RESEARCH ARTICLE



Cyphoderus (Cyphoderidae) as a major component of collembolan cave fauna in Thailand, with description of two new species

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Abstract

Distinguishing features of *Cyphoderus* Collembola of the *bidenticulati* group are described. Taxonomic problems in the *bidenticulati* group of *Cyphoderus* are emphasized, and new characters of taxonomic value are introduced and discussed. Two new species are described from caves of Thailand, differing mainly in claw morphology.

Keywords

Taxonomy, chaetotaxy, pseudopores, guano

Introduction

The species richness of Thai cave faunal communities are poorly known. Most studies in Thailand have focused on low-energy cave habitats, and large regions of the country have seldom been sampled. Consequently, the taxonomy, evolution, and biogeography of Thai cave Collembola are insufficiently known. Surveys of the Thai cave invertebrates revealed that Collembola in the family Cyphoderidae were the dominant arthropods in non-oligotrophic habitats of the dark zone. All examined specimens belong to the *bidenticulati*-group of the genus *Cyphoderus* (*sensu* Delamare-Deboutteville 1948), which previously included 16 species worldwide. Cyphoderidae are typically myrme-cophilous or termitophilous, with few records outside of ant and termite nests (Imms 1912, Folsom 1927, Delamare-Deboutteville 1948, Yoshii 1987). The abundance of *Cyphoderus* in caves in the absence or rarity of ants, and the striking morphological similarity of cave forms with myrmecophilous species raises several evolutionary and ecological questions.

Börner (1906) created Cyphoderini as a tribe of Entomobryinae to include *Cyphodeirus albinos* Nicolet, 1842 and three other species that he described in the same paper. In 1913, he upgraded Cyphoderini to subfamily rank, which he placed in Entomobryidae, a concept followed by Delamare-Deboutteville (1948). Subsequently, the taxon was upgraded yet again and was considered a family by most authors (Absolon and Kseneman 1942, Szeptycki 1979, Yoshii 1980, 1987, 1992, Deharveng 2004, Fanciulli et al. 2006). Soto et al. (2008) considered the group to be a subfamily in the Paronellidae on the basis of their non-annulated dens. However, the dens of cyphoderids is clearly reduced in length compared to that of all other Paronellidae *sensu stricto* and always bears characteristic feathered scales (more accurately termed feathered chaetae) consisting of a strong rachis with two symmetrical vanes made of long parallel barbs, a unique structure unknown from other Collembola. On this basis alone, we believe that Cyphoderidae deserve family rather than subfamily status.

Twelve genera have been described in Cyphoderidae (Bellinger et al. 2013): Calobatinus Silvestri, 1918 (4 species), Cephalophilus Delamare-Deboutteville, 1948 (3 species), Cyphoda Delamare-Deboutteville, 1948 (10 species), Cyphoderinus Denis, 1942 (1 species), Cyphoderodes Silvestri, 1910 (7 species), Cyphoderus Nicolet, 1842 (64 species), Delamareus Mitra, 1976 (2 species), Megacyphoderus Delamare-Deboutteville, 1948 (4 species), Mimoderus Yoshii, 1980 (5 species), Paracyphoderus Delamare-Deboutteville, 1948 (1 species), Pseudocyphoderus Imms, 1912 (4 species) and Serroderus Delamare-Deboutteville, 1948 (26 species). The genus Cyphoderus is the largest in the family and has a worldwide distribution. Like most cyphoderid species, most Cyphoderus species are termitophilous or myrmecophilous (Delamare-Deboutteville 1948, Christiansen 1957, Yoshii 1980, 1987, 1990, 1992). In his extensive revision of Cyphoderidae, Delamare-Deboutteville (1948) divided Cyphoderus into 5 groups according to the shape of the mucro (tridenticulati, bidenticulati, inermes, quadridenticulati and multidentati), to accommodate the 42 species known at that time.

Cyphoderus "bidenticulati-group" created by Delamare-Deboutteville (1948) and studied in this paper are easily recognized by their long, thin, and yellow mucro ending in two subequal small teeth. This group includes a large number of forms described as species, several only known from a single location, and a few species given as wide-spread on the account of numerous literature records. However, most of these records are doubtful because most species in this complex lack conspicuous morphological features, and are therefore difficult to distinguish.

Not only the so-called species are difficult to separate, but the description of the taxon's widespread type species, Cyphoderus albinus Nicolet, 1842, is poor by modern standards. In fact, the original description of Nicolet (1842) is so vague that it could apply to almost any species in the *bidenticulati* group. The most reliable, recent information comes from three sources: Delamare-Deboutteville (1948), whose description is probably based on French material; Yoshii (1990) based on material of Macaronesia; and Fjellberg (2007) describing material from Scandinavia. However, these contradictory accounts add further confusion, as there are disagreements about major diagnostic characters. According to Delamare-Deboutteville (1948), the species has no unpaired inner tooth on claw; the other two descriptions mention one unpaired tooth, but not at the same level. Fjellberg (2007) stated that there is no sublobal hair on outer maxillary lobe; Yoshii figured one. These contradictions may represent variability among populations, different species placed under the same name, or inaccurate observations. The only certainty is that the *bidenticulati* group of *Cyphoderus* is a complex of extremely similar forms after Delamare-Deboutteville (1948), where morphological examination reaches its limit for delimiting species. In this paper we describe new morphological characters, beyond those introduced by Yoshii, and provide detailed descriptions that could serve as references for future taxonomic works. The redescription of type material or topotypes will be necessary to extend the present work. In parallel, the use of molecular taxonomy might be the easiest way to assess the status of populations.

Materials and methods

Collembola were extracted from cave substrate samples using Berlese funnels and pitfall traps and stored in 90% ethanol at 5°C. Caves were sampled throughout Thailand (Fig. 1). The two described species come from two caves that yielded abundant populations, one from eastern Thailand and the other from the peninsula. Specimens were cleared in lactic acid and mounted on slides in Marc Andre II gum. The morphological analyses used a Leica DMLB light microscope. Images taken on a Cambridge 600 scanning electron microscope (SEM) were used for intepretating fine morphology of some chaetae. Figures were improved with Photoshop CS5 (Adobe Inc.).

Material deposition

PSU Prince of Songkla University, Hat Yai, Songkhla, Thailand**MNHN** Muséum national d'Histoire naturelle, Paris, France

Abbreviations used in the descriptions: Abd.= abdominal segment; Th.= thoracic segment; Ant.= antennal segment; AIIIO = Ant.III organ; M (in figures) or mac (in text) = macrochaeta(e); mes = mesochaeta(e); mic = microchaeta(e); sens = S-chaeta; T (in figures) = trichobothria; Man = manubrium (in tables). Chaetae notation: frontal



Figure 1. Sampling locations of cave Cyphoderidae in Thailand. Blue empty circles = caves without cyphoderids; red half–circles = caves with cyphoderids; C1, *Cyphoderus songkhlaensis* sp. n.; C2, unidentified species (Tham Nam Pray, Huay Yod District, Trang Province); C3, unidentified species (Tham Phung, Kiri Rat Nikhom District, Surat Thani Province); C4, unidentified species (Tham Phra, Patil District, Chumphon Province); C5, *Cyphoderus khaochakanus* sp. n., C6, unidentified species (Tham Kaeo, Pakdee Chumphon District, Chaiyaphum Province); C7, unidentified species (Tham Phupha Yatha Wararam, Muang Loei District, Loei Province); C8, unidentified species (Tham Mae U-Su, Tha Song Yang District, Tak Province).

chaetae of head and ventral tube chaetae after Yoshii (1980), tergite chaetotaxy after Szeptycki (1979), labial palp after Fjellberg (1999), AIIIO and ventral cephalic chaetae after Chen and Christiansen (1993).

Systematics

Cyphoderus Nicolet, 1842 http://species-id.net/wiki/Cyphoderus

Type species. Cyphoderus albinus Nicolet, 1842

Character assessment. Several characters of taxonomic importance were discovered or re-appraised in the course of this study.

1) All antennal segments were examined on both dorsal and ventral sides, revealing 10 types of chaetae (Fig. 3A). Their distribution pattern on the antennae is complex, but similar in the two species. Similarities are also obvious with the few Entomobryoidea where antennal chaetotaxy has been described. For instance, sens 1 to 5 and 8 of AIIIO as figured in *Sinella* by Chen and Christiansen (1993) were easily retrieved in our *Cyphoderus* (Fig. 3G). Several of the chaetal types recognized here are also found in other genera of Entomobryoidea. However, patterns are very complex and their comparisons would require detailed analyses beyond the scope of this paper.

2) S-chaetae can be grouped in four types (Fig. 4A), with chaeta S4 difficult to distinguish from type-5 mes. The S-chaetae formula observed in our species, as well as in other unidentified ones of the *bidenticulati* group, is 0/2,1/1,2,3,4,3,0 from head to Abd.VI (Figs 4–6), including 0/1,0/1,0,1,0,0 for S1; 0/1,1/0,1,0,0,0 for S2, 0/0,0/0,1,2,2,3 for S3 and 0/0,0/0,0,2,0 for S4. This S-chaetae pattern is similar to that of Entomobryoidea, except for the position of chaetae S1 and S2 on Th.II. In Entomobryoidea, S1 and S2 (=ms and S in Zhang et al. 2009) are close each other antero-laterally on the tergite (see Zhang et al. 2009). In the examined *Cyphoderus*, S2 is not close to S1, but intermediate between the position of antero-lateral S2 and of the postero-lateral S2 as observed in several Entomobryidae.

3) Pseudopores on tergites are arranged as in the Entomobryoidea species where they have been recorded (Jordana 2012 for instance): 1,1/1,1,1,1,0,0 from Th.II to Abd.VI. The presence of dorso-distal pseudopores on manubrium (2+2 in the studied *Cyphoderus*, Fig. 7H, 8D) is also characteristic of Entomobryoidea. Special to *Cyphoderus* described here are the 2+2 pseudopores behind the posterior row of chaetae of Abd.IV, found also in other unidentified *Cyphoderus* of the *bidenticulati* group (Fig. 4B). This pseudopore location is only known in Troglopedetinae, *i.e.*, in *Troglopedetes* (Deharveng 1988), in *Cyphoderopsis* (Jantarit et al. 2013) and in *Trogolaphysa* (Soto-Adames and Taylor 2013), with a number of pseudopores different for each genus. A ventral pseudopore is present on antennal area, in the same location as in Isotomidae (Deharveng 1979), Neanuridae (Deharveng 1983) and Onychiuridae (Pomorski 1998, Martinez et al. 2004). At least, the presence of 1+1 or 2+2 pseudopores on head anteriorly to the antenno-basal line (Fig. 2H) is a new pseudopore location for Collembola, unnoticed as far as we know in other genera of the class.

