified Afghani species of *M. robertsii*. In the laboratory, all isolates caused significant different mortality rate (F=104.08, df= (2, 30), $p<0001$) in comparison to control groups. All concentration caused significant different mortality rate in comparison to each other's (F=28.98, df= (4, 30), $p<0.0001$). The interaction between fungal species and concentrations were significantly different (F=7.10, df= (8, 30), $p<0.0001$). Conidia from the *M. robertsii* were highly virulent for *A. vagans* causing approximately 100 % mortality 4 days after inoculation in the concentration of 1×10^8 conidia/ml^{-1}; other concentration respectively caused 100% mortality approximately 5-6 days post inoculation.

The significant effect of fungal filtrates, observation time intervals were recorded in laboratory assay for

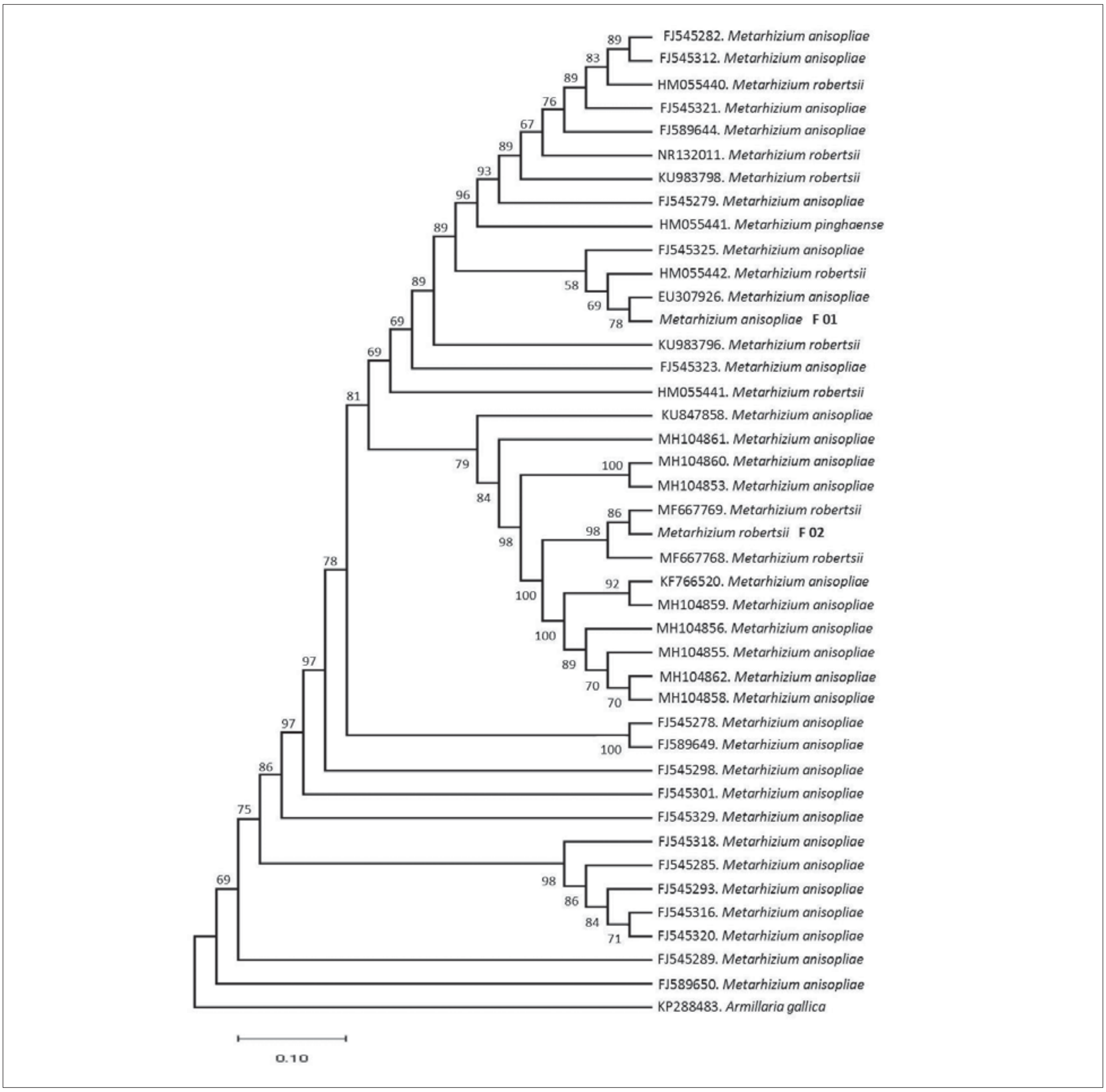

Fig. I – Phylogenetic analysis of new Afghan strains of *M. robertsii* and *M. anisopliae* and closely related species/strains based on ITS gene sequences using maximum likelihood.

concentration of 1×10^8 6 days of treatment of *B. bassiana*. Other concentration respectively caused 100% mortality approximately 7-10 days post inoculation. On the other hand, minimum mortality (so less than others) was recorded on 10th days by filtrate application of *B. varroae* (table 2). The results showed that indigenous *M. robertsii* was very virulent, it had significant highly mortality 4 day post inoculation (F=33.34, df= (4, 30), p<.0001), *B. bassiana* also significant effect 4 days post inoculation (F=9.47, df= (4, 30), p<.0001) and *B. varroa* was not significant effected 4 days post inoculation (F=.0.38, df=(4, 30), p<0.8246). *Metarhizium robertsii* and *B. bassiana* were selected for semi-field bioassay for their high pathogenicity.

