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EVOLUTION OF SENSORY ANTENNAL STRUCTURES IN THE ENSIGN SCALE INSECTS
(HEMIPTERA COCCOMORPHA ORTHEZIIDAE) (¹)

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Kaydan M.B., Vea I.M., Szita É. – Evolution of sensory antennal structures in the ensign scale insects (Hemiptera Coccomorpha Ortheziidae).

The Ortheziidae (Hemiptera: Sternorrhyncha: Coccomorpha) are considered one of the most ancient families of Coccoidea. In this study, the antennal structures of species belonging to the Ortheziidae are examined and combined with data from a previous phylogenetic study as well as habitat preference data. Illustrations of the antennal structures (with a focus on the distribution of setae and specialized sensilla) for the type species of all genera in the Ortheziidae are provided. The three main ortheziid lineages can be recognized by their sensilla: (i) the Ortheziinae all possess *coeloconic sensilla*; (ii) the Newsteadiinae, which only includes *Newsteadia*, is characterized by a different type of *coeloconic sensilla*, here named *Newsteadia-type coeloconic sensilla*, and (iii) a clade that includes the Mixortheziinii, Nipponortheziinii and Ortheziolini, all possess only *basiconic sensilla*.

KEY WORDS: thermoreceptor, hygroreceptor, leaf litter, hypogean insect, habitat.

INTRODUCTION

The Ortheziidae, or ensign scale insects, are considered one of the most ancient families of Coccoidea (KOTEJA, 1986; VEA & GRIMALDI, 2012). Females are distinctive, possessing well-developed legs and antennae, and have much of the body cloaked in bundles of extravagant, white wax secretions, giving them a peculiarly ornate appearance. There are about 208 described species of Ortheziidae to date, classified within 21 genera (including four extinct genera) (VEA & GRIMALDI, 2012). Although a few species of ortheziids are serious plant pests, such as the greenhouse ensign scale (*Insignorthezia insignis* (Browne)), most occur sporadically in leaf litter (presumably feeding on roots and fungal mycelia), and some are assumed to feed on mosses and lichens, habitats that are putatively the most primitive in Coccoidea (KOTEJA, 1986; KOZÁR, 2004; VEA & GRIMALDI, 2012).

KOTEJA (1985), based on an intuitive analysis of morphological variation and habitat of recent scale insects, hypothesized that the evolution of Coccoidea occurred in two main ecological stages. The first occurred after the split from the “homopteran” stock (probably during the Carboniferous), scale insects first lived in the forest litter on a “mixed” diet, sucking out liquid from the surface and from living and decaying plant tissues. During this period of soil habitat, the leg became a digging organ (one claw, one-segmented tarsus, functional tibio-tarsal joint), females lost their wings and became paedomorphic and with reduced life stages; males became dipterous and poly-

morphic without functional mouthparts, and acquired a different life cycle (quiescent stages reminiscent of complete metamorphosis) that shortened that developmental time. These transformations probably led to a primary radiation creating numerous new families during the Permian or Triassic. The second stage of evolution started during the early evolution of flowering plants (Jurassic) and continued to the present and the evolutionary trends radically changed: coccoids became true plant parasites. Most of the groups started to climb on plants and each of them settled and integrated their own endosymbionts. The switch from soil litter to plant habitat took place during a long period of time and independently within various groups (KOTEJA, 1985); as a result, recent scale insects are extremely differentiated and some groups still continue the primary mode of life (hypogean insects such as some Margarodidae *sensu stricto*, Rhizoecidae and some members of Ortheziidae).

Insect antennae possess various types of specialized sensilla that can function as chemoreceptors, thermoreceptors and/or hygroreceptors (KEIL, 1999) and play an important role in orientation (SCHNEIDER, 1964). Their number and distribution on antennal segments are potentially significant taxonomically (LARINK, 1986) and phylogenetically (WALther, 1983). Two morphological types, namely *basiconic sensilla* (*bs*) and *coeloconic sensilla* (*cs*), are present in many insect groups and may be responsible for humidity and thermo-detection (SCHNEIDER, 1964). *Basiconic sensilla* (sensory pegs or cones) are trichoid sensilla without any specialized basal membrane. *Coeloconic sensilla* (sensory pit-peg) are thin walled cones on the floor of depressions in the antennal cuticle. In cross-section this structure appears as double-walled, with pores and peg attached to several neurons. Despite their obvious structural differences, KOTEJA (1980) suggested a homology

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between *bs* and *cs*, with *bs* probably being the primary type because they occur mostly in archaeococcoid families, and *cs* being a secondary transformation of the basiconec-type and occurring generally in neococcoids (KOTEJA, 1980). Nevertheless, *cs* are found within the archaeococcoid family Ortheziidae, more specifically in the genera *Orthezia*, *Arctorthelia* and *Newsteadia*, while *bs* have been identified in *Ortheziola* (KOTEJA, 1980).

The non-overlapping presence of the two main types of antennal sensilla and the variety of habitats within the Ortheziidae provide a good model to examine the evolution of these sensilla in relation to habitat. As such, this study aims to determine: (i) whether distribution of *bs* and *cs* across ortheziid genera, (ii) whether there is any association between the types of sensilla and the currently recognized generic concepts, (iii) whether these antennal characters affect the phylogenetic hypotheses for the major ortheziid clades proposed by VEA & GRIMALDI (2012), and (iv) whether there is an association between the sensilla and habitat of ortheziid species. We also provide illustrations of the antennal structures for the type-species of all genera within the family Ortheziidae, showing the distribution of setae and specialized sensilla on all the antennal segments.

MATERIAL AND METHODS

TAXA AND SPECIMENS EXAMINED

Here we provide illustrations of the antennae of the type species of each of the known genera of the family Ortheziidae. Several species were studied for the Newsteadiinae, even though it contains just one genus, because of variation in antennal segment number among species.

All examined specimens mounted on microscope slides that are deposited at the Hungarian Natural History Museum (HNHM), Budapest, Hungary, and the Plant Protection Institute (PPI), Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary. These collections were acquired mostly by the late Ferenc Kozár

PHYLOGENETIC ANALYSIS

Taxon sampling and morphological characters

The morphological matrix of VEA & GRIMALDI (2012) was used as the basis of our analysis. In VEA & GRIMALDI (2012), the character about the microsetae was defined and coded based on KOZÁR (2004) definition. However, this character refers to the *bs* of KOTEJA (1980). We therefore removed microsetal as a character from the original matrix and added the following new character:

Type of antennal sensilla. According to Koteja (1980), *bs* and *cs* are two types of the same *sensilla*, being located as they occur on the same parts of the antennae and their occurrence does not overlap across species. States: 0= thin-walled *cs* (Fig. I, 1, 2); 1= thick-walled *cs* (*Newsteadia* type) (Fig. I, 3); 2= *bs* (Fig. I, 4, 5).