4) Important features of dorsal head chaetotaxy have been discovered by Yoshii (1980, 1987, 1992), useful for characterizing the family Cyphoderidae and several taxa of lower rank. The number and arrangement of post-labial chaetae as well as the presence of one mic among them are the same in the two species described here. However, they differ when compared with other species and might provide another promising set of taxonomic characters.

5) Body chaetae of various types were detected and tentatively grouped in categories. The mes of type-5 are the most numerous chaetae dorsally. They are seen as smooth under microscope examination, but serrated under SEM, Fig. 4A5; distinguishing them from S4 sens is especially difficult on Abd.IV where both are present, and the same confusion may arise for many other Entomobryoidea. As patterns of these mes as well as those of S4 sens seem to be stable inside population and different between species, further investigations will have to re-examine this character for its use in taxonomy.

6) The chaetotaxy of dorsal side (Figs 4–6) matches in most cases that given by Szeptycki (1979) for *Cyphoderus albinus*, and is very similar to that of Entomobryoidea (see Zhang et al. 2009). Main differences include the relative position of S1 and S2 on Th.II (see above), and chaeta "as" of Th.III as a mes in our material versus a short S-chaetae in Szeptycki (1979).

7) One of the most important characters for differentiating species of the *biden-ticulati* group is claw morphology, and it is the most diagnostic feature of the species described here. Although some variability in size and position of the various dental teeth has been noticed by other authors, it has not been taken into account in previous descriptions, leaving doubts about the validity of several species.

Cyphoderus songkhlaensis sp. n.

http://zoobank.org/99107FAB-981B-4F23-9D87-B962FEA5DB7A http://species-id.net/wiki/Cyphoderus_songkhlaensis Figs 2–7

Type locality. Thailand: Songkhla Province, Rattaphum District, Tham Khao Nui (12°12.227'N, 99°59.524'E), 120 m. above sea level, dark zone in cave, pitfall traps and Berlese extraction, S. Jantarit leg, 05 May 2012 (Sample #Songkhla-SJ.001).

Type material. Holotype, male adult (#PSUZC2011.SONG-001H) and 44 paratypes (6 males, 3 females, 35 of unknown gender) mounted on slides. Holotype and 29 paratypes at PSU (25 slides, 4 males, 2 females and 23 subadults, collection #PSUZC2011.SONG-001P-030P) and 15 paratypes at MNHN (12 slides, 2 males, 1 female and 12 subadults).

Description. Habitus thick (Fig. 2A), not troglomorphic, body length about 1.2 mm excluding antennae and furca. Furca well developed, about 2.5 times shorter than



Figure 2. *Cyphoderus songkhlaensis* sp. n. **A** habitus **B** outer maxillary lobe **C** maxilla head and ventral complex of the labrum **D** mandible **E** labial palp: proximal chaetae and external papilla E **F** labrum, dorsal view **G** chaetotaxy of labial basis; frontal chaetae **H** frontal chaetae and pseudopores of head **I** dorsal chaetotaxy of head.

body. Body color white. Eyes absent, no ocular patch. Dense cover of scales on head, body and furca (ventrally on manubrium, both sides on dens); scales present on Ant.I– II dorsally, absent on legs and ventral tube. Four categories of chaetae: ordinary chaetae (mac, mes and mic), scales, trichobothria and S-chaetae (= sens), described below separately for antennae and body.

Mouthparts. Outer maxillary lobe with one basal chaeta, a simple palp and one sublobal hair (Fig. 2B). Maxilla with 3-toothed capitulum and complex of 5 pad-shaped lamellae not analyzed in detail (Fig. 2C). Mandible head stocky, asymmetrical with 5 (left) and 4 (right) teeth (Fig. 2D). Labial palp with 5 papillae (A-E) and 13 guards, exactly as figured by Fjellberg (1999: fig. 72) (A and C without guards, B with 5 guards, D and E with 4 guards each); three hypostomal chaetae present with H longer than h1 and h2; 4 proximal chaetae (Fig. 2E). Labial basis formula m, e, 11, 12, with all chaetae smooth or indistinctly serrated, and 12 reduced to a minute but thick mic (Fig. 2G). Labral formula 4/5,5,4 with all chaetae smooth; two chaetae of the mid-row stronger and longer than others; dorso-distal limit of primary granules with a deep central incision (Fig. 2F); labral edge without structure; ventro-distally, two asymmetrical combs with many teeth variously developed and two central tubules (Fig. 2C).

Antennae. Less than 2 times the length of the head, segmentations I: II: III: IV as 1: 2.7: 1.6: 3.9. Sens and sens-like chaetae present on all antennal segments, of 10 morphological types (Fig. 3A); type-3 mes rather long, smooth under microscope examination but ciliated under SEM as in Fig. 3A (type-3*). Scales present dorsally on Ant.I and II (Fig. 3A11). Ant.I dorsally (Fig. 3B) with scales and ciliated mes (type-1), except 3 basal mic (type-9); ventrally (Fig. 3C) with various types of chaetae (types-1,2,3,5,6 and 9). Ant.II (Fig. 3D, E) dorsally and ventrally with numerous slender sens and chaetae (types-1,3,4,5,6,7); scales present dorso-basally; distally, 3–4 dorso-external swollen sens of type-7 and one ventro-external pseudopore. Ant. III (Fig. 3F, G) with various types of chaetae (types-1,3,4,5,6,7,9,10) not analyzed in detail; dorso-externally, AIIIO (Fig. 3H) typical of Entomobryoidea, with sens 1 to 5 and 8easily recognized, 2 and 3 being swollen sens of type-7; ventro-externally, one subapical pseudopore. Ant.IV (Fig. 3I, J) devoid of apical bulb, with various types of chaetae (including all types of sens except type-10); subapical organ present dorso-externally as a short and thick rod.

Body chaetae (Fig. 4A).

- 1) trichobothria, ciliated, very long and thin
- 2) weakly serrated, spiny mes
- 3) serrated or ciliated chaetae, of various length (mes to mac) and thickness
- 4) short and thickened mes in trichobothrial areas
- 5) thin mes, smooth under microscope examination, but ciliated under SEM
- 6) thick minute mic f0 and X on head
- 7) thin minute mic of anal valves
- S1) smooth, dark, short, straight, pointed sens
- S2) smooth, hyaline, short, subcylindrical, blunt sens
- S3) smooth, hyaline, longer, thinner sens



Figure 3. *Cyphoderus songkhlaensis* sp. n. continued **A** chaetae of antenna drawn from optical microscope, except 3* derived from SEM image **B** dorsal side of right Ant.I **C** ventral side of right Ant.II, the apical swollen sens of type-7 are indicated by arrows **E** ventral side of right Ant.II with apical pseudopore **F** ventral side of right Ant.III with apical pseudopore **G** dorsal side of right Ant.III **H** distal organite of Ant.III **I** ventral side of Ant.IV **J** dorsal side of Ant.IV with separate view of the subapical organite (left).

S4) smooth, rather long, rather thick, blunt sens

Scales oval to rectangular in shape, of various size, covering the whole body dorsally.

Dorsal chaetotaxy and pseudopore patterns (per side).

Macrochaetae: 0/0,0/0,0,1,2 from head to Abd.IV (excluding the antenno-basal lines on head and the 7–8+7–8 lateral mac on Abd.IV) (Fig. 4B).

Type-5 mes: 24–25 (and 1 uneven) /16,6/5,4,6,22,0,0 from head to Abd.VI (approximate numbers) (Fig. 2I for the head).

Trichobothria: 1/0,0/0,2,3,3,0,0 from head to Abd.VI.

S-chaetae (sens of types S1, S2, S3 and S4): 0/2,1/1,2,3,4,3,0 from head to Abd.VI. Possibly more on Abd.IV where type-5-like mes are often difficult to separate from S4.

Pseudopores: 1–2/1,1/1,1,1,1+2,0,0 from head to Abd.VI.

Chaetotaxy and pseudopores on head. As in Fig. 2I (dorsal side). No dorsal mac except the antenno-basal line of 5 mac (f1-f5); f0 as a minute thick uneven mic of type-6 between f1 chaetae; five ciliated clypeal mes and 1–2+1–2 pseudopores anteriorly to f1 (Fig. 2H). About 24 dorsal cephalic mes of type-5, subequal, short (Fig. 4A5). Cephalic trichobothria present dorsally at the middle of head with 1+1 mes internally near trichobothria, short and feebly ciliated (Fig. 2I, similar to Fig. 4A3). Ventrally, 4+4 post-labial mes smooth or very finely serrated along linea ventralis, and one mic of type-6 between G3 and H3 probably homologous with X (Fig. 2 in Chen and Christiansen 1993).

Chaetotaxy and pseudopores per tergite. (Figs 4B–F); values for type-5 mes are indicative). Th.II without mac; with a row of subequal spiny mes anteriorly and laterally, and several rows antero-laterally (type-2), 1+1 antero-lateral sens S1, 1+1 lateral sens S2 not close to S1, about 16+16 mes of type-5, and 1+1 pseudopores close to axis. Th.III without mac; with 1+1 antero-lateral sens S2, about 6+6 mes of type-5, and 1+1 pseudopores.

Abd.I without mac; with 1+1 lateral sens S1, about 5+5 mes of type-5 and 1+1 pseudopores.