PATHOGENICITY OF *M. ROBERTSII* AND *B. BASSIANA* UNDER SEMI-FIELD CONDITION

Metarhizium robertsii and *B. bassiana* were tested for insecticidal activity against *A. vagans* in semi-fields bioassays. There were significant mortality differences between both isolates (F= 64.22, df= (1, 20), p<0001) in comparison to control groups. Mortality of *A. vagans* at different doses of the selected *M. robertsii* and *B. bassiana* isolates was significantly different (F= 65.22, df= (4, 20), p<0001) and the interaction between fungal species and concentration were not significant different (F=7. 2.56, df= (4, 20), p<0.0706). The results showed that the Afghan *M. robertsii* was much virulent with highly mortality 5-day post inoculation (F= 45.72, df=(4,30), p<.0001), *B. bassiana* also with a significant effect 5 days post inoculation (F= 22.06, df=(4,30), p<.0001). In both species the higher concentration (1 ×10^8 conidia/ ml^{-1}) had achieved maximum mortality compared to 1 × 10^5, 1 × 10^6 and 1 × 10^7 conidia/ml^{-1} (Fig. III). Dead termite infected by entomopathogenic fungi would develop mycosis in five to six days after they were placed in wet condition. For the dead termites infected by *M. robertsii* they were covered by green conidia, while for *B. bassiana* the white conidia were fully grown on termite cadavers. In pathogenicity test,

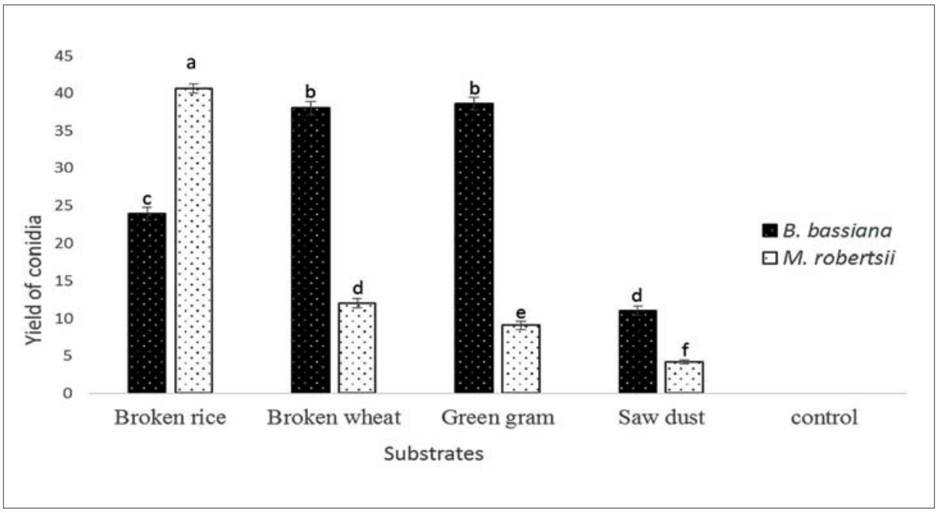

Fig. II – Mean number of conidia (± SE) produced by four solid substances (broken rice, broken wheat, green gram and saw dust) for *M. robertsii* and *B. bassiana.* Letters denotes significant differences between interactive fungal species and substance; Turkey's test; p<005.

Table 2 – Pathogenicity of three entomopathogenic fungi strains *Beauveria bassiana, B. varroae* and *Metarhizium robertsii* on termite *Anacanthotermes vagans* under laboratory condition.

Days after treatment	2	4	6	8	10
Fungal species [Concentration (conidia/ml-^{1})]	% Mortality (± SE)				
B. bassiana					
0.05% Tween-80	0.0 ± 0.0	0.0 ± 0.0	3.33 ± 3.3	6.7 ± 3.3	6.7 ± 3.3 ^{f}
1× 10^{5} conidia ml^{-1}	3.3 ± 3.3	13 ± 6.7	43.3 ± 6.7	63 ± 3.3	100 ± 0.0 ^{ef}
1× 10^{6}	3.3 ± 3.3	20 ± 5.7	63.2 ± 3.3	90 ± 5.7	100 ± 0.0 ^{efd}
1× 10^{7}	6.7 ± 3.3	23 ± 8.8	70 ± 11.5	100 ± 0.0	100 ± 0.0 ^{efd}
1× 10^{8}	13.4 ± 3.3	40 ± 5.7	100 ± 0.0	100 ± 0.0	100 ± 0.0 ^{bcd}
B. varroae					
0.05% Tween-80	0.0 ± 0.0	0.0 ± 0.0	3.3 ± 3.3	6.67 ± 3.3	6.67 ± 3.3 ^{f}
1× 10^{5} conidia ml^{-1}	3.3 ± 3.3	3.3 ± 3.3	3.3 ± 3.3	10.0 ± 3.3	23.3 ± 3.0 ^{f}
1× 10^{6}	3.3 ± 3.3	3.3 ± 3.3	6.7 ± 3.3	13.3 ± 3.3	26.7 ± 3.3 ^{f}
1× 10^{7}	3.3 ± 3.3	3.3 ± 3.3	10.0 ± 5.7	23.3 ± 3.3	30.0 ± 0.0 ^{f}
1× 10^{8}	0.0 ± 0.0	10.0 ± 3.3	20.0 ± 5.7	30.0 ± 7.7	40.0 ± 7.7 ^{ef}
M. robertsii					
0.05% Tween-80	5.0 ± 5.0	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0 ^{ef}
1× 10^{5} conidia ml^{-1}	10.0 ± 6.7	40 ± 10.0	100 ± 0.0	100 ± 0.0	100 ± 0.0 ^{ecd}
1× 10^{6}	13.3 ± 3.3	56 ± 10.0	100 ± 0.0	100 ± 0.0	100 ± 0.0 ^{bc}
1× 10^{7}	23.3 ± 8.8	76 ± 8.8	100 ± 0.0	100 ± 0.0	100 ± 0.0 ^{ab}
1× 10^{8}	53.3 ± 12	100 ± 8.8	100 ± 0.0	100 ± 0.0	100 ± 0.0 ^{a}