Phylogenetic and ancestral reconstruction methods

The final morphological matrix of 47 taxa and 70 characters was analyzed using Maximum Parsimony (MP) as the optimality criterion, using TNT (GOLOBOFF *et al.*, 2008) (command lines: hold 10000; mult=tbr replic 10000 hold 100), with all characters treated as unordered and *Matsucoccus* set as the outgroup. Bootstrap supports were calculated with 10000 replicates (command line: resample boot replic 10000).

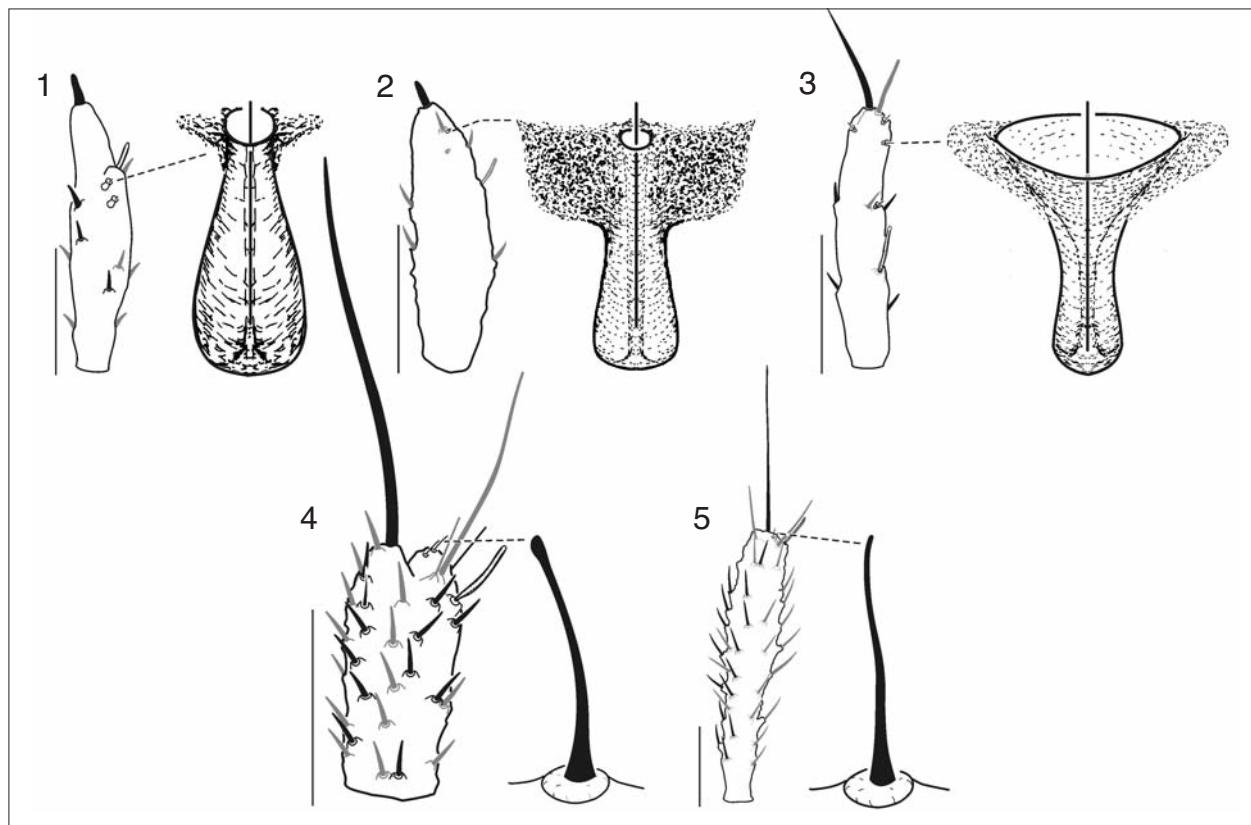


Fig. I – Sensilla types, 1, 2 -*Cococonic sensilla*, 3 – *Newsteadia*-type *cococonic sensilla*, 4 –*Basiconic sensilla* with capitate apex, 5 – *Basiconic sensilla* with a blunt apex. Black setae on ventral side, grey setae on dorsal side. Bar = 100 µm.

To examine any relationship between the type of antennal sensilla and habitat within the family, an ancestral reconstruction of character 70 was performed using the parsimony criterion in Mesquite version 3.01 (MADDISON & MADDISON, 2014) using a separate matrix (Matrix 2) from which habitat information based on KOZÁR (2004) was also coded as follows:

Aerial plant parts (0): species found feeding on stems and plant leaves.

Roots or ground dwelling (1): species found feeding on plant roots, or in leaf litter or on lichen (Character 70).

The new morphological matrix is available in Morphobank (O'LEARY & KAUFMAN, 2012) under Project 2181 (reviewer login at www.morphobank.com, login: 2181, password: Ortheziidae2015).

RESULTS

ORTHEZIIDAE

Description. Antennae 3 to 8 segmented; 7 or 8 segments in Ortheziinae, 3 in Ortheziolinae and Nipponortheziini, 4 in Mixortheziini and 3-7 in Newsteadiinae. Antennal bases separated from eyes except for Ortheziolinae. Setae spine-like, hair-like or clavate. Apical and subapical setae strongly

blunt or hair-like. Sensory pore (campaniform sensillum) dorsally on second segment (pedicel). Sensory fleshy setae on apical segment(s). Both *bs* and *cs* generally present on apical segment and pedicel ventrally.

ORTHEZIINAE

Description. Antennae 7 or 8 segmented. Setae spine like or hair-like. Apical seta short, strong, blunt; subapical setae hair-like. Sensory fleshy setae present on apical segments. *Cs* may be present on any segment ventrally. *Cs* thin, with hardly visible seta, inserted at bottom of invagination with narrow opening. *Bs* absent.

ARCTORTHEZIINI

Arctorthezia occidentalis (Douglas) (Fig. II, 1)

Material examined – 1 ♀, USA, New Mexico, Magdalena Mts., ex. Compositae, 15.ix.1994, leg. F. Kozár [4324 PPI]; 4 ♀♀, Canada, Ferry Creek, ex. moss, 7.vii.1988, leg. F. Kozár [3309 PPI].