Abd.II without mac; with 2+2 trichobothria, 6+6 modified mes around the trichobothria (type-4, Fig. 4C), 1+1 sens S2 (Fig. 4C) and 1+1sens S3 (Fig. 4C), about 4+4 mes of type-5, and 1+1 pseudopores. Abd.III with 3+3 trichobothria, 1+1 mac, 9+9 modified mes of type-4 on trichobothrial areas (3+3 near the internal trichobothria and 6+6 near the two external trichobothria, Fig. 4D), 3+3 sens in trichobothrial areas (1+1 S1 and 2+2 S3, Fig. 4D), about 6+6 mes of type-5, and 1+1 pseudopores. Abd.IV with 3+3 trichobothria, 2+2 mac, 4+4 modified mes of type-4 in the anterior trichobothrial area (none in the posterior trichobothrial area, Figs 4E and 6), 2+2 sens S3, 2+2 sens S4 near axis, about 22–23+22–23 mes, 2+2 sens S4 ahead pseudopores, in tandem with 2+2 short probably type-5 mes (Fig. 4F), 1+1 serrated mes of type-3 in tandem with 1+1 sens S3 posteriorly, and 3+3 pseudopores (1+1 in the middle of Abd.IV, 2+2 in the posterior margin of the tergite, behind a posterior row of 4+4 mes). Abd.V without pseudopore or mes of type-5; with 3+3 sens S3 and several short mac and mes.



Figure 4. *Cyphoderus songkhlaensis* sp. n. continued **A** chaetae of tergites drawn from optical microscope, except 5* derived from SEM image **B** chaetotaxy of tergites with types of S-chaetae S1 to S4 **C** trichobothrial complexes of Abd.II **D** trichobothrial complexes of Abd.III **E** anterior trichobothrial complexes of Abd. IV **F** tandem of chaetae on Abd.IV; the smallest is a short type-5 mes and the largest a S4 sens.



Figure 5. *Cyphoderus songkhlaensis* sp. n. continued **A** Szeptycki's notation of tergal chaetae on Th.II-Abd.III (Szeptycki 1979) **B** detail of Abd.II trichobothrial area **C** detail of Abd.III trichobothrial area.

Legs. Without scales. Trochanteral organ with 11–22 simple, straight, smooth chaetae arranged in V-form (Fig. 7C). Tibiotarsus chaetotaxy mostly composed of strong ciliated mes, with one thick smooth ventro-subapical chaeta on hind tibiotarsus. Each tibiotarsus with one tenent hair rather stout, apically spatulated, 4/5 as long as inner edge of claw; distal row of 9–10 serrated chaetae irregularly arranged on all tibiotarsi (Fig. 7D). Claw broad, not slender, with a weak or inconspicuous tunica; with one tooth at 40% of inner edge from the tip of the claw, a small dorsal tooth basally and a pair of inner basal teeth of unequal size, the outer one much larger than the inner one (Fig. 7D). Unguiculus pointed and broad, more than half as long as claw, lanceolate, with a strong outer tooth (Fig. 7D).

Ventral tube. Without scales. Anterior face with 2+2 long serrated chaetae (Fig. 7F). Posterior chaetae arranged typically for the genus, with L1 and L2 ciliated, L2 shorter than L1, M elongate and smooth, accompanied by 2+2 small peg-like microchaetae, and two long smooth distal chaetae; lateral flaps each with 2 small smooth mes (Fig. 7G).

Furca. Tenaculum with 4 teeth on each branch, anteriorly with strong, densely serrated, bent uneven chaeta (Fig. 7E). Furca with three types of chaetae (Fig. 7A) and 5 types of scales (Fig. 7B). Manubrium about 1.2 times as long as mucrodens. Dens about 2.3 times as long as mucro. Dorsal side of manubrium (Fig. 7H) with



Figure 6. *Cyphoderus songkhlaensis* sp. n. continued, Szeptycki's notation of tergal chaetae on Abd.IV (Szeptycki 1979).

2+2 pseudopores distally, and about 32–35 mes (fallen in most cases) arranged in two longitudinal stripes, including rather flexible and strongly ciliated mes and a few lateral ones slightly stronger, more straight, feebly serrated (Fig. 7A type-1), and baso-laterally 2+2 short serrated mes (Fig. 7A type-3); ventral side covered with oval scales (Fig. 7B type-5). Dens (Fig. 7H) elongate, dorsally with 2 rows of feathered scales (Fig. 7B type-1), 6 external and 5 internal, and 4 ciliated mes (Fig. 7A type-1) between two rows; proximal outer part of dens with 3 chaetae, two ciliated (Fig. 7A type-1) and the most external one smooth (Fig. 7A type-2); proximal inner part of dens with 2 slightly serrated mes (Fig. 7A1) close to dens-manubrium articulation; apical outer part of dens with one short serrated mes (Fig. 7A type-3); long dorso-distal feathered scales fallen in our specimens. Dens ventrally with oval scales (Fig. 7B types-4, 5), the distal internal one almost as long as mucro (Fig. 7B2, 3). Mucro straight, elongate, bidentate apically, with one minute external tooth almost at the level of the ante-apical normal tooth (Fig. 7I).



Figure 7. *Cyphoderus songkhlaensis* sp. n. continued **A** chaetae of furca **B** scales of furca **C** trochanteral organ **D** claw and distal part of tibiotarsus III **E** tenaculum **F** anterior face of the ventral tube **G** posterior face of the ventral tube; the peg-like setulae are indicated by arrows **H** furca; encircled by dotted lines are the 2+2 latero-basal mesochaetae of manubrium (**a**) the 3 outer basal mesochaetae of dens (**b**) and the 2+2 inner basal mesochaetae of dens (**c**) (I) mucro in lateral view (right) and in dorsal view (left) showing a third minute external tooth **J** female genital plate **K** male genital plate.

Genital plate. Male genital plate of the circinate type (*sensu* Christiansen 1958), with 6 genital mic and 15–16 circumgenital short, thin, smooth mes (Fig. 7K). Female genital plate with 2+2 mic (Fig. 7J).

Measurement. in µm (from type specimen #PSUZC2011.SONG-001H, male).

Body	Ant	Head	Ant.I	Ant.II	Ant.III	Ant.IV	Th.II	Th.III
1243	464	300	50	135	82	197	178	129
Abd.I	Abd.II	Abd.III	Abd.IV	Abd.V	Abd.VI	Man	Dens	Mucro
111	100	129	407	89	100	264	161	68

Etymology. From the name of the province "Songkhla" where this species was discovered.

Distribution. Only known from the type locality.

Ecology. Collected on guano in the dark zone of a cave developed in a karst covered with rainforest.

Discussion. The new species is similar to *Cyphoderus javanus* Börner, 1906 and to *C. sumatranus* Yoshii, 1987. The only detailed description of *C. javanus* is that of *C. borneensis* by Yoshii (1980, 1987), which was synonymized with *C. javanus* by the same author in 1992. *C. songkhlaensis* sp. n. differs from *C. borneensis* as described by Yoshii in the following combination of characters: the posterior face of its ventral tube with chaetae L1 and L2 ciliated but M smooth (given however as ciliated in Yoshii 1980) (*versus* L1, L2, M all ciliated chaetae), anterior mac of ventral tube serrated *versus* smooth, spatulate *versus* blunt tenent hairs, no *versus* a few smooth basal chaetae on manubrium and claw with two inner teeth versus one inner tooth on claw.

C. songkhlaensis sp. n. differs from *C. sumatranus* by its ciliated clypeal chaetae (*versus* smooth in *C. sumatranus*), the presence of one sublobal hair on outer maxillary lobe (*versus* none in *C. sumatranus*) and the posterior face of its ventral tube with chaetae L1 and L2 ciliated but M smooth (*versus* L1, L2, M all ciliated chaetae). The new species is known from caves like *C. sumatranus*, but *C. javanus* has been reported from diverse habitats: termite nests, forest soil and caves.

Cyphoderus khaochakanus sp. n.

http://zoobank.org/D18CFF8F-3003-4937-8DB3-C7CFF0C14E6D http://species-id.net/wiki/Cyphoderus_khaochakanus Fig. 8

Type locality. Thailand: Sa Kaeo Province, Khao Chakan District, Tham Meud (Dark Cave) (13°39.541'N, 102°05.414'E), 73 m. above sea level, dark zone in cave, pitfall traps and Berlese extraction, S. Jantarit leg, 29 July 2012 (Sample #Sakaeo-SJ.001).

Type material. Holotype, male adult (#PSUZC2011.SAK-001H) and 11 paratypes (1 male and 10 of unknown gender) mounted on slides. Holotype and 5 paratypes at PSU (5 slides, 5 subadults, collection #PSUZC2011.SAK-001P-005P) and 6 paratypes at MNHN (6 slides, 1 males, 5 subadults).

Description. Habitus thick, not troglomorphic, body length about 1.3 mm excluding antennae and furca. Furca well developed, about 2.4 times shorter than body. Body color white. Eyes absent, no ocular patch. Dense cover of scales on head, body and furca (ventrally on manubrium, both sides on dens); scales present on Ant.I–II dorsally, absent on legs and ventral tube. Types of chaetae as in *C. songkhlaensis* sp. n.

Mouthparts. Outer maxillary lobe with one basal chaeta, a simple palp and one sublobal hair. Maxilla with 3-toothed capitulum and a complex of 5–6 pad-shaped lamellae not analyzed in detail. Mandible head stocky, asymmetrical with 5 (left) and 4 (right) teeth. Labial palp with 5 papillae (A–E) and 13 guards, as in *C. songkhlaensis* sp. n.; hypostomal chaetae (H, h1, h2) present; 4 proximal chaetae. Labial basis formula m,e,l1,l2, with all chaetae smooth or indistinctly serrated, and l2 reduced to a minute but thick mic. Labral formula 4/5, 5, 4 with all chaetae smooth; two chaetae of the mid-row stronger and longer than others (similar to *C. songkhlaensis* sp. n.); dorso-distal limit of primary granules with a deep central incision; labral edge without structure; ventro-distally, two asymmetrical combs with many teeth variously developed and two central tubules.

Antennae. About 1.7 times the length of the head, segmentations I:II:III:IV as 1:3.6:2.5:4.8. Sens and sens-like chaetae present on all antennal segments, of 10 morphological types like in *C. songkhlaensis* sp. n. (Fig. 3A); type-3 mes rather long, apparently smooth under microscopic examination. Scales present dorsally on Ant.I and II (like Fig. 3A11). Ant.I dorsally like Fig. 3B, with scales and ciliated mes (type-1), except 3 basal mic (type-9); ventrally like Fig. 3C, with various types of chaetae (types-1,2,3,5,6 and 9). Ant.II like Fig. 3D, E, both dorsally and ventrally with numerous slender sens and chaetae (types-1,3,4,5,6,7), with scales present dorso-basally; distally, 3–4 dorso-external swollen sens of type-7 and one ventro-external pseudopore. Ant.III like Fig. 3F, G, with various types of chaetae (1,3,4,5,6,7,9,10) not analyzed in detail; dorso-externally, AIIIO like Fig. 3H, typical of Entomobryoidea, with sens 1 to 5 and 8 easily recognized, 2 and 3 being swollen sens of type-7; ventro-externally, one subapical pseudopore. Ant. IV like Fig. 3I, J, devoid of apical bulb, with various types of chaetae (including all types of sens except type-10); subapical organ present dorso-externally as a short and thick rod.