Insecticidal effects of fungal species against termite at four different conidial concentrations. Values expressed as mean of three replicates. Different upper case letter indicates the significant difference (F= 27.21, df= 30, P<0.0001) at Tukey HSD.

melanization spots were observed around the thoracic and abdominal segments after inoculation of conidial suspension. The fungal infection changed the color of the insect body with the progressive symptom of sluggishness (slow movement) when compared to control. The highest concentration (1 ×10^{8} conidia/ ml-^{1}), caused quick sporulation when exposed to individual termites.

DISCUSSION

In recent years, some studies have focused on developing fungal insecticides to control insect pest. Four to five hundred species of fungi have pathogenic effects on insects. For the basal fungi the largest numbers of EPF species occurs in the *phylum* Entomophthoromycota. Most species within this group are obligate entomopathogens, occurring in five of the six known families. These fungi are often highly specialized parasites that show distinct eco-morphological adaptation to the life cycles of their hosts, including the production of conidia (spore's production from asexual reproduction), discharged actively from host cadavers and timed to take advantage of favorable environmental condition, manipulation of host behavior, and the production of resting spores to survive adverse condition (Chandler, 2017). This fungal virulence mostly has been associated with intra and extracellular synthesis of different substances including cuticle-degrading enzyme, and low molecular weight toxic compounds (Mishra *et al.*, 2015; Luangsa-ard *et al.*, 2017). However, most of the

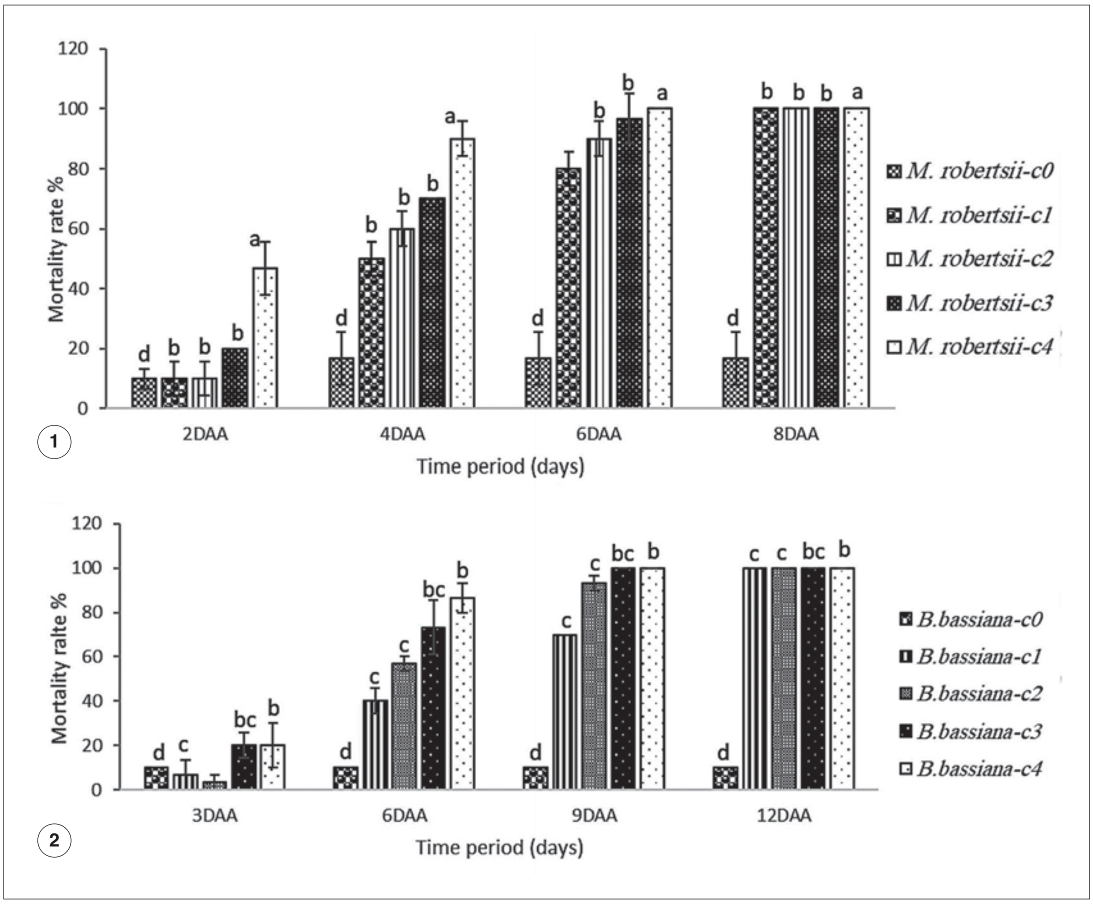

Fig. III – Virulence of difference concentrations (c0, c1, c2, c3, c4=concentrations) of *M. robertsii* (1) and *B. bassiana* (2) on the termite, *Anacanthotermes vagans* at 1-12 days (DAA: Days After Application). Bars indicate standard deviation of mean. Different letter indicates significant differences between effect of species and concentrations according Turkey's test.