Description – Antennae 7 or 8 (rarely 6) segmented; length of antennal segments: I 189-257, II 144-227, III 102-134, IV 64-123, V 61-113, VI 67, VII 70-102, and apical 176-250 µm long; all segments covered with 2-4 very robust, spine-like setae, longest spine 11-16 µm

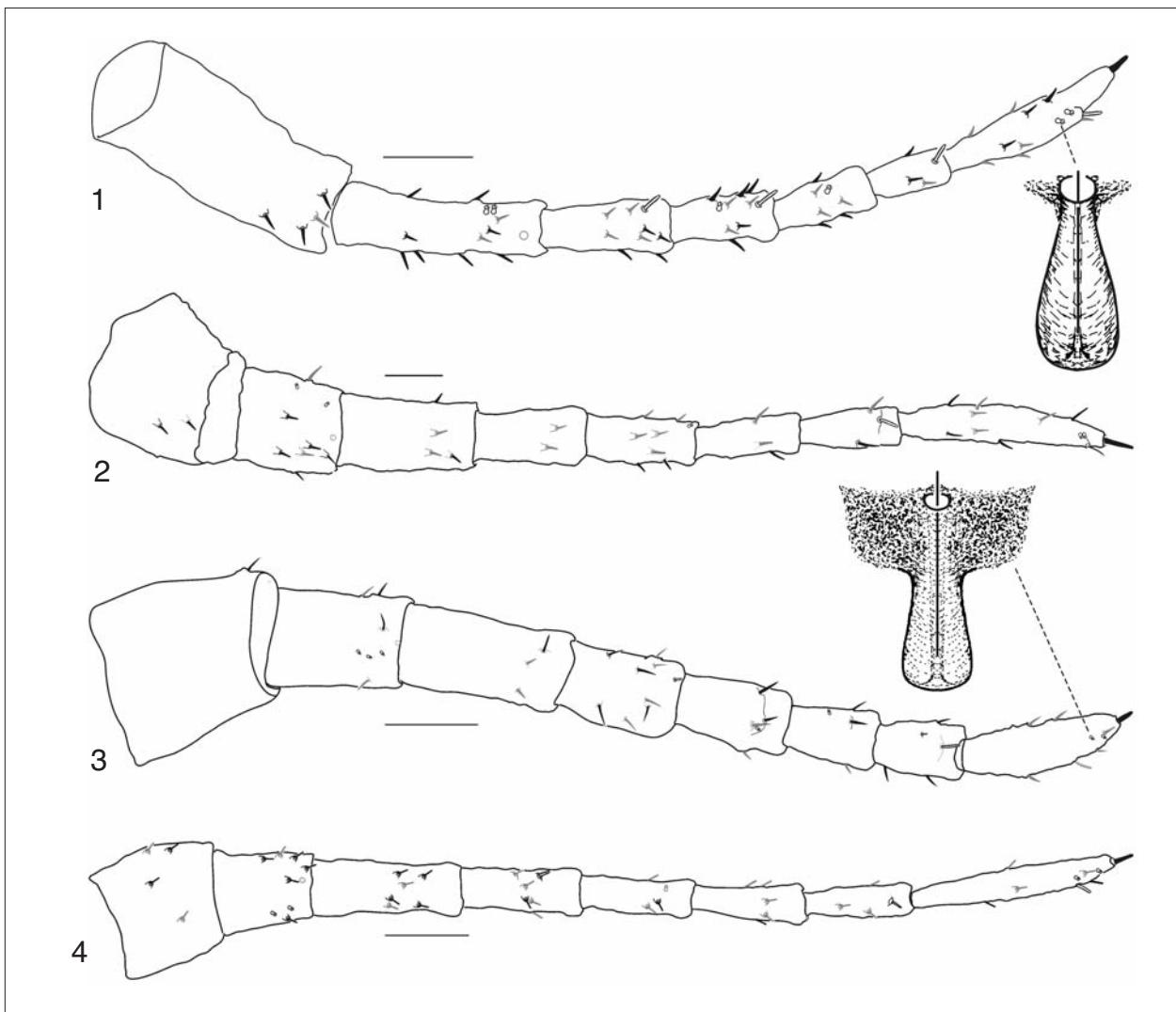


Fig. II – Coeloconic sensilla found in Ortheziinae. 1 - *Arctorthezia occidentalis* (Douglas), 2 - *Insignorthezia insignis* (Browne), 3 - *Orthezia urticae* (Linnaeus), 4 - *Praelongorthezia praelonga* (Douglas). Black setae on ventral side, grey setae on dorsal side. Bar = 100 µm.

long; apical spine of antennae thick and blunted, 11-20 μm long; subapical seta absent; sensory fleshy seta near apical seta and on preapical segment, 13-20 μm long each; 1 *cs* on segments III, IV, V, VI and 3 on apical segment. *Bs* absent.

ORTHEZIINI

Insignorthezia insignis (Browne) (Fig. II, 2)

Material examined – 1 ♀, South Africa, Nelspruit, Nat. Reserve, 25.x.1986, leg. Endrődy [4882 PPI]; 1 ♀, Tanzania, USA River, 1-25.iv.1965, leg. Szunyoghy [99 PPI]; 3 ♀, Tanzania, leg. unknown [634 PPI]; 1 ♀: Tanzania, 4.ii.1985, leg. Peregovits [590 PPI]; 3 ♀♀: USA, U.S. Dept. Agr., greenhouses, on *Coleus* sp., 21.i.1919, leg. N. Perrine; 1 ♀: Kenya, 26.iii.1978 [471 PPI].

Description – Antennae 7 or 8 (rarely 6) segmented; length of antennal segments: I 112-123, II 87-93, III 107-118, IV 97-103, V 102-115, VI 82-98, VII 77-87, and apical 154-180 μm long; all segments covered with 2-6 spine-like, straight setae, longest seta 6-10 μm long; apical seta 24-30 μm long; subapical seta absent; sensory fleshy seta on apical segment and on preapical segment, each 16-19 μm long; 1 or 2 *cs* on antennal segment II, 1 on V, 2 on apical segment. *Bs* absent.

Orthezia urticae (Linneaus) (Fig. II, 3)

Material examined – 1 ♀, Germany, Thuringia, Jena Distr., Pine forest near Zöllnitz, ex. *Melampyrum pratense*, 29.vii.1989, leg. G. Köhler; 2 ♀♀ Hungary, Csobánctető, ex. Umbelliferae, 22.viii.2002, leg. Kozár [6922 PPI]; 1 ♀, Hungary, leg. Kozár [4982 PPI]; 1 ♀, Hungary, Nagykovácsi, Nagyszénás Mt., ex. *Thesium* sp., 9.viii.2004, leg. B. Nagy [7068 PPI]; 1 ♀, Hungary, Szin, ex. *Dorycnium germanicum*, 30.vii.1987, leg. Kozár [3097 PPI]; 1 ♀, Switzerland, Romoos, Wiese, 13.v.1994, leg. Rézbányai [6147 PPI].

Description – Antennae 8 (rarely 6 or 7) segmented; length of antennal segments: I 160-175, II 144-185, III 188-237, IV 140-192, V 128-170, VI 108-144, VII 103-144, apical 185-237 μm long; all segments covered with 3-6 spine-like, straight setae; apical seta a strong blunt spine, 12-15 μm long; subapical seta absent; sensory fleshy seta present on apical and subapical segments, each 13-19 μm long, plus a smaller seta on segment IV; 2 or 3 *cs* on segment II, 1 on V and VI, 2 on apical segment. *Bs* absent.

Praelongorthezia praelonga (Douglas) (Fig. II, 4)

Material examined – 3 ♀♀, Congo, Brazzaville, ex. *Citrus* sp., 2.v.2008, leg. A. Kiyindou [8195 PPI].

