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THE SECOND INSTAR OF *BREVENNIA PULVERARIA* (NEWSTEAD, 1892)
(HEMIPTERA COCCOMORPHA PSEUDOCOCCIDAE) WITH NOTES
ON THE TAXONOMIC AFFINITIES OF THE SPECIES ⁽¹⁾

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Kalandyk-Kołodziejczyk M. – The second instar of *Brevennia pulveraria* (Newstead, 1892) (Hemiptera Coccoomorpha Pseudococcidae) with notes on the taxonomic affinities of the species.

The second instar of *Brevennia pulveraria* is described and illustrated. The characteristic features common to both the adult female and second-instar nymph are presented. The characters of immature instars may be useful for identification to species level. Notes on the taxonomic affinity of *B. pulveraria* are presented.

KEY WORDS: *Brevennia pulveraria*, second instar, Pseudococcidae.

INTRODUCTION

The genus *Brevennia* Goux, 1940 comprises 8 species. Six of them occur only in Asia or in Europe and Asia, and only *Brevennia filicita* (De Lotto, 1967) occurs in South Africa, whereas *B. rehi* (Lindinger, 1943) is distributed worldwide and is considered pest of e.g. *Cynodon dactylon* L., *Dactyloctenium australe* Steud. (GARCÍA MORALES *et al.*, 2016).

The only representative of genus *Brevennia* found in Poland is *B. pulveraria* (Newstead, 1892), commonly called bluegrass mealybug or Goux mealybug (ŁAGOWSKA, 2004). This species belongs to group of species whose range is limited to two zoogeographical regions (Palearctic and Oriental) (ŁAGOWSKA, 2001); it has been reported also from France, Hungary, Pakistan, South Korea, Sri Lanka, Sweden, Taiwan, Ukraine and United Kingdom (GARCÍA MORALES *et al.*, 2016).

B. pulveraria has been recorded from several zoogeographical regions in Poland (e.g. Pieniny Mountains, Świętokrzyskie Mountains, Małopolska Upland and Lublin Upland) (KAWECKI, 1985; ŁAGOWSKA & GOLAN, 2002), but in spite of intensive research it has not been found in other regions e.g. Upper Silesia (SIMON & KALANDYK-KOŁODZIEJCZYK, 2011).

According to NEWSTEAD (1892), almost all individuals of *B. pulveraria* occurred on isolated plants growing in warm, dry environments; the infested plants did not present any external symptoms, such as swelling or decayed leaves, and the specimens could only be found by accurately checking all parts of the plant.

Brevennia pulveraria has been found between the stems and leaf sheaths of grasses e.g. *Agropyron* sp. and *Agrostis capillaris* L., also on the roots of *Dactylis* sp. (KOSZTARAB & KOZÁR, 1988). The species seems to tolerate a wide range of

environments, because in Poland it occurs inside the leaf sheaths of grasses in different types of forests, water-meadows, pastures, xerothermic and psammophilous grasslands, and ruderal plant communities (KOTEJA & ŻAK-OGAZA, 1989; ŁAGOWSKA & KOTEJA, 1996; GOLAN *et al.*, 2001). The specimens collected by NEWSTEAD (1892) were so heavily infested with Dipteran larvae that the author was able to find only a very few unparasitized individuals. *B. pulveraria* is parasitized also by species of Hymenoptera: Encyrtidae (KOSZTARAB & KOZÁR, 1988). The biology of *B. pulveraria* is not known (KOSZTARAB & KOZÁR, 1988).

The first descriptions of the adult female and immature instar given by NEWSTEAD (1892) (as *Ripersia pulveraria*), were rather brief and do not contain information on cuticular structures such as pores or ducts. Descriptions and illustrations of the adult female were given also by GOUX (1940); TEREZNIKOVA (1962; 1975); BEN-DOV & MATILE-FERRERO (1989); DANZIG & GAVRILOV-ZIMIN (2013; 2014); MILLER & MCKENZIE (1970) and TANG (1992). The first instar of *B. pulveraria* was illustrated and described by GOUX (1940) as *B. tetrapora*.