literature has focused on low molecular Weight fungal metabolites (secondary metabolites), whereas the high-molecular Weight compounds, such as proteins, remain poorly studied (Keppanan *et al*., 2018a; Nishi *et al*., 2011). The host can be infected by direct treatment and by the transportation of inoculums from treated insects to untreated ones (Singha *et al*., 2011). In the present study, we identified indigenous isolates of *M. robertsii* and *M. anisopliae*. Identification based on molecular and morphological species identification of the fungal isolates were confirmed by phylogenetic analysis (Fig.1). From two indigenous isolates which were identified, only *M. robertsii* was applied with *B. bassiana* and *B. varroae* as a biological agent to replace chemical insecticides for control of *A. vagans* in laboratory. *M. robertsii,* with *B. bassiana* were tested against *A. vagans* under semi-field conditions. In laboratory, all isolates caused different mortality rate in comparison to control groups (Habtegebriel *et al*., 2016). All isolates caused different mortality rate in comparison to each other's.

The highest mortality rate was obtained by indigenous isolate *M. robertsii,* more than 95%, the second mortality obtained by *B. bassiana* and the lowest mortality recorded by *B. varroae,* confirming the data obtained by Singha *et al*. (2011) with the termite *Microtermes obesi* Holmgren. While others have reported the fungal effect of termites under laboratory conditions, this assessment shows that these fungi have also effect under semi-field condition. In semi-field condition only *M. robertsii* and *B. bassiana* were applied and both isolates caused different mortality confirming what Azmi *et al*. (2011) reported for the termite *Globitermes sulphureus* Haviland. We examined conventional parameter used to produce conidia on different solid substrates, with optimized sporulation (Keppanan *et al*., 2018a; Lopes *et al*., 2018).

The *Galleria* bait method's, *i.e.* using *G. mellonella* for EPF recover from soils, was described by Zimmermann (1986). Since then it has been used quite often in numerous studies as the only method for EPF isolations, in the past three decades (Sharma *et al*., 2018). The result of this study showed that the isolate *M. robertsii* infected most of the *Galleria* larvae in insect baiting assay and the progression of external symptoms was found at 48h of post inoculation when compared to the other EPFs. This data suggest that soil is an important reservoir of entomopathogenic fungi, with potential effect for controlling insect pests (Keppanan *et al*., 2018b).

Entomopathogenic fungi can control insect pests and, as a result, have the potential to be used in the biological control system (Nazir *et al*., 2018; Groth *et al*., 2017; Oreste *et al*., 2016; Tarasco *et al*., 2016). They offer the advantages of no pollution, safety and avoiding pesticide resistance; and they can sustainably control insects during their reproductive phase, thereby reducing pest population and the risks of epidemics. However, in the long-term, the effectiveness of entomopathogenic fungi as biological agents can decline as a result of strain degradation, variation, rejuvenation, and contamination (Meng *et al*., 2017; Migiro *et al*., 2010).

Most of the basic studies in the literature have suggested that solids fermentation has emerged as an appropriate technology for the self-enhancement of fungal virulence and killing speed of the host (Keppanan *et al*., 2018b). Four different solid-substrates were used in the present study with the mass production of new isolate *M. robertsii* comparing an Iranian isolate of *B. bassiana* under *in vitro* conductions (Gurlek *et al*., 2018). We observed that the activities of endogenous isolate (*M. robertsii*) had a more potent effect on killing fast and quick sporulation on *A. vagans* insect cadaver. A time of killing of 4-6 days post inoculation in laboratory condition for *M. robertsii* and 6-10 for *B. bassiana* was observed. In semi-field condition the potential effect of killing 5-8 days post inoculation for *M. robertsii* and 8-12 days for *B. bassiana* was observed. Time of killing at four concentrations was different and quick mortality was seen at a concentration of 1×10^8.

ACKNOWLEDGMENTS

The work is part of first author MSc theis in Ferdowsi University of Mashhad which financially supported by a grant to JK which approved on July 10, 2018 as p3/47254. The authors appreciate from research deputy of Ferdowsi University of Mashhad as well assistance of Ms. Shokoofeh Kamali, Dr. Reyhaneh Darsouei and Javad Al-Zidawi.

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REDIA, 102, 2019: 171-176 http://dx.doi.org/10.19263/REDIA-102.19.24

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SAPERDA TRIDENTATA OLIVIER (COLEOPTERA CERAMBYCIDAE LAMIINAE): CONTINUOUS INTERCEPTIONS AT THE ITALIAN PORT OF LIVORNO REPRESENT A GROWING CHALLENGE FOR PHYTOSANITARY SERVICES

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Binazzi F., Del Nista D., Sabbatini Peverieri G., Marianelli L., Roversi P.F., Pennacchio F. – *Saperda tridentata* Olivier (Coleoptera Cerambycidae Lamiinae): continuous interceptions at the Italian port of Livorno represent a growing challenge for phytosanitary services.

Trade of plant material poses a potential risk for accidental introductions of alien pests. Despite prevention and control measures, many exotic species have been unintentionally introduced into the Italian peninsula in the last decades. From April 2016 to March 2019, infested wood material from several areas of the USA was intercepted by phytosanitary authorities at the port of Livorno (Tuscany, Italy). The longhorn beetle *Saperda tridentata* was detected in six shipments containing heavily infested elm logs (*Ulmus rubra)* with bark. All the interceptions were made thanks to a national monitoring network including the Italian Ministry of Agriculture and Forests (MIPAAF), the Regional Phytosanitary Services, the Research Centre for Plant Protection and Certification (CREA-DC) and other Italian scientific institutions. Our findings suggest that monitoring of timber shipments should be further developed not only at the entry ports but also, and particularly, in the countries of origin. Moreover, a shift in phytosanitary policies such as introducing mandatory wood treatments (e.g. debarking, fumigation or heat treatments) prior to shipment would be advisable as it could drastically reduce introductions of alien pests and streamline all inspection procedures.