Description – Antennae 8 (rarely 6 or 7) segmented; length of antennal segments: I 102-123, II 87-103, III 107-175, IV 92-129, V 102-123, VI 102-123, VII 97-113, and apical segment 206-237 μm long, all segments covered with 4-10 spine-like, straight setae, longest seta 7-12 μm long; apical seta short, blunt, 19-25 μm long; subapical seta absent; sensory fleshy seta present on apical and subapical segments and on segment IV, each 22-24 μm long; also 2 *cs* present on segment II, 1 on V, 2 on apical segment. *Bs* absent.

NEWSTEADIINAE NEWSTEADIINI

Description – Antennae 3 to 8 segmented. Setae on antennae spine-like or hair-like. Apical and subapical setae long hair-like. One sensory fleshy seta present on apical

segment. *Cs* structurally different from Ortheziinae: thick-walled, named here *Newsteadia*-type *cs*. Each sensilla with a thin seta, inserted at bottom of invaginations with wide thick-walled cone-shaped opening (Figures I, 3, III, 3), located on ventral side of antennae, with 2-4 on apical segment and on 1 or 2 on segment II, occasionally on segment III. *Bs* absent.

Newsteadia africana Miller & Kozár (Fig. III, 1)

Material examined – 1 ♀, Holotype, South Africa, Nelspruit Nature Reserve, litter, 18.xii.1986, leg. Y.S. Endrődy [4898 PPI]; 1 ♀, Paratype: South Africa, Kitsuk Forest St., 26.x.1968, leg. Y.S. Endrődy [4895 PPI]; 3 ♀♀, Paratypes: South Africa, Dweza Forest, 27.ii.1986, leg. Y.S. Endrődy [4897 PPI]; 1 ♀, Paratype: South Africa, Dweza Forest, 6.xii.1978, leg. Y.S. Endrődy [4833 PPI].

Description – Antennae 7 segmented; length of antennal segments: I 145-205, II 155-168, III 31-41, IV 31-41, V 36, VI 41, and apical 175-242 μm long; with 6-8 hair-like setae on segment I; 4 hair-like setae on segment II; without setae on segment III, IV, V, and VI; 6 hair-like setae present on apical segment, longest seta 24-31 μm long; apical seta hair-like, 100-118 μm long, subapical seta hair-like, 55-58 μm long; with 1 sensory fleshy seta on apical segment, 26-38 μm long; 1 *Newsteadia*-type *cs* present on segment II, 3 on apical segment. *Bs* absent.

Newsteadia caledoniensis Kozár & Konczné Benedixty (Fig. III, 3)

Material examined – 1 ♀ Holotype, 3 ♀♀ Paratypes: New Caledonia, Ile de Pius, litter, 18.v.1986, leg. J. Balogh [B.12 HNHM].

Description – Antennae 4 segmented; length of antennal segments: I 93-108, II 67-82, III 82-98, and apical 180-191 μm long, with 4-8 spine-like setae on all segments, longest setae 6 μm long; apical seta thick and blunt, 70-74 μm long, subapical seta absent, sensory fleshy seta on apical segment, 8 μm long; with 2 *Newsteadia*-type *cs* present on apical segment. *Bs* absent.

Newsteadia floccosa (De Geer) (Fig. III, 3)

Material examined – 1 ♀, Armenia, Dilizhan, 1600, 28.ix.1982, leg. O. Merkl [As 566 HNHM]; 1 ♀, Finland, Helsinki, ex. moss, 5.v.2005, leg. Pócs [E-1555 HNHM, 11039 PPI]; 1 ♀, Hungary, Estergom, Strázsza Mt., D-vac, 23.ix.2011, leg. É. Szita [10088 PPI]; 1 ♀, Hungary, Lébény, soil sample from horn beam (*Carpinus*) forest, 02-05.ix.1997, leg. E. Horváth [6265 PPI]; 1 ♀, Hungary, Körös-Maros National Park, Bányarét, ex. moss, 17-18.viii.1999, leg. Kozár [6020 PPI]; 1 ♀, Hungary, Nagykovácsi, Júlia-major, rocky grassland, 9-11.ii.2000, leg. F. Samu [6069 PPI]; 1 ♀, Romania, Oltenia, litter, 25.x-5.xi.2007, leg. J. Kentschán [10821/a PPI]; 1 ♀, Romania, Maramures Mts., 24.v.2007, leg. J. Kentschán [10922 PPI]; 1 ♀, Switzerland, Valais, s/Venayaz, 700 m a.s.l., 7.x.1980, leg. C. Besuchet [8864 PPI].

Description – Antennae 6 or 7 segmented, length of antennal segments: I 227-237, II 154-165, III 62-37, IV 41-51, V 46-51, VI 46-51, and apical 186-216 μm long; with 2-8 spine-like setae present on all segments, longest seta 12-13 μm long; apical seta 80-90 μm long; subapical seta 41-47 μm long; sensory fleshy seta present on apical segment, each 36-41 μm long; with 2 *Newsteadia*-type *cs* present on segment II, 1 on III, 4 on apical segment. *Bs* absent.