Brevennia pulveraria and *B. tetrapora* used to be considered separate species (e.g. KOSZTARAB & KOZÁR 1988; TANG, 1992). According to KOSZTARAB & KOZÁR (1988), *B. pulveraria* was characterized by possessing 3 or 4 pairs of cerarii; claws with a small denticles, and 6- or 7-segmented antennae, whereas characteristic features of *B. tetrapora* were 2 pairs of cerarii, claws without denticles and 6-segmented antennae. The latter species was reported only from France. According to TANG (1992) *B. pulveraria* possesses 5-ocular pores of two sizes, whereas *B. tetrapora* has 5-ocular pores of only one size.

However, DANZIG & GAVRILOV-ZIMIN (2013) recognized *B. tetrapora* as a junior synonym of *B. pulveraria*, so *B. pulveraria* is now the valid name (GARCÍA MORALES *et al.*, 2016).

Brevennia pulveraria has often been confused with *Heterococcus nudus* (Green, 1926) (KOSZTARAB & KOZÁR, 1988). MILLER & MCKENZIE (1970) included *Brevennia* in the genus *Heterococcus* FERRIS, 1918 but later, MILLER (1975) treated *Brevennia* as a distinct genus.

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In *Heterococcus*, 5-locular pores are abundant and trilobular pores are absent. The genus *Brevennia*, most closely related to *Heterococcus*, comprises species in which some trilobular pores remain near the spiracles (e.g. in the type species), near the spiracles and in the cerarii [in *B. lonicerae* (Borchsenius, 1948)], or near the spiracles, in the cerarii, and on the dorsum (DANZIG & GAVRILOV, 2013). Moreover, adult female *Heterococcus* possess two pairs of ostioles, whereas females of *Brevennia* have only the posterior pair of ostioles developed (MILLER, 1975).

The first instars of *Heterococcus* differ from those of *Brevennia* in possessing ostioles on both the abdomen and the head, claw usually with (rarely without) a denticle, and are devoid of trilobular pores. Crawlers of *Brevennia* have only abdominal ostioles, claw without a denticle, and swirled trilobular pores present (MILLER, 1975). The second and third female immature instars have not been described.

The purpose of this study was to describe and illustrate the second instar of *B. pulveraria*. This paper presents the first part of ongoing studies on the immature stages of mealybugs.

MATERIALS AND METHODS

The material studied consisted of four second instars of *B. pulveraria* collected and determined by Professor Jan Koteja and deposited in his collection located at the University of Silesia (Katowice, Poland).

The collection data is given in Table 1. The microscope slides contain specimens that are in rather bad condition. A compound light microscope (Olympus) with a micrometer eyepiece was used to take measurements and prepare illustrations. Body size (length and width in mm), and the length of antennal segments, leg segments, apical setae, and the width of anal ring were measured in μm . The morphological description below was based on two specimens. The poor condition of the specimens made it impossible to distinguish between female and male specimens. The method of description of immature instars was adopted from KAYDAN *et al.* (2015).

RESULTS

SYNONYMY

Brevennia pulveraria was described as *Ripersia pulveraria* by NEWSTEAD (1892). Subsequently it was incorporated into different genera as *Dactylopius pulverarius*, Newstead, 1903, *Trionymus pulverarius*, Goux, 1933, *Erium pulverarium*, Lindinger, 1935, *Heterococcus pulverarius*, Boratynski & Williams, 1964, and *Heterococcus tetraporus*, Miller & Mckenzie, 1970.

DESCRIPTION

Slide-mounted second instar (Fig. 1): body elongate, 0.60–0.72 mm long, 0.22–0.34 mm wide. Eye distinct, located on margin, 28.5–30.1 μm wide. Antenna 6-segmented, first segment 27.3–37.0 μm long, second 24.7–26.4 μm , third 19.1–17.7 μm , fourth 14.1–15.3, fifth 15.4–17.0 μm , and apical segment 49.2–51.6 μm long and 30.2–32.0 μm wide. Clypeolabral shield 102.8–103.3 μm long and 103.3–104.5 μm wide. Labium 52.7–60.7 μm long and 73.5–77.6 μm wide. Anterior spiracle 31.5 μm long, 14.6 μm wide across atrium; posterior spiracle 37.1–38.4 μm long, 12.7 μm wide across atrium. Circulus absent. Legs robust: hind trochanter + femur 114.8–118.0 μm long, hind tibia + tarsus 122.2–130.7 μm long, hind claw 17 μm long. Hind coxae enlarged. Only few translucent pores visible on hind femur and trochanter. Tarsal digitules setose; claw digitules capitate. Claw with small but distinct denticle. Only posterior ostioles developed, each with 1 or 2 5-locular pores on the lips. Anal ring 27.5 wide, with 6 setae. Anal lobes indistinct, bearing setae each 97.3–102.9 long.