KEY WORDS: exotic species, timber trade, monitoring activities, pest risk assessment, alien species.

INTRODUCTION

Timber trade and the widespread use of wood as packaging material represent a potential risk for accidental introductions of xylophagous beetles belonging mainly to the families Cerambycidae, Buprestidae and Curculionidae (CAVEY, 1998; HAACK, 2001, 2006; HAACK *et al*., 2010; LIEBHOLD *et al*., 1995; MCCULLOUGH *et al*., 2006; AUKEMA *et al*., 2010, HAACK & RABAGLIA, 2013; PENNACCHIO *et al*., 2003, 2004, 2012a, 2012b, 2016; RASSATI *et al*., 2017; STRANGI *et al*, 2017; MEURISSE *et al*., 2019; TORRINI *et al*., 2019).

In the last decades, despite prevention and control measures, many alien species have been unintentionally introduced into the Italian peninsula. Some of them often spread and established in new territories with negative consequences for agriculture and biodiversity conservation (INGHILESI *et al*., 2013, PENNACCHIO *et al*., 2012a; HERARD *et al*., 2009; BULLAS-APPLETON *et al*., 2014; TORRINI *et al*., 2016; MAZZA *et al*., 2017). Nonetheless, national control networks recently established in high-risk areas such as airports, customs, ports and nurseries allowed the detection of hundreds of xylophagous species, belonging mainly to the families Cerambycidae, Buprestidae, Bostrichidae and Curculionidae (particularly Scolytinae). In particular, thanks to the monitoring activities implemented at the Italian port of Livorno, several interceptions of infested wood material were made in recent years. Among them, the longhorn beetle *Saperda tridentata* Olivier (Cerambycidae, Lamiinae) has been continuously recorded in elm wood shipped from the USA since the spring of 2016. The adults of this woodborer feed on elm leaves and twigs while larvae colonize trunks forming extensive galleries under tree bark. In all inspections, logs with bark resulted heavily colonized by mature larvae of *S. tridentata* often associated with bark beetles (Scolytinae) at various development stages.

MATERIALS AND METHODS

Inspection methods performed by phytosanitary services may vary according to context and type of shipped material. During the inspections of elm wood the following standard procedure was carried out: Metal containers were opened at the port in special facilities and phytosanitary authorities examined the entire content. Since insects are attracted to light, particular attention was paid to the potential presence of live adults able to escape through the openings. If free-walking insects or any flying activity were observed, containers were immediately closed and all wood material subjected to fumigation and/or thermal treatments before performing further investigations. If no potentially dangerous insect activity was detected, all items including packaging material and/or plastic envelop or other synthetic elements used to wrap wood pallets were screened for the presence of live organisms. Pavement and all the inner frame of containers were also carefully inspected.

All inspections were carried out using specific cutting tools (e.g. hatchets, knives, gouges, chisels), inspection

– Received 23 September 2019 Accepted 20 November 2019

tools (e.g. magnifying glasses, mirrors, electric torches) and other electronic devices such as portable field USB-microscopes. All live organisms and more generally all suspected material including wood pieces, sawdust, frass and any type of debris were collected from logs and/or wood items and placed into tubes or metal containers for further laboratory evaluations. Moreover, all debris including earth, sand, sawdust and frass scattered on the container pavement was equally collected, stored with the same procedure and finally screened in the laboratory for potential biological material. When logs with bark were inspected, a special focus was placed on detecting holes and insect galleries. In fact, their presence may indicate infestations due to bark beetles, ambrosia beetles and other woodborers. Moreover, when holes and galleries on the inner wood appeared dark and/or clearly surrounded by abundant darkish frass, particular care was taken in the inspections. In fact these characteristics might be effective indicators of the presence of insect-associated phytoparasitic nematodes or fungi such as *Ambrosia* spp. (Fig. I, 1, 2, 3).

All collected material was then placed into robust plastic bags and stored in climatized rooms or refrigerators. Subsequently, material was observed in the laboratory with a stereomicroscope equipped with a cold light source. Bark and wood material were thoroughly inspected to detect the presence of insect larvae, pupae and adult stages. When insects were found, specimens were morphologically identified with keys and/or stored in 96% ethanol fur further molecular evaluation (Pennacchio *et al*., 2012b; Sabbatini Peverieri G., 2012). All waste material useless for the identification was autoclaved and then discarded.

RESULTS

From April 2016 to March 2019, the abovementioned procedures allowed phytosanitary authorities at the port of Livorno (Tuscany, Italy) to inspect six shipments containing wood material from several areas of the USA. Once containers were opened, no flying activity was detected but several logs of *Ulmus rubra* presented on the outer bark clear symptoms of insect infestations such as penetration holes and/or frass due to intense activity of xylophagous species.

Subsequent wood debarking evidenced the presence of insect pupae and larvae at various development stages, with particular abundance of *S. tridentata* mature larvae. In the same infested logs, larvae, pupae and adults of the scolytid *Scolytus multistriatus* Marsham coexisting with *S. tridentata* were also observed (Tab. 1) (EUROPHYT, 2019). Pupae and several mature larvae of both *S. tridentata* and

Fig. I – 1. Detail of a *Saperda tridentata* mature larva; 2. Adult male of *Saperda tridentata*. 3. Inspection procedures of *Ulmus rubra* logs shipped in metal containers.