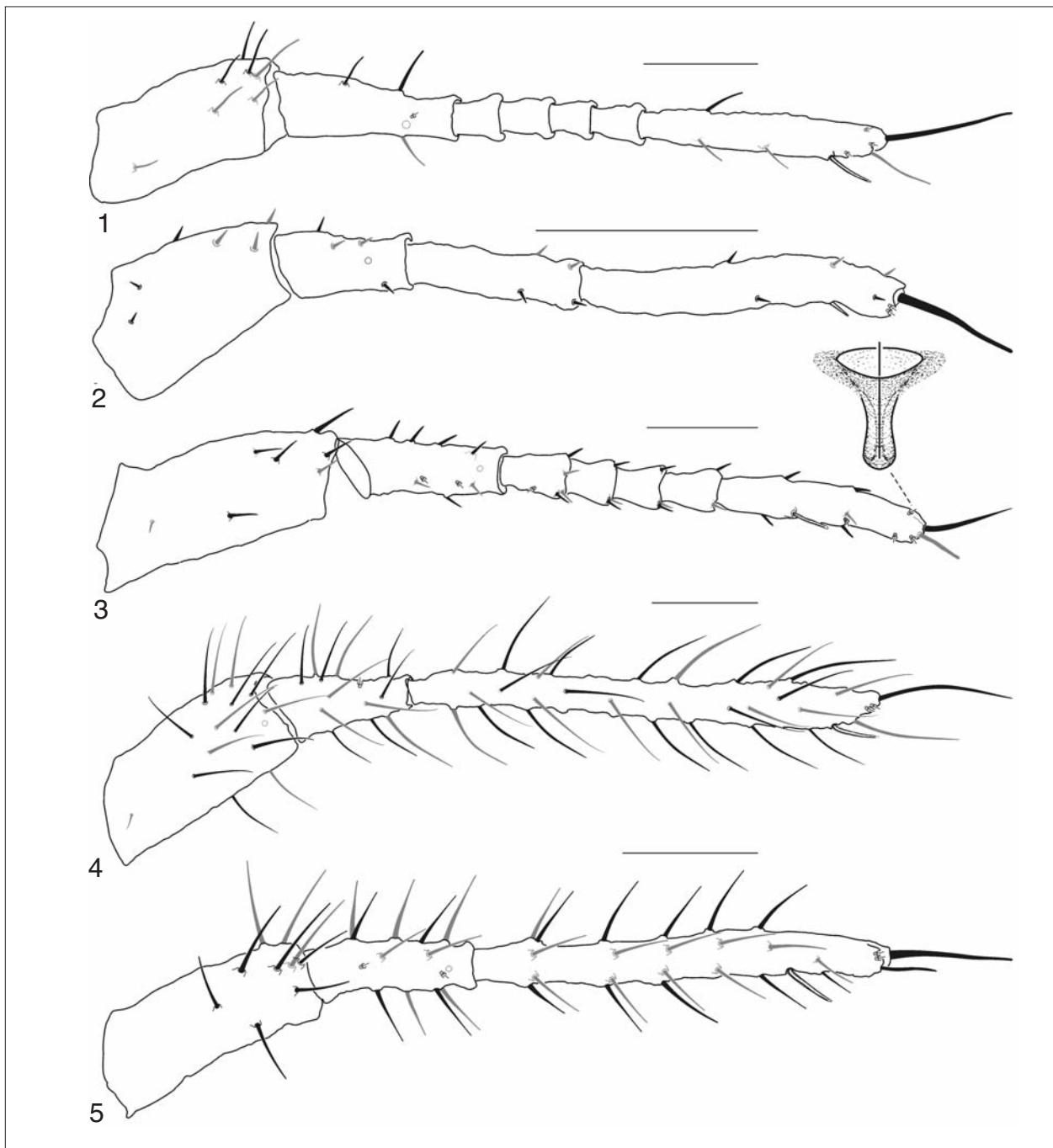


Fig. III – *Newsteadia* type *coeloconic sensilla* (*Newsteadiinae*). 1 - *Newsteadia africana* Miller & Kozár, 2 - *Newsteadia caledoniensis* Kozár & Konczné Benedicty, 3 - *Newsteadia floccosa* (De Geer), 4 - *Newsteadia monikae* Kozár & Konczné Benedicty, e - *Newsteadia morrisoni* Kozár & Konczné Benedicty. Black setae on ventral side, grey setae on dorsal side. Bar = 100 μ m.

***Newsteadia monikae* Kozár & Konczné Benedicty**
(Fig. III, 4)

Material examined – 1 ♀ Holotype, 3 ♀ Paratypes: New Caledonia, Nouméa, Mt. Koghi, 11.vii.1987, leg. J. Balogh [B.36 HNHM].

Description – Antennae 3 segmented, length of antennal segments: I 192-226, II 128-134, and apical 463-606 μ m long; with long hair-like setae present on all segments, 12 on segment I and II, more than 20 on apical segment, longest seta 80-92 μ m long; apical seta 96-108 μ m long, subapical seta 82-90 μ m long; sensory fleshy seta present on apical segment, each 37-50 μ m long; with 1

Newsteadia-type cs on segment II, 2 on apical segment. Bs absent.

***Newsteadia morrisoni* Kozár & Konczné Benedicty**
(Fig. III, 5)

Material examined – 1 ♀ Holotype, Costa Rica, Tarbaca, 1550, 14.i.1993, leg. J. Balogh [B98]; 8 ♀ Paratypes with the same data as Holotype [B98, B95, B96]; 7 ♀♀, Costa Rica, Sierra de la Muerte, 1800, 24.i.1993, leg. J. Balogh [B54, B55].

Description – Antennae 3 segmented; length of antennal segments: I 173-186, II 128-150, and apical 314-350 μ m

long; with long hair-like setae on all segments; with 10 setae on segment I and II and 19 setae on apical segment, longest seta 50-65 μm long; apical seta 99-106 μm long; subapical seta 43-49 μm long; sensory fleshy setae present on apical segment, each 41-51 μm long; 2 *Newsteadia*-type *cs* on II and 2 on apical segment. *Bs* absent.

NIPPONORTHEZIINAE

Description. Antennae 3 or 4 segmented. Setae on antennae spine-like or hair-like. Apical and subapical setae long and hair-like. With 1-4 sensory fleshy setae present on apical segment, sometimes also on subapical segment. *Bs* relatively thick, conical seta with a pointed or slightly clubbed apex (Figure I, 4), present ventrally, with 2 on apical segment and 1 or 2 on antennal segment II, occasionally also on segment I. *Cs* absent.

MIXORTHEZIINI

Description – Antennae 4-segmented. 2 *bs* present on apical segment, with a slightly capitate apex. 1 sensory fleshy seta present on apical segment and sometimes also on subapical segment. *Cs* absent.

Jermycoccus boliviensis Kozár & Konczné Benedicty (Fig. IV, 1)

Material examined. 1 ♀, Holotype, Bolivia, La Paz-P. Lineares, 14-16.xi.1971, leg. J. Balogh [B.13 HNHM].

Description. Antennae 4 segmented; length of antennal segments: I 51 μm , II 45 μm , III 38, and apical 94 μm long; with 4-8 thick seta on each segment; apical seta 90 μm long; subapical seta 43 μm long; with 1 sensory fleshy seta present on apical segment, 35 μm long; 2 *bs* present on apical segment each with a capitate apex. *Cs* absent.

Mixorthezia morrisoni Konczné Benedicty & Kozár (Fig. IV, 2)

Material examined – 1 ♀ Holotype: Cuba, Sierra de la Gran Piedra, 27.v.1979, leg. Pócs [D.Am.145 HNHM].

Description – Antennae 4 segmented; length of antennal segments: I 96, II 64, III 160, and apical 128 μm long, setae of antenna spine-like, longest setae 20 μm long; apical seta 192 μm long; subapical seta 112 μm long; with 1 sensory fleshy seta on apical segment, 29 μm long, plus a shorter seta on subapical segment; also with 2 *bs* with capitate apex and 2 hair-like sensory seta present on apical segment. *Cs* absent.

Neomixorthezia brasiliiana Konczné Benedicty & Kozár (Fig. IV, 3)

Material examined – 1 ♀ Holotype, 1 ♀ Paratype: Brasil, 1995, leg. J. Balogh [B.21 HNHM].

Description. Antennae 4 segmented; length of antennal segments: I 103-107, II 56-65, III 168-175, and apical 155-170 μm long; setae of antenna hair-like, each 23-30 μm long setae present on apical and subapical segments, and spines each 10 μm long on the first 2 segments; apical seta 156-168 μm long; subapical seta 103-132 μm long; with 1 sensory fleshy seta on apical segment, 32-36 μm long, 2 shorter setae present on subapical segment; with 2 *bs* with a capitate apex on apical segment. *Cs* absent.