Venter: body setae flagellate, the longest (18.7–22.5 μm long), situated in the middle of the head, others not very numerous and located mostly on the abdomen. Multilocular pores absent; 5-locular pores of two sizes (the larger 4.8–5 μm in diameter; the smaller 3.5–3.7 μm in diameter). Larger 5-locular pores numerous on head, in the proximity of antennae and on the body margin, also scattered on abdomen. Smaller 5-locular pores more numerous than the larger ones, present over entire ventral surface, forming transverse rows across abdominal segments and associated with spiracles. A few trilobular pores located near spiracles. Some simple disc pores situated near antennal bases. A few oral collar tubular ducts (each 7.4–8.0 μm long) present near body margin only.

Dorsum: three pairs of cerarii present, on margins of last 3 abdominal segments. Anal lobe cerarii each with 2 conical setae, 1 trilobular pore, 4 or 5 larger 5-locular pores and 1 auxiliary seta. Dorsal body setae spine-like, short (each 3.4–4.6 μm long). Larger 5-locular pores occurring in small numbers on margins of abdomen and on head. Smaller 5-locular pores scattered over entire surface, but not as numerous as on venter. Only few tubular ducts present, in middle of dorsum.

DISCUSSION

The current classification of the Coccoomorpha is mainly based on the morphology of the adult females, which is often highly derived and many morphological features are absent (COOK *et al.*, 2002; GULLAN & COOK, 2007). The immature stages are less studied, despite the presence of conserved morphological features, so they have the potential to resolve evolutionary relationships (COOK *et al.*, 2002). Often, structures such as ostioles, circuli, conical

Table 1 – Collection data of the specimens studied from Professor Jan Koteja's collection.

Species name	Host plant	Date of collection	Region of collection	Country
<i>Brevennia pulveraria</i>	<i>Agrostis</i> sp.	28.viii.1963	Nowotarska Valley	Poland
<i>Brevennia pulveraria</i>	Poaceae	-.ix. 1981	Świętokrzyskie Mountains	Poland
<i>Brevennia pulveraria</i>	Poaceae	14.viii.1986	Roztocze	Poland
<i>Brevennia pulveraria</i>	Poaceae	27.viii.1988	Roztocze	Poland