Table 1 – Shipments of *Ulmus rubra* logs infested with *Saperda tridentata* and *Scolytus multistriatus.*

Interception Dates	Country of origin	Quant. (m^3)	Intercepted Species	Development Stages
04/08/2016	USA, Ohio	3,8	*Saperda tridentata*	Larvae at various development stages
01/02/2017	USA, Virginia	7,8	*Saperda tridentata*	Larvae at various development stages
08/05/2017	USA, Illinois	18,2	*Saperda tridentata*	Larvae at various development stages
11/10/2017	USA, Iowa	18,3	*Saperda tridentata*	Larvae at various development stages
23/10/2018	USA, Ohio	18,8	*Saperda tridentata, Scolytus multistriatus*	*S. tridentata* larvae at various development stages; larvae and adults of *S. multistriatus*
27/03/2019	USA, Iowa	19,6	*Saperda tridentata, Scolytus multistriatus*	*S. tridentata* larvae at various development stages; larvae and adults of *S. multistriatus*

S. multistriatus were reared in climatic chambers set at 26 ± 2 °C and 75 % RH until adult emergence.

BIOLOGY

The first account on the biology of the Lamiid, *S. tridentata* on elm in southeastern New York dates back to the first half of the 20th century (Pechuman, 1940).

As the vast majority of saperdini, *S. tridentata* has an annual life cycle in temperate climates. Adult males are blackish fading to reddish, subparallel and 10-14 mm long, with antennae almost as long as the body. A dense grey pubescence with three orange oblique crossbars covers the elytra while narrow stripes are visible on each side of pronotum and base of the elytra. Adult females are similar to males but they are more robust and slightly longer (13 to 17 mm) (Fig. I, 2). Adult emergence starts at the beginning of the spring (early May) and continues until the mid of July, with peaks in the last week of May and in the first of June. This pest has an unbiased sex ratio with males emerging earlier than females (Pechuman, 1940; Linsley & Chemsak, 1995; Eppo, 2019). Adults are active mainly at night while during the day they rest on both tree bark and foliage. Mating occurs one or two weeks after the emergence and females, who can live up to 1 month in the field, start oviposition only after feeding on leaves and twigs. Each female can lay up to 50-60 eggs, which are deposited singly into incisions made with mandibles in bark crevices (Hoffmann, 1939; Pechuman, 1940).

After hatching, larvae penetrate into the phloem where they form extensive feeding galleries. In already weakened plants larvae may migrate from already colonized parts to healthy ones often causing death of the upper part of tree canopies. Mature larvae are white-yellowish, limbless and approximately 30 mm long even though their size may vary considerably depending on food sources and other environmental factors (Fig. I, 1). In fresh wood, larvae are generally active in the space between the inner bark and the external layer of the sapwood. From August to October, they start to dig chambers for overwintering and at the end of the winter they penetrate deeper to form pupal cells (Pechuman, 1940; Eppo, 2019). These structures are usually constructed 5-6 mm below the sapwood surface or in thick bark and are plugged with frass. Pupal stage develops typically between March and April and may last from 15 to 33 days (Pechuman, 1940; Eppo, 2019). Newly formed adults often remain in the pupal cells for about a week before emerging through the larval tunnel or digging their way out. Emergence is generally affected by temperature and insects tend to follow the shortest route for reaching the surface. However, in case of sawed wood, adults have been sometimes observed to emerge at the end of logs depending on the position of pupal chambers.

In its native range, the life cycle of *S. tridentata* is highly variable even among individuals originating from the same group of eggs. The entire development period is usually one year but in some circumstances it was reported to be up to more than three years depending on temperature and moisture content in the wood (Hoffmann, 1939; Pechuman, 1940; Eppo, 2019).

DISTRIBUTION

The genus *Saperda* Fabricius, 1775 includes the following 8 species in continental Europe: *Saperda carcharias* (L., 1758), *S. similis* Laicharting, 1784*, S. punctata* (L., 1767)*, S populnea* (L., 1758)*, S. scalaris* (L., 1758)*, S. octopunctata* (Scopoli, 1772)*, S. perforata* (Pallas, 1773)*, S. quercus* Charpentier, 1825. All of them have been recorded in Italy except for *S. quercus*.

Conversely, *S. tridentata* is native to central Eastern North America and its range is wide, extending approximately from southeastern Canada, to Texas (Salomon, 1995; Boberg & Björklund, 2018). In particular, records have been reported from the following North American states: in Canada (Manitoba, New Brunswick, Nova Scotia, Ontario, Québec, Saskatchewan) and in the USA (Alabama, Arkansas, Colorado, Connecticut, Florida, Georgia, Idaho, Illinois, Indiana, Iowa, Kansas, Kentucky, Maine, Maryland, Massachusetts, Michigan, Minnesota, Mississippi, Missouri, Montana, Nebraska, New Hampshire, New Jersey, New York, North Carolina, North Dakota, Ohio, Oklahoma, Pennsylvania, Rhode Island, South Carolina, South Dakota, Tennessee, Texas, Vermont, West Virginia, Wisconsin) (Eppo, 2019). It is worth noting that this species has been frequently observed in the northeastern states of United States (e.g. New York and Massachusetts) and that no records have been reported outside its native range so far (Salomon, 1995; Boberg & Björklund, 2018; Eppo, 2019).