NIPPONORTHEZIINI

Description – Antennae 3 or 4 segmented. Two *bs* with pointed apex present on apical segment and 0-2 on segment II, occasionally on basal segment. 1-4 sensory fleshy seta present on apical segment.

Neonipponorthezia regina Konczné Benedicty (Figure IV, 4)

Material examined – 1 ♀ Holotype, 1 ♀ Paratype, New Guinea, Wau, Nami Creek valley, ex. mosses, 15-19.viii.1968, leg. J. Balogh [B.13. HNHM].

Description – Antennae 3 segmented; length of antennal segments: I 128-144, II 176-185, and apical 336-360 μm long; setae on antennae strong, spine-like, each 26-30 μm long; apical seta 166 μm long; subapical seta 77 μm long; with 4 sensory fleshy seta present on apical segment, each 32-64 μm long; 2 *bs* with a pointed tip present on apical segment, absent from pedicel; 2 hair-like sensory seta on apical segment. *Cs* absent.

Nipponorthezia koreana Konczné Benedicty & Kozár (Fig. IV, 5)

Material examined – 1 ♀ Holotype, 3 ♀♀ Paratypes, Korea, Pyongyang city, Mt. Daesong-san, 11.ix.1979, leg. T. Vásárhelyi [As 437 HNHM].

Description – Antennae 3 segmented; length of antennal segments: I 82-92, II 102-122, and apical 250-275 μm long; setae of antenna strong and spine-like, each 12 μm long; apical seta 158-168 μm long, subapical seta absent; with 2 sensory fleshy seta on apical segment, each 26-41 μm long; 2 *bs* present on apical segment and on segment II and 1 on basal segment; 2 hair-like sensory setae each with slightly capitate apex on apical segment. *Cs* absent.

Nipponorthezinella guadalcanalia (Morrison) (Fig. IV, 6)

Material examined – 3 ♀♀, New Caledonia, Ile de Pius, leaf litter, 18-24.v.1987, leg. J. Balogh [B. 4-5 HNHM]; 2 ♀♀, New Caledonia, Farino, soil, 19.vii-25.viii.1982, leg. J. Balogh [82.B.2. HNHM]; 2 ♀♀, New Caledonia, Maré, on roots of epiphytons, 26.v-9.vi.1978, leg. J. Balogh [B.19. HNHM]; 2 ♀♀, Thailand, 1994, leg. S. Mahunka [As 712 HNHM]; 3 ♀♀, Seychelles, Cascade East, 10.xii.1975, leg. Fjellberg [431 PPI].

Description – Antennae 3 segmented; length of antennal segments: I 72-82, II 108-144, and apical 227-298 μm long; setae strongly curved and hair-like, each 25-30 μm long with a long cylindrical basal collar; apical seta 130-150 μm long; subapical setae 35-41 μm long; with 1 (rarely 2) sensory fleshy seta on apical segment, each 40-45 μm long; 2 *bs* present on apical segment and 3 on pedicel; 2 hair-like sensory seta present on apical segment. *Cs* absent.

Orthezinella hispanica Silvestri (Fig. IV, 7)

Material examined – 3 ♀♀: Spain, Algeciras, 03.i.1923, leg. F. Silvestri.

Description – Antennae 4 segmented; length of antennal segments: I 82-103, II 113-122, III 154-187 μm long, and apical 133-150 μm long; setae spine-like, each 20 μm long; apical seta 134-140 μm long; subapical seta 38-41 μm long; with 1 sensory fleshy seta on apical segment and 2 on subapical segment, each 31-48 μm long; 2 *bs* present on apical segment and 2 on segment II; 2 hair-like sensory seta on apical segment. *Cs* absent.

ORTHEZIOLINI

Description – Antennae 3 or 4 segmented. Pseudo-basal (first) segment fused with eye. Setae on antennae spine-like or hair-like. Apical and subapical setae long and hair-like. One sensory fleshy seta present on apical segment, occasionally also on basal segment. *Bs* present on ventral