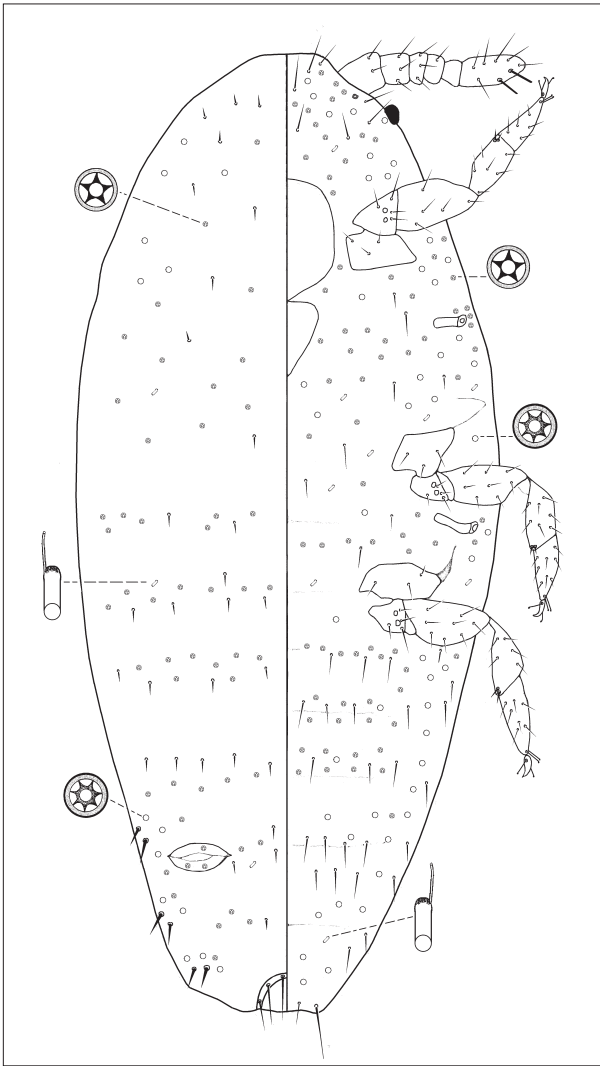


Fig. 1 – *Brevennia pulveraria* (Newstead), second instar, original.

setae and (more rarely) trilocular pores are present in the larvae, but absent in the imago and *vice versa*. In general, amongst descriptions of Palearctic mealybugs, information about the larvae is known from no more than 60 species, and in most of these cases only one of the larval stages has been described (DANZIG & GAVRILOV-ZIMIN, 2014). Recently some keys for the identification of immature instars of mealybugs have been published (e.g. GULLAN, 2000, KAYDAN *et al.*, 2015; WAN & WU, 2016).

The morphology of immature stages has proven helpful in determining relationships within the scale family Diaspididae (HOWELL & TIPPINS, 1990). Therefore, there is a need to adequately describe and illustrate the nymphs of more species in the Pseudococcidae to enable comparison of the immature stages of other genera/more species, resulting in interpretation of possible relationships (AHMAD *et al.*, 2013).

Immature instars possess some morphological features similar to adult females that can be useful in identification. Both adult females and second instars of *B. pulveraria* have only the posterior ostioles developed, and 5-locular pores of two sizes numerous on venter and less so on the dorsum. These characters are considered to be diagnostic features of *B. pulveraria* (MILLER, 1975; DANZIG & GAVRILOV-ZIMIN, 2013). Larger 5-locular pores are numerous on the ventral

body margin in adult and nymph. Each spiracle is associated with 2 or 3 trilocular pores and the smaller type of 5-locular pore. Neither the adult female nor the second-instar nymph possess a circulus. The number of cerarii may be different (from 3 to 4 pairs), due to infraspecific variability. The similarity of these features between the adult female and the second instar makes species identification possible, even if only second-instar nymphs are collected in the field.

Many authors (e.g. KOTEJA & ŽAK-OGAZA, 1983; TANG, 1992; KOTEJA, 1996; DANZIG & GAVRILOV-ZIMIN, 2013; 2014) assigned *Brevennia* to the subfamily Phenacoccinae similarly as in the case of related genus *Heterococcus*. Both genera possess 5-locular pores, and according to KOTEJA (1996) and DANZIG & GAVRILOV-ZIMIN (2013, 2014) quinelocular pores are characteristic of the subfamily Phenacoccinae (they are missing in the subfamily Pseudococcinae). HARDY *et al.* (2008) placed *Heterococcus* in the subfamily Phenacoccinae and the related genus *Brevennia* in the subfamily Pseudococcinae. According to HARDY *et al.* (2008), adult females of species of Phenacoccinae can be recognized by the following features (given in order of decreasing utility): (i) all tarsal digitules setose; (ii) claw with denticle; (iii) quinelocular pores present; and (iv) nine-segmented antennae; whereas adult females of species in the Pseudococcinae can be recognized by the following features: (i) tarsal digitules usually knobbed; (ii) claw without denticle, or, if a denticle is present, it is poorly developed and situated near the claw apex; (iii) quinelocular pores usually absent; and (iv) antennae usually with fewer than nine segments. As one can see, adult females of *Brevennia pulveraria* possess features characteristic for both subfamilies: 5-locular pores, tarsal digitules setose and claw with distinct denticle (characteristic of the Phenacoccinae) and antennae with fewer than nine segments (characteristic of the Pseudococcinae). It seems that further morphological studies and molecular analysis of adult females and immature stages of the genus *Brevennia* are needed to elucidate its taxonomic affinity.

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