Dorsal chaetotaxy and pseudopores. Patterns and types of chaetae similar to those of *C. songkhlaensis* sp. n. (Fig. 4). Dorsal chaetotaxy and pseudopore patterns (per side) as follows: macrochaetae: 0/0,0/0,0,1,2 from head to Abd.IV (excluding the antenno-basal lines on head and the 7–8+7-8 lateral mac on Abd.IV) (Fig. 4B). Type-5 mes: not analyzed in detail. Trichobothria: 1/0,0/0,2,3,3,0,0 from head to Abd.VI. S-chaetae (sens of types S1, S2, S3 and S4): 0/2,1/1,2,3,4,3,0 from head to Abd.IV where type-5-like mes are often difficult to separate from S4. Pseudopores: 1-2/1,1/1,1,1,1+2,0,0 from head to Abd.VI.

Ventral chaetotaxy of head. 4+4 post-labial mes smooth or very finely serrated along linea ventralis, and one mic between G3 and H3 probably homologous with X (Fig. 2 in Chen and Christiansen 1993).



Figure 8. *Cyphoderus khaochakanus* sp. n. **A** trochanteral organ **B** claw and distal part of tibiotarsus III **C** posterior face of the ventral tube **D** furca; feathered chaetae in lateral view, only one of the two vanes attached to the rachis is visible **E** mucro.

Legs. Without scales. Trochanteral organ with 18 to 30 simple, straight, smooth chaetae arranged in V-form (Fig. 8A). Tibiotarsus chaetotaxy mostly composed of strong mes, with one thick smooth ventro-subapical chaeta on hind tibiotarsus. Each tibiotarsus with one tenent hair rather stout, apically spatulated, 3/4 to 4/5 as long as inner edge of claw; distal row of 9–10 serrated chaetae irregularly arranged on all tibiotarsi (Fig. 8B). Claw broad, not slender, without tunica; with 2 small teeth at 12% and 25% of inner edge from the tip of the claw, a small dorsal tooth basally and a pair of inner basal teeth of unequal size, the outer one much larger than the inner one (Fig.

8B). Unguiculus pointed and broad, more than a half as long as claw, lanceolate, with a strong outer tooth (Fig. 8B).

Ventral tube. Without scales. Anterior face with 2+2 long serrated chaetae (like Fig. 7F). Posterior chaetae arranged typically for the genus, with all 5 proximal chaetae (L1, L2 shorter than L1, M) ciliated, accompanied by 2+2 small peg-like microchaetae, and two long smooth distal chaetae; lateral flaps each with 2 short smooth mes (Fig. 8C).

Furca. Tenaculum with 4 teeth on each branch, and a strong, densely serrated, bent uneven chaeta anteriorly (like Fig. 7E). Furca with the same types of chaetae and scales as C. songkhlaensis sp. n. (see Figs 7A, B). Manubrium slightly shorter or as long as mucrodens. Dens about 2.3 times as long as mucro. Dorsal side of manubrium (Fig. 8D) with 2+2 pseudopores distally, and about 32-35 mes (fallen in most cases) arranged in two longitudinal stripes, including rather flexible and strongly ciliated mes and a few lateral ones slightly stronger, more straight, feebly serrated (like Fig. 7A type-1), and baso-laterally 2+2 short serrated mes (like Fig. 7A type-3); ventral side covered with oval scales (like Fig. 7B5). Dens (Fig. 8D) elongate, dorsally with 2 rows of feathered scales (like Fig. 7B type-1), 6 external and 5 internal, and 4 ciliated mes (like Fig. 7A type-1) between the two rows; proximal outer part of dens with 3 chaetae, two ciliated (like Fig. 7A type-1) and the most external one smooth (like Fig. 7A type-2); proximal inner part of dens with 2 slightly serrated mes (like Fig. 7A type-1) close to the dens-manubrium articulation (like Fig. 7H); apical outer part of dens with a short serrated mes (like Fig. 7A type-3); long dorso-distal feathered scales fallen in our specimens. Dens ventrally with oval scales (like Fig. 7B types-4, 5), the two long distal ones fallen in our specimens. Mucro straight, elongate, bidentate apically, with an additional minute outer tooth almost at the level of the ante-apical normal tooth (Fig. 8E).

Body	Ant	Head	Ant.I	Ant.II	Ant.III	Ant.IV	Th.II	Th.III
1316	545	328	46	164	114	221	221	121
Abd.I	Abd.II	Abd.III	Abd.IV	Abd.V	Abd.VI	Man	Dens	Mucro
93	86	107	528	96	64	328	243	96

Measurements. In µm (from type specimen #PSUZC2011.SAK-001H, male).

Etymology. From the locality "Khao Chakan" district, in SaKaeo province, where this species is found.

Distribution. Only known from type locality.

Ecology. Abundant on guano in the dark zone of a karstic cave.

Discussion

C. khaochakanus sp. n. differs from *C. songkhlaensis* sp. n. by: 1) the claw with two inner unpaired teeth (*versus* one); 2) posterior face of ventral tube with all chaetae ciliated (L1, L2, M) (*versus* L1 and L2 ciliated, M smooth); and 3) manubrium slightly shorter than

or subequal to mucrodens (*versus* manubrium longer than mucrodens). The number of teeth on the claw has been confirmed on 5 specimens of C. *khaochakanus* sp. n. and 8 specimens of *C. songkhlaensis* sp. n. Characters 2 and 3 are more difficult to observe, and their variability need to be assessed more firmly. In any case, these very slight differences are those usually reported in the literature between the species of the *albinus* group of *Cyphoderus*. Whether they indicate species-status would require re-examination of many species of the genus, especially for testing the variability of inner teeth on claw. We surmise that there are too few consistently different morphological characters in this group to further describe new species based only on morphology. We believe that molecular data will be helpful in providing additional information relevant to alpha taxonomy.

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References

- Absolon K, Kseneman M (1942) Troglopedetini. Vergleichende Studie über eine altertümliche höhlenbewohnende Kollembolengruppe aus den dinarischen Karstgebieten. Bericht über eine naturwissenschaftliche forschungsreise und biospeologische Erforschung der Insel Brac (Brazza) in Dalmatien. Studien aus dem Gebiete der Allgemeinen Karstforschung, der Wissenschaftlichen Höhlenkubde, der Eiszeitforschung und den Nachbargegebieten, Biologische Serie 16: 1–57.
- Bellinger PE, Christiansen KA, Janssens F (2013) Checklist of the Collembola of the World http://www.collembola.org
- Börner C (1906) Das system der Collembolen neuer Collembolen des Hamburger Naturhistorischen Museums. Mitteilungen aus den Naturhistorischen Museum in Hamburg 23: 147–188.
- Chen JX, Christiansen K (1993) The genus *Sinella* with special reference to *Sinella* s. s. (Collembola: Entomobryidae) of China. Oriental Insects 27: 1–54. doi: 10.1080/0030-5316.1993.10432236
- Christiansen K (1957) The collembolan of Lebanon and western Syria part II families Cyphoderidae and Oncopoduridae. Psyche 64: 119–133.
- Christiansen K (1958) The Entomobryiform Male Genital Plate. Proceedings of the Iowa Academy of Science 65: 474–476.

- Deharveng L (1979) Note sur un type d'organites tégumentaires originaux rencontré chez les Isotomidae (collembola). In: Dallai R (Ed) first International Seminar on Apterygota, Siena 1979: 59–62.
- Deharveng L (1983) Morphologie évolutive des Collemboles Neanurinae, en particulier de la lignée Néanurienne. Travaux du Laboratoire d'Ecobiolologie des Arthropodes Edaphiques, Toulouse 4: 1–63.
- Deharveng L (1988) A new troglomorphic Collembola from Thailand: *Troglopedetes fredstonei* new species (Collembola: Paronellidae). Occasional Papers of the Bernice Pauahi Bishop Museum 28: 95–98.
- Deharveng L (2004) Recent advances in Collembola systematic. Pedobiologia 48: 415–433. doi: 10.1016/j.pedobi.2004.08.001
- Delamare-Deboutteville C (1948) Recherches sur les Collemboles termitophiles et myrmécophiles (Ecologie, Ethologie, Systématique). Archivesde Zoologie Expérimentale et Générale 85: 261–425.
- Fanciulli PP, Caruso D, Dallai R (2006) On some Collembola from a Sicily cave, with the description of a new species of *Serroderus* Delamare, 1948 (Collembola, Cyphoderidae). Journal of Natural History 40: 1241–1251. doi: 10.1080/00222930600803241
- Fjellberg A (1999) The labial palp in Collembola. Zoologischer Anzeiger 237: 309–330.
- Fjellberg A (2007) The Collembola of Fennoscandia and Denmark. Part II: Entomobryomorpha and Symphypleona. Fauna Entomologica Scandinavica 42: 266 pp.
- Folsom JW (1927) Insect of the subclass Apterygota from Central America and the West Indies. Proceedings of the United States National Museum 72: 1–16.
- Jantarit S, Satasook C, Deharveng L (in press) The genus *Cyphoderopsis* Carpenter (Collembola: Paronellidae) in Thailand and a faunal transition at the Isthmus of Kra in Troglopedetinae. Zootaxa.
- Jordana R (2012) Synopses on Palaearctic Collembola: Capbryinae and Entomobryini. Senckenberg Museum of Natural History, Görlitz, 7(1): 390 pp.
- Imms AD (1912) On some Collembola from India, Burma, and Ceylon, with a Catalogue of the Oriental Species of the Order. Proceedings of the Zoological Society of London 82: 80–125. doi: 10.1111/j.1469-7998.1912.tb07006.x
- Nicolet H (1842) Recherches pour servirà l'histoire des podurelles. Nouveaux Mémoires de la Société Helvétique des Sciences Naturelles 6: 1–88.
- Martínez M, Baquero E, Barranco P, Ariño AH, Jordana R (2004) A new genus and species of Collembola from caves of south Iberian Peninsula (Collembola, Poduromorpha, Onychiuridae). Zootaxa 734: 1–15.
- Soto-Adames FN, Barra JA, Christiansen K, Jordana R (2008) Suprageneric Classification of Collembola Entomobryomorpha. Annals of the Entomological Society of America 101: 501–513. doi: 10.1603/0013-8746(2008)101[501:SCOCE]2.0.CO;2
- Soto-Adames F, Taylor S (2013) The dorsal chaetotaxy of *Trogolaphysa* (Collembola, Paronellidae), with descriptions of two new species from caves in Belize. ZooKeys 323: 35–74. doi: 10.3897/zookeys.323.4950