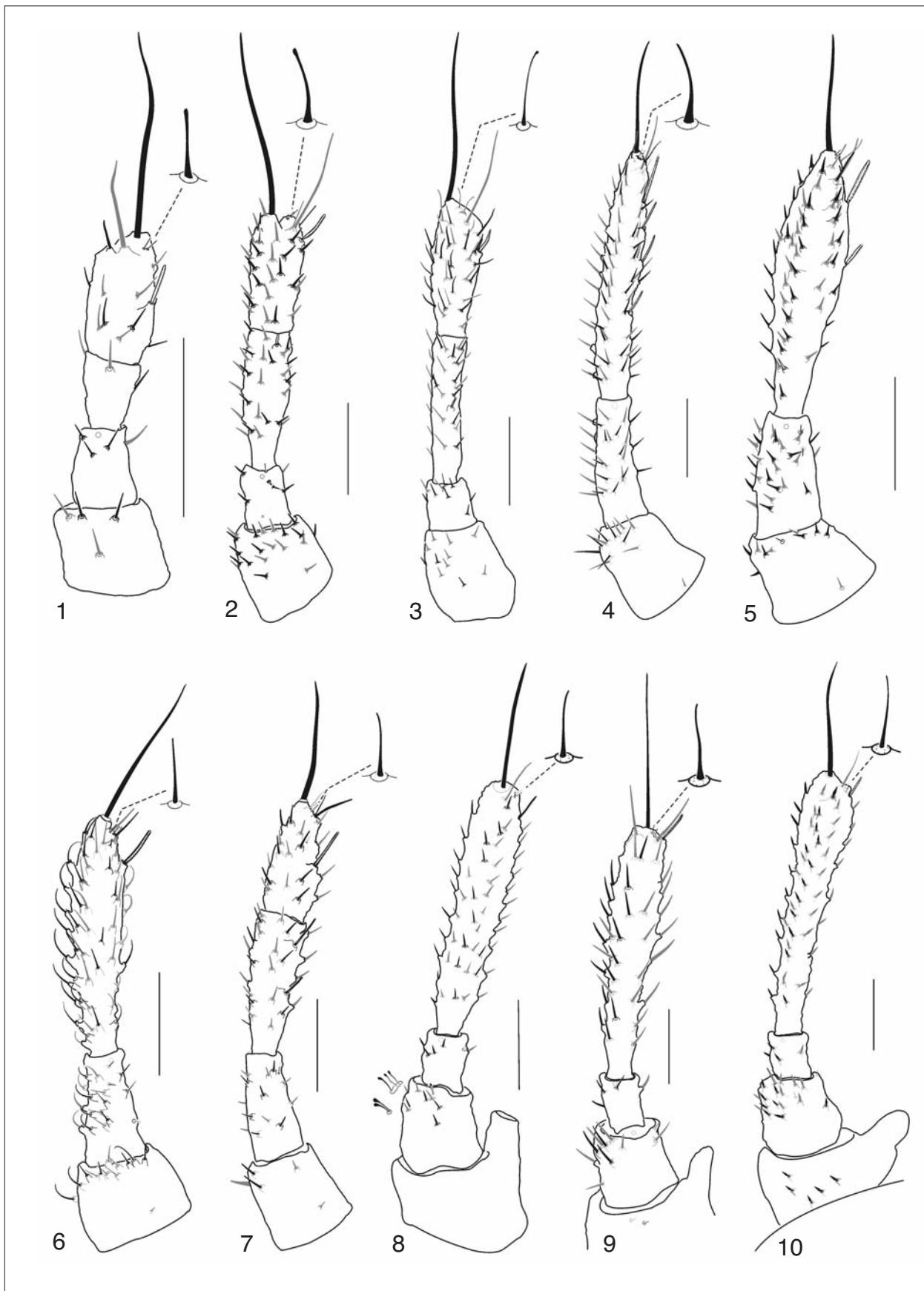


Fig. IV – Basiconic sensilla (Nipponortheziinae). a–*Jermycoccus boliviensis* Kozár & Konczné Benedixty, b–*Mixorthezia morrisoni* Konczné Benedixty & Kozár, c–*Neomixorthezia brasiliensis* Konczné Benedixty & Kozár, d–*Neonipponorthezia regina* Konczné Benedixty, e–*Nipponorthezia koreana* Konczné Benedixty & Kozár, f–*Nipponorthezinella guadalcanalis* (Morrison, 1952), g–*Orthezinella hispanica* Silvestri, h–*Ortheziola vejvodkyi* Šulc, i–*Ortheziolacoccus matileferreroae* Kozár & Miller, j–*Ortheziolamameti guinensis* (Morrison). Black setae on ventral side, grey setae on dorsal side. Bar = 100 µm.

side of antennae as relatively thick, conical setae with a pointed apex, with 2 on apical segment and on 1 or 2 on segment II and 0 or 1 on basal segment. *Cs* absent.

***Ortheziola vejvodkyi* Šulc (Fig. IV, 6)**

Material examined – 1 ♀, Austria, Vienna, soil with mosses, 26.vii.2013, leg. K. Fetykó [11132 PPI]; 1 ♀, Hungary, Nagykovácsi, Júlia-Major, rocky grass land, D-vac, 12.x.1999, leg. É. Szita & E. Botos [6061 PPI]; 1 ♀, Hungary, Báróliget, ösláp, D-vac, 7.xi.2008, leg. F. Samu [8521 PPI]; 1 ♀, Hungary, Pátka, Aszal-Völgy, D-Vac, 24.ix.2012, leg. É. Szita & E. Botos [10660 PPI]; 1 ♀, Hungary, Aggteleki NP, Jósvafő, Haragistya, mosses, 15.vii.2009, leg. Kozár [8816 PPI]; 1 ♀, Armenia, Tsakhkadzor, 1800-2300, 1.x.1982, leg. O. Merkl [As 569 HNHM]; 2 ♀♀, Slovenia, Bohinjska Bela, leaf litter, 22.viii.2009, leg. J. Kontschán [10925 PPI]; 1 ♀, Romania, Békás-szoros, 20.vii.1983, leg. K. Sim [10905 PPI]; 1 ♀, Romania, Mehadia, leaflitter, 25.x-5.xi.2007, leg. J. Kontschán [10825 PPI]; 2 ♀♀, Romania, Tordaihasadék, 28.ix.2001, leg. T. Szűts [10844 PPI]; 1 ♀, Switzerland, Valais, s/Venayaz, 700, 7.x.1980, leg. C. Besuchet [8864 PPI].

Description – Antennae 4 segmented; length of antennal segments: I 84-113, II 62-84, III 309-346 µm long; all segments of antennae covered with a moderate number of spine-like setae, each straight and apically acute; apical segment with 35-45 setae, longest seta 12-16 µm long; apical seta 154-163 µm long; subapical seta 51-59 µm long; with 1 sensory fleshy seta on apical segment, each 30-48 µm long; 2 *bs* present on apical segment and 1 on pedicel; a group of 2 or 3 blunted sensory setae present on ventral side of basal segment. *Cs* absent.

***Ortheziolacoccus matileferreroae* Kozár & Miller (Fig. IV, 9)**

Material examined – 1 ♀ Holotype, 4 ♀♀ Paratypes, Tanzania, Poroto, ex. *Arundinaria alpina*, 22.i.1972, leg. Pócs [160 PPI].

Description – Antennae 4 segmented; length of antennal segments: I 99-131, II 58-96 µm, III 347-390 µm long; all segments of antennae covered with small number of robust, straight, apically acute setae; apical segment with 32-40 setae, longest setae each 34-44 µm long; apical seta 212-232 µm long; subapical seta 67-74 µm long; sensory fleshy seta present on apical segment, each 32-38 µm long; 2 *bs* present on apical segment and 1 on basal segment; 2 hair-like sensory seta present on apical segment. *Cs* absent.

***Ortheziolamameti guinensis* (Morrison) (Fig. IV, 10)**

Material examined – 1 ♀: Ghana, Nyankapala, 5.viii.1968, leg. Y.S. Endrődy.

Description – Antennae 4 segmented; length of antennal segments: I 110-124, II 61-82, III 398-422 µm long; all segments of antennae covered with moderate number of robust, slightly lanceolate setae, apical segment with 48-52 setae, longest setae each 14-25 µm long; apical seta 153 µm long; subapical seta 49-54 µm long; with 1 sensory fleshy seta on apical segment and 1 on basal segment, each 31 µm long; 2 *bs* present on apical segment and 2 on basal segment. *Cs* absent.

PHYLOGENETIC RELATIONSHIP

By adding the antennal characters to the morphological matrix of VEA & GRIMALDI (2012), the MP analysis retrieved 461 most parsimonious trees of 296 steps (Fig. I). Modifying the above morphological matrix did

not result in a significant change of topology with almost all groupings remaining the same as in VEA & GRIMALDI (2012). The only difference is a loss of resolution within the clade including the *Ortheziolini* and *Mixortheziini*.

Three main lineages are distinguished by their type sensilla (Fig. V): (1) the *Ortheziinae* (including both *Arctortheziini* and *Ortheziini*) all possess *cs*, which according to character optimization originated in this group; (2) the *Newsteadinae*, which includes only the genus *Newsteadia*, is characterized by a different type of *cs*, the *Newsteadia*-type *cs*, and (3) the last group includes members of the *Mixortheziini*, *Nipponortheziini* and *Ortheziolini*, which all possess only *bs*.

When optimizing the type of *sensillum* (character 70) on the strict consensus (Fig. V) using parsimony, *bs* are inferred as the ancestral type of *sensillum* at least within the *Ortheziidae*. Therefore, despite their similarity, the two types of *cs* have a different evolutionary origin, one originating in the *Ortheziinae* and the other in the *Newsteadinae*.