HOST PLANTS AND POTENTIAL RISKS

Elms are emblematic and valuable trees highly prized for landscaping and wood production in all the EPPO countries. Regrettably, in the last decades, these plants have been gradually decimated by epidemics of Dutch elm disease (i.e. *Ophiostoma ulmi, O. novo-ulmi*) primarily vectored by *Scolytus* spp.

S. tridentata is associated with several North American *Ulmus* species. Its main host is *U. americana*, but infestations have been also observed on *U. rubra* and *U. crassifolia*. To date, there is very limited knowledge on the potential susceptibility of all the other North American elms (KRISCHIK & DAVIDSON, 2013). Equally controversial is the possible vulnerability of Palearctic elms located in the EPPO regions. Among the European elms, there is particular concern for the native *U. glabra*, *U. laevis*, *U. minor* and the introduced *U. pumila* (BOBERG & BJÖRKLUND, 2018). The latter one, native to East Asia, is resistant to Dutch elm disease and has been widely used for landscaping in countryside areas or as a street tree in urban environments.

S. tridentata, though normally regarded as a secondary pest of weakened trees in North America, may become lethal for elms in case of high infestation levels (FELT & JOUTEL, 1904; TUCKER, 1907; HOFFMANN, 1939; PECHUMAN, 1940; EPPO, 2019). Recently-cut logs and weakened trees suffering from root injury, diseases, water deficiency or other stress factors are usually the first choice for ovipositing females even though attacks have been sometimes reported also on apparently healthy plants (HOFFMANN, 1939; PECHUMAN, 1940).

The first symptom of *S. tridentata* attack in apparently vigorous trees is the appearance of a generalized yellowing and thinning of foliage followed by dieback and death of some branches (often scattered over the tree). At the beginning of insect colonization, reddish frass extruded from tiny openings in bark crevices, can be observed on either tree trunks or branches. As the infestation progresses, bark tends to lose adherence to wood until large pieces can be easily stripped from the main trunk and widespread larval galleries can be detected in the inner bark (PECHUMAN, 1940; EPPO, 2019). During the peak of infestation, larvae boring galleries may gradually girdle elm branches or trunks ultimately causing tree death (HOFFMANN, 1939; PECHUMAN, 1940).

Unlike other longhorn beetles such as *Trichoferus* spp. or *Clytus* spp., which preferentially exploit dehydrated material and repeatedly colonize the same wood portions for more generations, *S. tridentata* is more attracted to fresh non-colonized areas of already attacked trees. Since weakened but still fully hydrated elms are so attractive, larvae of *S. tridentata* and occasionally *Scolytus multistriatus* rapidly exploit the suitable wood depleting all the available food sources.

Elms already declining due to infestations of *Ophiostoma ulmi* or *O. novo-ulmi* are particularly exposed to *S. tridentata* attack and may be gradually colonized by this pest as Dutch Elm disease progresses. Furthermore, since *S. tridentata* has been also reported as a vector of *Ophiostoma ulmi*, European elms might be threatened not only directly by this longhorn beetle but also indirectly by vector-borne infections (EPPO, 2019). This scenario represents thus a further matter of concern, as this beetle, once introduced into Europe, might not only extensively damage elms already weakened by Dutch elm disease but also contribute to the spread of the disease itself. Therefore, in 2019 this beetle was included in the EPPO alert list.

PHYTOSANITARY MEASURES AND POLICIES

Biological invasions have been often reported to have negative impacts on newly colonized environments with important phytosanitary implications. In some cases, though, damages to crop production and natural ecosystems due to accidentally introduced species could be theoretically predicted based on previous knowledge on their biology, behaviour and ecology in the native ranges. Check lists of organisms potentially harmful to newly invaded ecosystems can be generated in order to improve long-term monitoring of high-risk areas particularly exposed to possible introduction of dangerous species such as alien phytophagous insects (Annex I Part A, of the COUNCIL DIRECTIVE 2000/29/EC, lists A1, A2 e Alert list EPPO).

It should be stressed anyway that it is often complex to evaluate a priori the potential threat posed by invasive organisms in new environments (SABBATINI PEVERIERI *et al*., 2017). In fact, there is growing evidence that even species apparently harmless in their native range might turn into more aggressive pests once established in new ecosystems (EL KHOURY *et al*., 2019).

In the last decades, the accidental introduction of several alien xylophagous insects evidenced the potentially negative impact of global trade in logs, woody plants, packaging material and wood pallets on newly colonized environments. Among the alien organisms recently introduced into Italy, *Anoplophora chinensis* Thomson, *A. glabripennis* (Motschulsky), *Aromia bungii* (Faldermann), *Psacothea hilaris* (Pascoe) (Coleoptera Cerambycidae), *Xylosandrus germanus* (Blandford), *X. crassiusculus* Motschulsky, *X. compactus* (Eichhoff), *Phloeotribus liminaris* (Harris), *Pityophthorus juglandis* Blackman, *Ambrosiodmus rubricollis* (Eichhoff), *Xyleborus atratus* Eichhoff, *Hypocryphalus scabricollis* (Eichhoff) (Coleloptera Curculionidae Scolytinae), *Megaplatypus mutatus* (Chapuis) (Coleloptera Curculionidae Platypodinae) and *Aclees* sp. (Coleoptera Curculionidae) deserve particular attention in terms of phytosanitary risk. Equally noteworthy for their potential phytosanitary implications are the interceptions of *Phryneta leprosa* (Fabricius) and *Coptops aedificator* (Fabricius) (Coleoptera, Cerambycidae Lamiinae) under the bark of *Milicia excelsa* (Welw.) C.C.Berg (Iroko) trunks shipped from Cameroon and the interceptions of *Tricoferus campestris* (Faldermann) captured by traps located in the port of Napoli and likely vectored through packaging material (PENNACCHIO *et al*., 2003; PENNACCHIO *et al*., 2004; SABBATINI PEVERIERI *et al*., 2012a; PENNACCHIO *et al*., 2012b; PENNACCHIO *et al*., 2016; STRANGI *et al*., 2017; FRANCARDI *et al*., 2017)