- Szeptycki A (1979) Chaetotaxy of the Entomobryidae and its phylogenetical significance: Morphosystematic studies of Collembola. IV. Polska Akademia Nauk, Zakład Zoologii Systematycznej i Doświadczalnej Państwowe Wydawnictwo Naukowe, Warszawa, Kraków, 219 pp.
- Yoshii R (1980) Cyphoderid Collembola of Sabah. Contributions of the Biological Laboratory of Kyoto University 26: 1–16.
- Yoshii R (1987) Notes on some Cyphoderid Collembola of the tropical Asia. Contributions of the Biological Laboratory of Kyoto University 27: 121–136.
- Yoshii R (1990) Miscellaneous notes on the Collembola of Macaronesia. Contributions of the Biological Laboratory of Kyoto University 27: 535–540.
- Yoshii R (1992) Interim report of the taxonomic researches toward the Collembolan family Cyphoderidae. Contributions of the Biological Laboratory of Kyoto University 28: 99–118.
- Zhang F, Chatterjee T, Chen JX (2009) A new species of the genus *Lepidocyrtus* Bourlet and a new record of *Seira delamarei* Jacquemart (Collembola: Entomobryidae) from the east coast of India. Zootaxa 2310: 43–50.

RESEARCH ARTICLE



A new species of *Tamarixia* Mercet (Hymenoptera, Eulophidae), parasitoid of *Trioza aguacate* Hollis & Martin (Hemiptera, Triozidae) in Mexico

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http://zoobank.org/FC8648E8-3DB1-44F1-B96B-033C4DFDF360
http://zoobank.org/640DF66D-8B7C-428B-BA70-6A406966E95A

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Abstract

Tamarixia aguacatensis Yefremova, **sp. n.** (Hymenoptera: Eulophidae: Tetrastichinae) is described from Mexico as a parasitoid of the avocado psyllid, *Trioza aguacate* Hollis & Martin (Hemiptera: Triozidae). *Trioza aguacate* is a serious pest of avocado, *Persea americana* Miller. A key to the species of *Tamarixia* Mercet in Mexico is given.

Keywords

Insecta, Chalcidoidea, Tamarixia aguacatensis, Trioza aguacate, Persea americana, Mexico

Introduction

The Mexican fauna of Psyllidae is poorly known, and even less known there are psyllid parasitoids. At least four *Tamarixia* Mercet (Eulophidae: Tetrastichinae) species have been recorded in Mexico as psyllid parasitoids: *T. leucaenae* Bouček from *Heteropsylla cubana* Crawford (Psyllidae: Ciriacreminae), *T. triozae* (Burks) from *Bactericera cockerelli* (Sulc) (Psyllidae: Triozinae) (Burks 1943), *T. radiata* (Waterston) from *Diaphorina citri* Kuwayama (Psyllidae: Diaphorinae) (Waterston 1922), and *T. schina* Zuparko from *Calophya schini* Tuthill (Psyllidae: Calophyidae) (McClay 1990; Lomeli-Flores and Bueno Partida 2002; Alvarez-Zagoya and Cibrian-Tovar 1999; Zuparko et al. 2011).

The most studied species is T. triozae, which was first recorded by Lomeli-Flores and Bueno Partida (2002) from a collection on tomato crops at Michoacán with a level of parasitism of 20-85% on B. cockerelli. This species is common as a B. cockerelli parasitoid in field crops of some solanaceous plants such as tomatillo (Physalis philadelphica Lam.), tomato (Solanum lycopersicum L.), potato (Solanum tuberosum L.), eggplant (Solanum melongena L.), and peppers (Capsicum annuum L.). Tamar*ixia radiata* was introduced to Mexico for the biological control program against D. *citri*; this parasitoid species has a wide distribution in Mexico and is now common in most Mexican citrus-growing areas (González-Hernández et al. 2009). This species is mass-reared for the augmentative biological control by Koppert México. As part of a federal program (Campaña Fitosanitaria de Prioridad Nacional contra el HLB: http://www.senasica.gob.mx/?id=4512) in Mexico there are two facilities designated for T. radiata mass rearing, one in Colima and the other one in Yucatán. In April, 2013, alone more than 465,900 parasitoids were released in the citrus areas in seven Mexican states. Elsewhere, the other two species (T. leucaenae and T. schina) were established as biological control agents of the invasive psyllids H. cubana in Africa and Asia (Day 1999; Rao et al. 2000) and C. schini in California (Zuparko et al. 2011). The latter species (T. schina) is now common in Mexico as a parasitoid of C. schini on peppertrees (Schinus molle L.). Apparently, this parasitoid disperseed to Mexico from California on its own, and no further studies on it have been conducted. In addition to these psyllid species as hosts of Tamarixia, another species of this genus was recovered from the avocado psyllid, Trioza aguacate Hollis & Martin (Hemiptera: Triozidae).

The avocado psyllid was discovered for the first time in Mexico in 1995, on avocado trees (*Persea americana* Miller) (Hollis and Martin 1997). This species was originally reported in Uruapan, Michoacán, and later dispersed in most of the avocado growing areas of that state. This pest affects the native Mexican avocado trees including those of the Hass variety. The main damage is caused by the nymphs. Adults deposit their eggs along the centre ribs of young leaves, and the nymphs establish themselves on the leaf ribs and on the tender stems, causing bud deformities. This species is abundant only in spring, when avocado trees develop most of the new buds. Recently, parasitoids in the host nymphs have been detected. From them a species of *Tamarixia* has been reared which does not correspond to any of the previously described taxa in the genus.



Figure 1. Mummy of Trioza aguacate nymph.

Material and methods

Periodic samples were taken in avocado groves in the town of Salvador Escalante, Michoacán, from January 2012 to January 2013; however, presence of the parasitoid was detected only in April and May 2012. To recover some of the parasitoids, buds and avocado leaves with parasitized nymphs of *T. aguacate* were collected; these are recognized by their ochre brown tone (Fig. 1). No more than 10 mummies per jar were collected.

The collected material was placed in glass jars covered with organza fabric to wait for the parasitoids to emerge. Overall parasitism of the nymphs was 14.6%; but when we recorded only the large nymphs the percent parasitism was 46.7%.

After the parasitoids were processed, pictures were taken of the diagnostic characteristics to compare this species with the already described species (Graham 1987; La-Salle 1994; Zuparko et al. 2011). The pictures were taken using the Scanning Electron Microscope (JEOL JSM 6390) and a stereomicroscope. Also, a sample of 36 buds was collected on 11 May 2012; the total number of nymphs and the parasitized nymphs were recorded per each 5 cm bud.

Morphological terminology follows that of Graham (1991). F1 – first segment of antennal funicle, F2 – second segment, F3 – third segment, F4 – fourth segment, C1 – first segment of clava, C2 – second segment of clava, C3 – third segment of clava; SMV – submarginal vein, MV – marginal vein, PMV – postmarginal vein, SV – stigmal vein, POL – the minimum distance between the posterior ocelli, OOL – the minimum distance between the eye margin and the adjacent posterior ocellus. Absolute measurements are given in millimetres (mm) for body and fore wing length; for other dimensions, relative measurements are used. Observations and measurements were made using a Nikon dissecting microscope (top magnification of $63 \times$) with a 100-division linear scale micrometer.

The following acronyms are used for the depositories of specimens:

CNIN	The National Insect Collection at the Instituto de Biología, Universidad
	Autónoma de Mexico, Mexico City, Mexico.
FSCA	Florida State Collection of Arthropods, Gainesville, Florida, USA.
USNM	United States National Museum of Natural History, Washington, D.C., USA.
TAUI	The National Collection of Insects, Zoological Museum, Department of
	Zoology, Tel Aviv University, Tel Aviv, Israel.

Taxonomy

Genus Tamarixia Mercet, 1924

http://species-id.net/wiki/Tamarixia

Type species. Tamarixia bicolor Mercet, 1924: 57 (original designation).

Diagnosis. *Tamarixia* can be distinguished by the following combination of features: fore wing with a single seta on the dorsal surface of the submarginal vein, propodeum without a Y-shaped carina; plicae and paraspiracular carinae absent, midlobe of mesoscutum with 2 pairs of long adnotaular setae (three pairs setae in *Tamarixia dahlsteni* Zuparko, 2011) and additional 2 pairs of short setae in the upper part in a horizontal row and 1 seta near notauli in *Tamarixia aguacatensis* sp. n. (Fig. 7). The anterior margin of the female hypopygium is almost straight, and the males have exceptionally long genitalia. An additional diagnostic character is that the toruli are closer to eye margin than to each other. Species are generally shiny black, but may have yellow markings on the gaster and/or head. The gaster of the female subcircular to ovate; one seta of each cercus 1.5 times or more the length of the next longest seta.

Biology. Species of *Tamarixia* are primary ectoparasitoids of psyllids (Graham 1987, 1991; Bouček 1988a, 1988b; LaSalle 1994; Brothers and Moran 1969; Moran et al. 1969; Noyes 2013) and parasitize immature stages of *Trioza* (Hemiptera, Psyllidae) (Mead 1994).

Distribution. *Tamarixia* is a cosmopolitan genus, with about 50 described species (Noyes 2013), most of them in Palearctic. Zuparko et al. (2011) listed 47 species of *Tamarixia* in the world but the authors missed 3 species: *Tamarixia krascheninnikovi* (Kostjukov, 1990), *T. fulvus* Yefremova & Yegorenkova, 2009 and *T. psyllae* Yefremova & Yegorenkova, 2009 (Kostjukov 1990; Yefremova and Yegorenkova 2009).