When mapping the habitat for each terminal, a trend linking the type of sensilla and evolution of habitat seems to appear. First, we have groups feeding on aerial parts and possessing *cs*, as in *Ortheziinae*. However, this excludes the tribe *Arctortheziini*, mainly found either in the leaf litter or feeding on plant roots. Second, the majority of species within *Newsteadia* are ground-dwelling, with some exceptions where species are found in the aerial parts of plants (e.g., *N. americana* Morrison, *N. floccosa*). Overall, the *Newsteadia*-type *cs* is linked to a ground-dwelling habitat. Third, the majority of the *Ortheziidae* which have *bs* either feed on plant roots or are ground-dwelling.

DISCUSSION

LARINK (1986) and WALTHER (1983) suggested that the number and distribution of sensilla on the antennal segments are probably of taxonomic and phylogenetic significance in insects. Here, we found that the type of antennal sensillum within the *Ortheziidae* is indeed taxonomically informative. The family can be divided into three main lineages based on their type of sensillum: (i) *coeloconic sensilla*, (ii) *Newsteadia*-type *coeloconic sensilla*, and (iii) *basiconic sensilla*. These three lineages are the same as those recovered in the phylogenetic analysis of VEA & GRIMALDI (2012). Based on the character optimization in our phylogenetic hypothesis, *bs* seem to be the ancestral state to the two types of *cs*. Although *Newsteadia*-type *cs* and regular *cs*, have some similarities, these are herein considered to be morphologically different. *Newsteadia*-type *cs* have thicker walls and longer setae than those on “typical” *cs*. It is thought that these differences led to different lineages in ortheziid evolution.

KOTEJA (1980) also described *bs* and *cs* of different families within the Coccoidea and did mention their phylogenetic importance. He put forward two conclusions: (i) the *bs* are probably the primary type, because they occur in *Phenacoleachia*, and partly in the Monophlebidae and Coelostomidiidae, (ii) the *cs* underwent a secondary transformation towards the *bs* in some specialized groups such as part of *Ortheziidae*, and in some neococcoid lineages. Although our study partly supports Koteja evolutionary views, we believe that this separation occurred not only because of the age of the families but also because of the habitat where the insects live. In this study, we

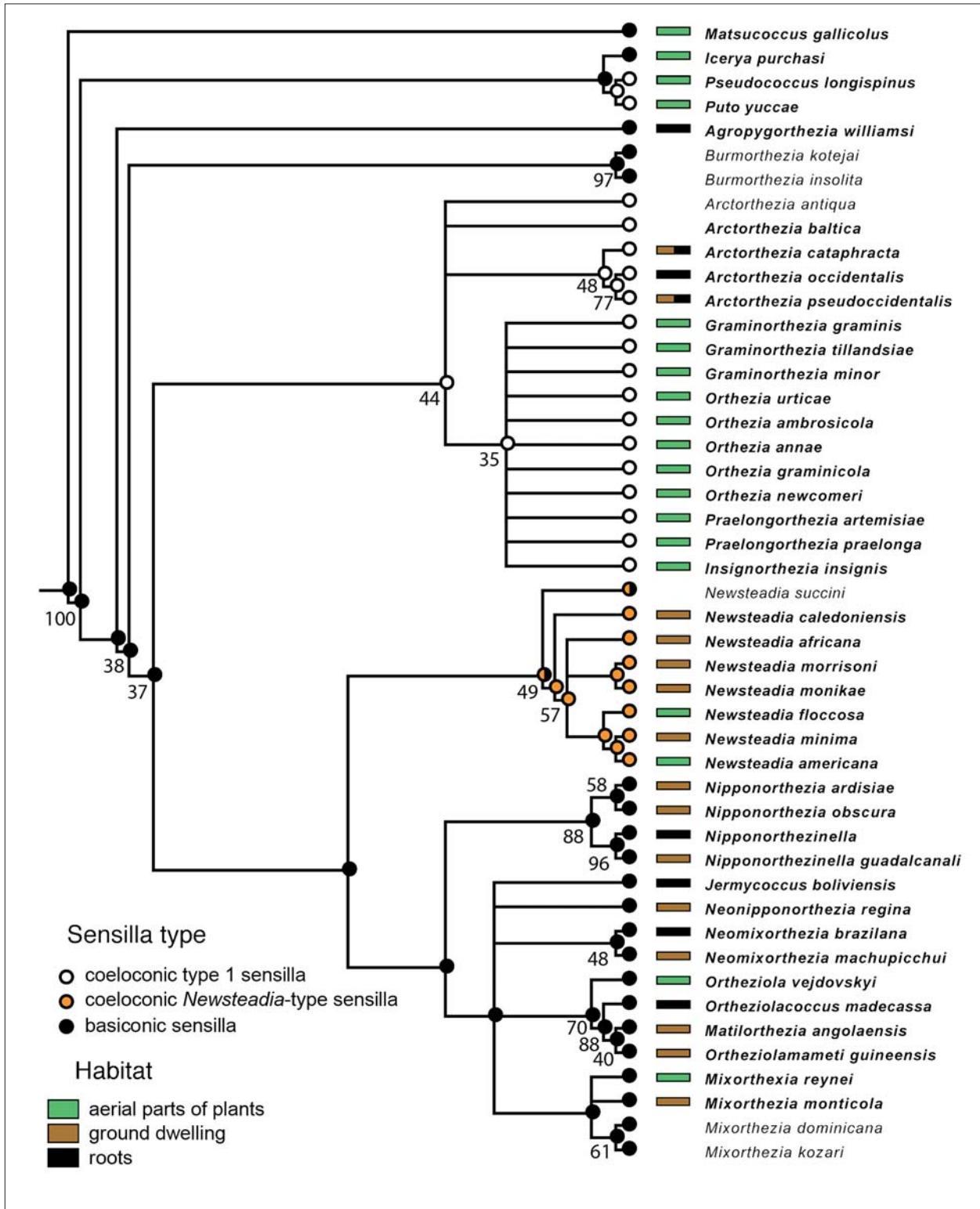


Fig. V – Strict consensus of the 461 most parsimonious trees obtained from a Maximum Parsimony analysis with TNT. $L=296$; $CI=0.35$, $RI=0.74$. Ancestral reconstruction of the type of antennal sensilla (Character 70) was performed with Mesquite using unordered parsimony and is shown on the tree as coloured circles. Habitat is indicated with the coloured rectangles. Bootstrap values $> 20\%$ are indicated at each node.

showed that *bs* occurs in insects (such as *Ortheziolini* and *Nipponorthaeziini*) that live on mosses, leaf litter and in the soil with a humid ecosystem. On the other hand, all insects that live on aerial plant parts in this study have *cs* (i.e., *Ortheziinae* and *Newsteadiinae*).

KOTEJA (1980) also indicated that the *bs* and *cs* represent organs of the same origin and function, being present in

similar number and situated on the same segments. In this study, it is shown that the location and number of the sensilla can be variable in different species, but the antennal apical segments always bear sensilla. To better understand their role, further studies are necessary to determine their number and location not only in the Ortheziidae but also in other families of Coccoidea.

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