Several of the abovementioned species resulted harmful in newly colonized areas, even though they had never been officially listed as noxious organisms before their introductions (e.g. *Xylosandrus crassiusculus*, *X. compactus*, *Pityophthorus juglandis*) or before their repeated interceptions (e.g. *Saperda tridentate*). Likewise, some species evidently harmful to *Ficus carica* such as *Aclees* sp. *Psacothea hilaris* and *Hypocryphalus scabricollis,* have never been included in any phytosanitary alert list so far.

These records suggest that trade of wood with bark represents a potential risk factor for all EU countries. Recent literature further highlights that Mediterranean areas are particularly exposed to potential introduction and establishment of alien pests native to temperate but also tropical and subtropical regions (RASSATI *et al.,* 2016; EL KHOURY *et al*., 2019). Moreover, Italy for its peculiar geographical location and extreme variety of habitats (e.g. mountain environments, plains, costal areas) is probably the Mediterranean state most at risk to accidental pest introduction, dispersal and establishment (PENNACCHIO *et al*., 2016).

The frequent interceptions of *S. tridentata* are in line with the trend described above. In fact the main vectors of this pest are either infested wood traded with bark or packaging material that may host larvae, pupae and adults. In particular, shipments of recently cut fresh wood represent a highly efficient network of pest transfer and should deserve special care from phytosanitary authorities (Pennacchio *et al*., 2016).

In its native range, *S. tridentata* is rarely a matter of concern and phytosanitary measures against this beetle are focused mainly on silvicultural practices aimed at maintaining trees vigorous such as removal and burning of severely infested trees to decrease insect populations (Hoffmann, 1939).

On the other hand, in Europe, regular trade of infested elms and the capacity of *S. tridentata* to transmit Dutch elm disease might represent a risk in case this beetle should be introduced into Italy. Nonetheless, there is still controversy on the actual susceptibility of elm species in the EPPO regions, whereas a general lack of information prevents a thorough evaluation of the economic impact of this pest even in its native range (Eppo, 2019).

Field experience and scientific literature suggest, though, that once alien species are established in a new ecosystem their complete eradication is often complex. Therefore, ideal phytosanitary measures should require accurate wood inspections aimed at diagnosing infestations due to xylophagous insects (e.g. presence of galleries and frass) or other dangerous organisms and/or diseases. Positive diagnosis should be then followed by complete wood debarking and/or other phytosanitary measures such thermal treatments or fumigation. Even more important, all inspections, diagnosis and eventual treatments should be performed in the countries of origin before shipments take place. That would not only benefit trade reducing the risk of infested material being refused at the entry ports, but would also protect the entire community and environment from potentially dangerous biological invasions (Pennacchio *et al*., 2012b, 2016).

Regrettably, according to the current regulations, most of broadleaved woody species shipped form North America, tropical and subtropical Asia, Africa and South America are not subjected to any debarking procedure nor to any standard phytosanitary treatment or compulsory inspections by phytosanitary authorities.

In accordance with Pennacchio *et al*., 2016 and Poland & Rassati, 2018, our findings underline the importance of implementing the active monitoring of areas highly exposed to accidental introductions of alien species and to improve the alert system designed to limit the spread of exotic pests in the Italian territory.

The abovementioned interceptions of *S. tridentata* were carried out thanks to a national monitoring network including MIPAAF, regional phytosanitary services, CREA and other Italian scientific institutions. Nonetheless, we believe that both prevention and monitoring activities should be further developed not only at the entry ports but also, and particularly, in the countries of origin. Moreover, we emphasize the importance of improving the compliance with the current international legislation concerning safety measures for packaging material, in agreement with the FAO requirements (ISPM No. 15) (2013). At the same time, a shift in phytosanitary policies should be highly recommended, as field experience suggests that introducing mandatory wood debarking prior to shipment could drastically reduce introductions of alien pests and effectively streamline all inspection procedures.

ACKNOWLEDGMENTS

This work was carried out as part of the National Project “ASPROPRI” – “Activities for the protection of plants”, funded by MIPAAF (Italian Ministry of Agricultural Food and Forestry Policies, DM n. 28635, 2015/12/28) and the National Project “PROTEZPIANTE – “Activities for plant protection”, funded by MIPAAF (DM n. 34140, 2017/12/29)

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- A concise and informative title (in block capitals, with species in italic capitals)
- The affiliation(s) and address(es) of the author(s)
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Abstract

Please provide an abstract of 150 to 250 words. The abstract should not contain any undefined abbreviations or unspecified references.

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- Manuscripts should be submitted in Word.
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Abbreviations should be defined at first mention and used consistently thereafter.

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Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section before the reference list. The names of funding organizations should be written in full.

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Please always use internationally accepted signs and symbols for units (SI units).

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Cite references in the text as follows: (Ramarkers, 1983) or Ramarkers (1983); (Riom and Gerbinot, 1977) or Deubert & Rhode (1971); (Robertson *et al*., 1989)

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Wallwork J.A., 1967 – *Acari*. In: Soil Biology, Burgers A. & Raw F. Ed., Academic Press, London, New York, pp.365-395.

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