Identification. Keys to *Tamarixia* species are available for Europe (Graham 1991), the European part of Russia and the Far East of Russia (Kostjukov 1978; Kostjukov 1995, 2000), India (Narendran 2007), North America (Burks 1943, two species as part of *Tetrastichus*), and Yemen (Yefremova and Yegorenkova 2009).

Key to Mexican species of Tamarixia

(Females)

F3 subquadrate or transverse (Figs 14, 16, 19, 21), F1 1.2-1.3 times as long
as F3 2
F3 1.8–2.0 times as long as broad (Fig. 6), F1 1.45–1.5 times as long as F3.
Mesoscutum with complete median line Tamarixia radiata (Waterston)
Mesoscutum with incomplete median line (Fig. 7)
Propodeum steeply inclined relative to longitudinal axis of the body
Propodeum inclined 45 degrees from longitudinal axis of the body (Fig.5)4
F2 as long as F3, F1 2.2 times as long as broad, clava 1.3 times as long as
funicle (Fig. 16)
F2 1.4 times as long as F3, F1 1.8 times as long as broad, clava 1.5 times as
long as funicle (Fig. 19) Tamarixia leucaenae Bouček

(Males)

Pedicel 1.5 times as long as F1 (Figs 15, 17, 20, 22)2
Pedicel as long as F1 or slightly longer (1.1 times as long as F1) (Fig. 8)
Clava 5.0 times as long as broad (Fig. 22) Tamarixia radiata (Waterston)
Clava 4.0 times as long as broad
F2, F3 1.3–1.4 times as long as broad (Fig. 15) Tamarixia schina Zuparko
F2, F3 1.8–2.0 times as long as broad
Whorled setae of F1 reaching the top of F3, whorls of F4 reaching top of C2
(Fig. 17) <i>Tamarixia triozae</i> (Burks)
Whorled setae of F1 reaching top of F4, whorls of F4 attach out apical sen-
sillum (Fig. 20) Tamarixia leucaenae Bouček

Description of new species

Tamarixia aguacatensis Yefremova, sp. n.

http://zoobank.org/2E77279C-F3E8-4C9F-97A8-4329A33AC45D http://species-id.net/wiki/Tamarixia_aguacatensis Figs 2–13

Holotype (female): MEXICO, Michoacán, Salvador Escalante, Ejido El Tarascon, 19°26'29.81N, 101°49'53.03W, 1,910 m, 2.iv.2012, G. González-Santarosa (deposited in TAUI). PARATYPES (same data): $3 \ Q$, $3 \ O$ (CNIN); $1 \ Q$, $1 \ O$ (USNM); $2 \ Q$, $4 \ O$ (TAUI).



Figures 2-3. Tamarixia aguacatensis, female and male (habitus).

Description. FEMALE (Fig. 2). Body length: 0.85–1.04 mm; fore wing length: 2.07–2.94 mm. Body shiny black, eye pink; antenna yellow, scape black except yellow ventrally and apically; pedicel dark dorsally and basally, yellow-brown on ventral surface; flagellar segments and clava sandy yellow; tegula yellow; legs brown dark, coxae brown, trochanters brown, trochantelli yellow, basal and distal apices of pro- and meso-femora and tibiae yellow, and metafemur and tibia brown; tarsi yellow except apical segment brown. Metanotum yellow. Gaster brown. Wings hyaline, venation brownish.

Head 2.2 times as wide as long (Fig. 4). POL 2.0–2.2 times OOL. Face smooth; vertex, frons, areas near orbits and lower face setose. Malar sulcus present. Toruli slightly above lower level of eyes. Mandible with upper long tooth and several lower short teeth. Scrobes depressed and sutured (inverted V-shaped). Eye bare. Antenna (Fig. 6) with scape 2.3 times as long as pedicel, 1 discoid anellus, pedicel as long as F1 and F2 combined, F1 2.2 times as long as broad and equal to F2, F2 2.0 times as long as broad and 1.3 times as long as F3, clava 3-segmented, 2.3–2.4 times as long as broad and 2.4–2.6 times as long as F3.

Mesosoma. Pronotum short, with 8 marginal setae (Fig. 5). Mesoscutum 1.5 times as long as broad with an incomplete median line (0.63 length of mesocutum) and with 2 pairs of long adnotaular setae (Fig. 7). Mesoscutum with additional 2 pairs of short setae in the upper part in a horizontal row and 1 seta near notauli (Figs 5, 7). Mesocutum, scutellum and dorsellum finely reticulate. Scutellum with two submedian lines closer to each other than to sublateral lines, with 2 pairs of setae; first pair of setae in the middle of scutellum. Mesosoma in lateral view higher than the plane of propodeum and inclined at an angle less than 45 degrees from the longitudinal axis of the



Figures 4–9. *Tamarixia aguacatensis*. Female: 4 Head, frontal view 5 Mesosoma, lateral view 6 Antenna 7 Mesosoma, dorsal view 9 Propodeum. Male 8 Both antennae on the head.

body (Fig. 5). Propodeum (Fig. 9) strongly reticulate, with a complete simple median carina; spiracle with a rim. Callus with 2 long setae in one row (Fig. 7).

Fore wing (Fig. 10) 2.6 times as long as broad. SMV with 1 seta. Speculum extending along half length of MV and closed. SMV 1.2 times as long as MV. MV with 8 setae (Fig. 11). STV 3.4 times shorter than MV. PMV absent. Hind wing acute at apex.

Gaster 1.16–1.27 times as long as broad. Ovipositor sheaths slightly visible (Fig. 2).

MALE (Figs 3, 12, 13). Body length 0.8–1.00 mm. Colour of body very similar to that of female except gaster with tergite 1 completely yellow. Antennal scape dorsally



Figures 10–13. *Tamarixia aguacatensis.* Female: **10** Fore wing **11** Marginal vein with setae. Male: **12** Fore wing **13** Marginal vein with setae.

dark brown; pedicel, and funicle sandy yellow. Coxae of all legs brown, trochanters brown, trochantelli yellow, pro- and meso- femora brown except yellow at apex, metafemur and tibia brown, tarsi yellow except apical segment dark brown. Tegula yellow. Eyes pink. Ocelli white.

Head. POL 1.6–1.8 times OOL. Antenna (Fig. 8). Scape with ventral plaque about 0.2 length in the basal half. Pedicel 1.0–1.2 times as long as F1, F2 1.1 times as long as F1, F3 1.18 times as long as F2 and equal to F4, C1 equal to C2 and C3 1.2 times as short as C2. Four funicle segments with whorled setae; whorls of F1 reaching middle of F3, whorls of F2 reaching base of F4, whorls of F3 reaching tip of C3, whorls of F4 reaching middle of C2, whorls of C1 reaching base of C3, whorls of C2 reaching middle of C3, whorls of C3 reaching apical placoid sensillum. Scutellum smooth between submedian lines, and submedian and sublateral lines. Fore wing 2.1 times as long as broad (Fig. 12). Speculum slightly larger than that in female and MV with 9 setae (Fig. 13). Metasoma. Gaster 1.65–1.8 times as long as broad. Genitalia with two long longitudinal digital sclerites. Aedeagus very long, 2.3 times as long as gaster (Fig. 3). Parameres triangular with one long parameral seta.

Diagnosis. Tamarixia aguacatensis resembles T. leucaenae (examined were two female paratypes (FSCA) with the following data: Trinidad and Tobago, Trinidad Island, "UWJ Field, stn. (Lab)", on *Leucaena* sp., det. by Z. Bouček, 1988) from which it differs by the colour of the female: legs dark brown except coxae and trochanters brown, trochantelli yellow (coxae yellow in T. leucaenae); in addition, the female of T.



Figures 14–20. *Tamarixia schina*: 14 Female antenna 15 Male antenna. *Tamarixia triozae*: 16 Female antenna 17 Male antenna 18 Female fore wing.

aguacatensis differs from that of *T. leucaenae* in having F1-F3 2.0–2.2 times as broad as long and clava 2.4 times as broad as long (F1 1.7 times as long as broad, F2 1.4 times as broad as long, F3 subquadrate and clava 2.0 times as broad as long in *T. leucaenae*).

The female antenna of *T. aguacatensis* differs from that of *T. schina* (Fig. 14) as follows: F1-F3 2.0–2.2 times as broad as long and clava 2.3–2.4 times as broad as long (F1 1.8 times as broad as long, F2 1.2 times as broad as long, F3 transverse, and clava 1.8 times as broad as long in *T. schina*). The male antenna of *T. aguacatensis* differs from that of *T. schina* (Fig. 15, illustrated here for the first time) as follows: pedicel equal in length to F1 (1.5 times as long as F1 in *T. schina*), F1 and F2 equal, F2 1.2 times as long as F3 (F1, F2 and F3 equal in *T. schina*), clava 2.5 as long as F3 (2.0 times as long as F3 in *T. schina*), clava 2.0 times as long as broad (1.5 times as long as broad in *T. schina*). Additionally, the metanotum and propodeum are inclined much less in *T. aguacatensis* than in *T. schina*.



Figures 19–22. *Tamarixia leucaenae*: 19 Female antenna 20 Male antenna 21 *Tamarixia radiata*: 21 Female antenna 22 Male antenna.

Female of *T. aguacatensis* differs from that of *T. triozae* (Fig. 16) by in having F1-F3 2.0–2.2 times as broad as long and clava 2.3–2.4 times as broad as long (F1 2.0 times as broad as long, F2 1.7 times as broad as long, F3 subquadrate, and clava 1.6–1.7 times as broad as long in *T. triozae*). The male antenna of *T. aguacatensis* differs from that of *T. triozae* (Fig. 17) as follows: pedicel equal to length F1 (1.6 times as long as F1 in *T. triozae*), F1 and F2 equal to each other, F2 1.2 times longer than F3 (F1 subquadrate, F2 1.17 times shorter than F3 in *T. triozae*), clava 2.5 as long as F3 (2.2 times as long as F3 in *T. triozae*).

Female of *T. aguacatensis* differs from that of *T. radiata* (Fig. 19) in having F1-F3 2.0–2.2 times as broad as long, clava 2.4 times as broad as long (F1 1.6 times as broad as long, F2 1.5 times as broad as long, F3 subquadrate, and clava 2.0 times as broad as long in *T. radiata*). The male antenna of *T. aguacatensis* differs from that of *T. radiata* (Fig. 20) as follows: F1 and F2 equal to each other (pedicel equal in length to F1 in both species), F2 1.2 times longer than F3 (F1 1.4 times as short as F2, F2 equal to F3 in *T. radiata*), clava 2.5 as long as F3 (5.0 times as long as F3 in *T. radiata*), whorled setae of F1 reaching middle of F3 (reaching top of F4 in *T. radiata*), whorls of F2 reaching base of F4 (Fig.8) (reaching middle of C2 (Fig. 22) in *T. radiata*).

The male antenna of *T. aguacatensis* resembles that of *T. psyllae* Yefremova & Yegorenkova from Yemen that was reared from *Trioza erytrea* (Del Guercio) (Yefremova and Yegorenkova 2009). In the former the whorled setae of F1 reaching middle

of F3 (reaching base of F2 in *T. psyllae*), whorls of F2 reaching base of F4 (reaching base of F3 in *T. psyllae*), whorls of F3 reaching top of C3 (reaching base of F4 in *T. psyllae*), whorls of F4 reaching middle of C3 (reaching C1 in *T. psyllae*).

The male antenna of *T. aguacatensis* resembles that of *T. dryi* (Waterston), reared from *Trioza citri* Laing in Kenya (Waterston 1922), from which it differs as follows: whorls of F4 reaching middle of C3 (not reaching C1 in *T. dryi*), whorls of C1 reaching base of C3 (whorls of C1 reaching 0.8 length of clava in *T. dryi*).

Tamarixia aguacatensis also resembles *T. flavigaster* (Brothers & Moran), described from South Africa from Psyllidae on *Calodendrum capense* (L.) (Brothers and Moran 1969), from which it differs as follows: mesoscutum with incomplete median carina, coxae brown (complete median carina and pale coxae in *T. flavigaster*), male antennal plaque about 0.2 length of scape (0.1 in *T. flavigaster*), whorled setae of F1 reaching middle of F3, whorls of F2 reaching base of F4, whorls of F3 reaching top of C3 (whorls of F1 reaching clava, whorls of F2 and F3 reaching base of C3 in *T. flavigaster*). Also, the species has a brown gaster (the gaster is almost yellow in *T. flavigaster*).

Distribution. Mexico.

Host. Known from Trioza aguacate, as a nymphal parasitoid.

Etymology. The species name is derived from its host, Trioza aguacate.

Tamarixia aguacatensis is the fifth known species of *Tamarixia* in Mexico. It can be distinguished from other congeneric species in the country by having two pairs of short setae in the horizontal row on mesoscutum (Fig. 7).

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References

- Alvarez-Zagoya R, Cibrian-Tovar D (1999) Biology of the peppertree psyllid *Calophya rubra* (Blanchard) (Homoptera: Psyllidae). Revista Chapingo, Serie Ciencia Forestales y del Ambiente 5(1): 51–57.
- Bouček Z (1988a) Australasian *Chalcidoidea* (Hymenoptera). CAB International, Wallingford, UK, 832 pp.
- Bouček Z (1988b) Tamarixia leucaenae sp. n. (Hymenoptera: Eulophidae) parasitic on the leucaena psyllid Heteropsylla cubana Crawford (Hemiptera) in Trinidad. Bulletin of Entomological Research 78: 545–547. doi: 10.1017/S0007485300013298

- Brothers DJ, Moran VC (1969) A new species of *Tetrastichus* Haliday, 1844 (Hymenoptera: Eulophidae) parasitic on the nymphs of *Paurocephala calodendri* Moran (Homoptera: Psyllidae). Proceedings of the Royal Entomological Society of London (B) 38(3/4): 40–46.
- Burks BD (1943) The North American parasitic wasps of the genus *Tetrastichus* —a contribution to biological control of insect pests. Proceedings of the United States National Museum 93: 505–608. doi: 10.5479/si.00963801.93-3170.505
- Day RK (1999) Integrated Control of Leucaena Psyllid. Final Technical Report of Project R6524, Funded by DFID, NR Integrational, Chayham, Kent, UK.
- Gibson GAP, Huber JT, Woolley JB (Eds) (1997) Annotated keys to the genera of Nearctic *Chalcidoidea* (Hymenoptera). NRC Research Press, Ottawa, Ontario, 794 pp.
- Graham MWR de V (1987) A reclassification of the European Tetrastichinae (Hymenoptera: Eulophidae), with a revision of certain genera. Bulletin of the British Museum (Natural History) 55: 1–392.
- Graham MWR de V (1991) A reclassification of the European Tetrastichinae (Hymenoptera: Eulophidae): revision of the remaining genera. Memoirs of the American Entomological Institute 49, 322 pp.
- Hollis D, Martin JH (1997) Jumping plantlice (Hemiptera: Psylloidea) attacking avocado pear trees, *Persea americana*, in the New World, with a review of Lauraceae-feeding among psylloids. Bulletin of Entomological Research 87(5): 471–480. doi: 10.1017/ S000748530004133X
- González-Hernández A, Arredondo-Bernal HC, Robles-González M, Martínez-Carrillo JL, Pérez J, López-Arroyo JI (2009) Determinación de especies de parasitoides del psílido asiático de los cítricos *Diaphorina citri* (Hemiptera: Psyllidae) en México. Entomología Mexicana 8: 373–377.
- Kostjukov VV (1978) [Podsem 5. Tetrastichinae]. In: Medvedev GS (Ed) Opredelitel' Nasekomykh Evropey'skoy Chasti SSSR, Tom III, Pereponchatokrylye, Vtoraya chast'. Nauka, Leningrad, 430–76. [in Russian]
- Kostjukov VV (1990) New species of the eulophid genus *Tetrastichus* Haliday (Hymenoptera, Chalcidoidea, Eulophidae) from far eastern Russia. In: Lelei AS (Ed) Novosti sistematiki nasekomikh Dalnego Vostoka. AN SSSR, Dalnevostochnoe Otdelenie biol. pochv. in-t, Vladivostok, 46–63. [in Russian]
- Kostjukov VV (1995) 46. [Family Eulophidae Subfamily Tetrastichinae]. In: Lehr PA (Ed) [Key to the insects of Russian Far East in six volumes]. 4. Dal'nauka, Vladivostok, Russia, 346–505. [in Russian]
- Kostjukov VV (1996) New species of the genus *Tamarixia* Mercet (Hymenoptera, Eulophidae). Buletinul Academiei de Stiinte a Republicii Moldova. Stiinte Biologice si Chimice 4(277): 27–31. [in Russian]
- Kostjukov VV (2000) [Nadsem. Chalcidoidea 46. Sem. Eulophidae]. In: Lehr PA (Ed) Opredelitel' nasekomykh dal'nego vostoka Rossii 4(4): 582–601. Dal'nauka, Vladivostok. [in Russian]
- LaSalle J (1994) North American genera of Tetrastichinae (Hymenoptera: Eulophidae). Journal of Natural History 28: 109–236. doi: 10.1080/00222939400770091
- León JH, Sétamou M (2010) Molecular evidence suggests that populations of the Asian citrus psyllid parasitoid *Tamarixia radiata* (Hymenoptera: Eulophidae) from Texas, Florida and Mexico

represent a single species. Annals of the Entomological Society of America 103: 100–120. doi: 10.1603/008.103.0113

- Lomeli-Flores JR, Bueno Partida R (2002) New record of *Tamarixia triozae* (Burks), parasitoid of the tomatoe [sic] psilid [sic] *Paratrioza cockerelli* (Sulc) (Homoptera: Psyllidae) in Mexico. Folia Entomológica Mexicana 41(3): 375–376.
- McClay AS (1990) Distribution of leucaena psyllid and its natural enemies in Mexico: implications for biological control. Leucaena psyllid: problems and management. In: Napomopeth B, MacDicken KG (Eds) Proceedings of an international workshop held in Bogor, Indonesia, January 16–21, 1989. Winrock International Institute for Agricultural Development, Bangkok. 139–143.
- Mead FW (1994) Eugenia psyllid, *Trioza eugeniae* Froggatt (Homoptera: Psyllidae). Entomology Circular, Florida Department of Agriculture, Gainesville, No 367: 1–3.
- Mercet RG (1924) Eulófidos de España (1.a nota). Boletín de la Real Sociedad Española de Historia Natural 24: 54–59.
- Moran VC, Brothers DJ, Case JJ (1969) Observations on the biology of *Tetrastichus flavigaster* Brothers & Moran (Hym., Eulophidae), parasitic on psyllid nymphs (Hem., Hom.). Transactions of the Royal Entomological Society of London 121: 41–58. doi: 10.1111/j.1365-2311.1969.tb00516.x
- Narendran TC (2007) Indian Chalcidoid Parasitoids of the Tetrastichinae (Hymenoptera: Eulophidae). Records of the Zoological Survey of India, Occasional Paper No. 272, 1–386 + 5 plates.
- Noyes JS (2013) Universal Chalcidoidea Database, World Wide Web electronic publication. The Natural History Museum, London. http://www.nhm.ac.uk/entomology/chalcidoids/ index.html [accessed on 23 August 2013]
- Patil NG, Baker PS, Pollard GV (1993) Life histories of *Psyllaephagus yaseeni* (Hym., Encyrtidae) and *Tamarixia leucaenae* (Hym., Eulophidae), parasitoids of the leucaena psyllid *Heteropsylla cubana*. Entomophaga 38: 565–577. doi: 10.1007/BF02373091
- Pluke RWH, Qureshi JA, Stansly PA (2008) Citrus flushing patterns, *Diaphorina citri* populations and parasitism by *Tamarixia radiata* in Puerto Rico. Florida Entomologist 91: 36–42. doi: 10.1653/0015-4040(2008)091[0036:CFPDCH]2.0.CO;2
- Rao MR, Singh MP, Day R (2000) Insect pest problems in tropical agroforestry systems: Contributory factors and strategies for management. Agroforestry Systems 50: 243–277. doi: 10.1023/A:1006421701772
- Schauff ME, LaSalle J, Coote LD (1997) Chapter 10. Eulophidae. In: Gibson GAP, Huber JT, Woolley JB (Eds) Annotated keys to the genera of Nearctic *Chalcidoidea* (Hymenoptera). NRC Research Press, Ottawa, Ontario, 327–429.
- Waterston J (1922) On the chalcid parasites of psyllids (Homoptera). Bulletin of Entomological Research 13(1): 41–58. doi: 10.1017/S0007485300045235
- Yefremova ZA, Yegorenkova EN (2009) The subfamily of Tetrastichinae (Hymenoptera: Eulophidae) in Yemen, with description of new species. Fauna of Arabia: 169–211.
- Zuparko RL, De Queiroz DL, La Salle J (2011) Two new species of *Tamarixia* (Hymenoptera: Eulophidae) from Chile and Australia, established as biological control agents of invasive psyllids (Hemiptera: Calophyidae, Triozidae) in California. Zootaxa 2921: 13–27.
RESEARCH ARTICLE



Genus Indiopius Fischer, 1966 (Hymenoptera, Braconidae, Opiinae) in Iran with a key to the world species

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Abstract

The Iranian species belonging to the genus *Indiopius* Fischer are reviewed. A description of the first recorded female of *I. cretensis* Fischer, 1966 is provided. A key to the world species of the genus *Indiopius* is given.

Keywords

Braconidae, Opiinae, Indiopius, new records, key, Iran

Introduction

The subfamily Opiinae contains approximately 2,000 catalogued species worldwide (Yu et al. 2012). These are strictly koinobiont parasitoids of the Diptera-Cyclorrhapha (Wharton 1999), mainly of larvae of leaf miners and those living in fruits. The hosts are known for only about 300 opiine species, mostly belonging to the dipterous families Agromyzidae, Anthomyiidae and Tephritidae (Fischer 1971, 1972, 1977, 1987; Shaw and Huddleston 1991).

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The genus *Indiopius* Fischer, 1966 is a small and rarely collected taxon, with only eight known species, despite its wide distribution throughout the Afrotropical, Oriental and Palaearctic regions (Yu et al. 2012). The main characters for diagnosis of this genus are: marginal cell of the fore wing widely open apically; veins m-cu, r-m and 2-SR of the fore wing absent; the first subdiscal cell of the fore wing open postero-apically; vein cu-a of the hind wing absent; clypeus wide, short and impressed ventrally; mandible long and slender; occipital carina completely absent; the first to third metasomal tergites more or less distinctly coriaceous or rugulose; fourth to six metasomal segments usually largely retracted (Li et al. 2013).

Our investigation of the braconid parasitoid wasps of the subfamily Opiinae in Iran allowed the discovery of the genus *Indiopius* Fischer; one species is described from Iran for the first time. The description of the female of *I. cretensis*, only the male was known until now, and a key for identification of the world species of *Indiopius* are included in this paper.

Material and methods

Specimens were collected using standard sweeping nets on semi-aquatic plants within a protected landscape in the Sistan area (31°02'N, 61°32'E, 485 m A.S.L). This small area is artificially irrigated to protect the endemic flora and fauna from the unfavorable dry climates of the recent decade.

A field emission gun environmental scanning electron microscope (Hitachi S-4100) at 2 kV was used for high-resolution imaging without gold coating.

For the terminology of the morphological features and sculpture, measurements and wing venation nomenclature, see van Achterberg (1988, 1993). Additionally, the following abbreviations are used: POL – postocellar line; OOL – ocular-ocellar line; and OD – maximum diameter of lateral ocellus. The specimens studied are deposited in the collections of the Faculty of Agriculture, University of Zabol, Iran (FAOUZ), in the Entomological Collection at the University of Valencia (Valencia, Spain; ENV), and in the Zoological Institute RAS (St. Petersburg, Russia; ZISP).

Taxonomy

Indiopius cretensis Fischer, 1983

http://species-id.net/wiki/Indiopius_cretensis Figures 1–15

Indiopius cretensis Fischer 1983: 1; Yu et al. 2012.

Material examined. 1 \Diamond (holotype), Greece, Crete, Biro, Canea, 1906/11 (Hungarian Natural History Museum, Budapest); 2 \bigcirc and 4 \Diamond , Iran, Zabol (31°02'28"N,



Figures 1–6. *Indiopius cretensis* Fischer (1, 3–6 female 2 male). 1, 2 Habitus, lateral view 3 Head, lateral view 4 Face in front view, mandible and maxillary palpi 5 Antenna 6 Basal segments of antenna.

61°32'02"E, 482 m A.S.L.), 26.iv.2013, sweeping on *Cyperus rotundus* (Z. Rahmani leg.) (ENV, ZISP); 7 \bigcirc and 20 \bigcirc , same locality, 22–24, 26 and 27.iv.2013 (Z. Rahmani leg.) (FAUOZ).

Diagnosis. This species resembles *Indiopius fischeri* Samiuddin et Ahmad from India and *I. turcmenicus* Tobias from Turkmenistan. *I. cretensis* differs from *I. fischeri* in has the maxillary palpi as long as head height (0.5 times in *I. fischeri*), the first flagellar segment of female 2.55–2.65 times as long as its width (2.1 times in *I. fischeri*), and the middle flagellar segments of female 2.25–2.65 times as long as their width (1.5 times



Figures 7–12. *Indiopius cretensis* Fischer (7, 8 male 9–12 female). 7 Antenna 8 Basal segments of antenna 9 Head, dorsal view 10 Mesosoma, lateral view 11 Mesoscutum 12 Propodeum.

in *I. fischeri*). Also, *I. cretensis* differs from *I. turcmenicus* in having the first flagellar segment of female 2.55–2.65 times as long as its width (3.0 times in *I. turcmenicus*), the middle flagellar segments of female 2.25–2.65 times as long as their width (2.0 times in *I. turcmenicus*), the first metasomal tergite 1.0–1.1 times as long as its apical width (0.8 times in *I. turcmenicus*), and vein 1cu-a postfurcal (interstitial in *I. turcmenicus*).

Description. Female (first record). Body length 1.0–1.1 mm; fore wing length 1.4–1.5 mm.



Figures 13–15. *Indiopius cretensis* Fischer (female). **13** Hind and middle legs, metasoma and ovipositor, lateral view **14** First metasomal tergite **15** Fore and hind wings.

Head. In dorsal view, 2.0 times as wide as median length, 1.4 times as wide as mesoscutum, smooth, with rounded temples behind eyes. Eye in lateral view 1.35 times as high as wide and twice as wide as temple in middle. POL 2.6 times OD; OOL 3.45 times OD. Face 1.25 times as wide as high; inner margins of eyes subparallel. Clypeus 3.35 times as wide as high, slightly curved ventrally. Mandible narrow, weakly and evenly widened towards base; upper tooth longer than lower tooth. Maxillary palpi as long as head height. Antenna thick, 18-segmented. Scape 1.40–1.45 times as long as maximum width, about twice as long as pedicel. First flagellar segment 2.55–2.65 times as long as its maximum width. Third to ninth flagellar segments 2.55–2.65 times and tenth to sixteenth segments 2.25–2.30 times as long as their maximum width.

Mesosoma. In lateral view, 1.05 times as long as high. Mesoscutum 0.75–0.80 times as long as its maximum width. Notauli mainly absent, finely developed on vertical anterior part. Mesoscutal pit absent. Prescutellar depression with numerous carinae. Precoxal suture present, very shallow, not reaching anterior and posterior margins of mesopleuron. Posterior mesopleural furrow smooth. Propodeum completely smooth. Propodeal spiracles relatively small.

Legs. Hind femur 3.60–3.65 times as long as its maximum width. Hind tibia weakly widened towards apex, about 10.0 times as long as its maximum subapical width, 1.1 times as long as hind tarsus. First segment of hind tarsus 1.3 times as long as second segment.

Wings. Length of fore wing 2.5 times its maximum width. Pterostigma almost triangular. Vein 1-R1 not reaching wing apex and as long as pterostigma. Veins r, 3-SR and SR1 not differentiated; 1-M straight; cu-a postfurcal, 1-CU1 widened. First subdiscal cell open. CU1b absent. M+CU1 only apically sclerotized. Hind wing 6.5 times as long as its maximum width; vein cu-a absent.

Metasoma. Distinctly depressed dorso-ventrally, apical segments rather distinctly protruding behind third tergite. First tergite weakly widened towards apex, 1.1 times as long as its apical width, finely rugose but basally smooth. Second tergite largely finely granulate. Third and following tergites smooth. Ovipositor 1.05 times as long as first tergite, 0.65 times as long as hind femur.

Colour. Body and legs uniformly brown to dark brown, second tergite yellowish brown. Wings hyaline. Pterostigma brown.

Male. Body length 1.4–1.5 mm; fore wing length 1.5 mm. First flagellar segment 2.7–2.8 times and second segment 2.5 times as long as their width accordingly. Third to sixteenth flagellar segments 2.20–2.75 times as long as their width. Otherwise similar to female.

Distribution. Cape Verde Islands, Greece, Turkey, and Iran (new record).

Key to the world species of the genus Indiopius Fischer

1	Precoxal suture long, reaching anterior or anterior and posterior margins of
	mesopleuron
_	Precoxal suture short, not reaching anterior and posterior margins of meso-
	pleuron
2(1)	Maxillary palpi 0.6 times as long as head height. Hind femur 3.3 times as
	long as its maximum width. First flagellar segment 2.2 times as long as its
	maximum width. Head in dorsal view 1.75 times as wide as median length.
	Antenna 19-segmented. Body length 1.3 mm. China
	<i>I. chenae</i> van Achterberg & Li (\bigcirc)
_	Maxillary palpi as long as head height. Hind femur 4.0 times as long as its max-
	imum width. First flagellar segment 2.5 times as long as its maximum width.
	Head in dorsal view 2.0 times as wide as median length. Antenna 19-segment-
	ed. Body length 1.4 mm. Turkey, Vietnam <i>I. saigonensis</i> Fischer (Q)
3(1)	Hind femur wide, 3.60–3.65 times as long as its maximum width4
_	Hind femur narrow, 4.0–4.5 times as long as its maximum width
4(3)	First flagellar segment 2.1 times as long as its maximum width. Middle flagel-
	lar segments 1.5 times as long as their width. Maxillary palpi 0.5 times as long

	as head height Vein 1 cu-a clearly postfurcal. Antennae 18-segmented.
	Body length 1.3 mm. India
_	First flagellar segment 2.65–3.00 times as long as its maximum width. Mid-
	dle flagellar segments 2.00–2.55 times as long as their width. Maxillary palpi
	as long as head height
5(4)	First flagellar segment 2.65 times as long as its maximum width. Middle
	flagellar segments 2.25-2.55 times as long as their width. First metasomal
	tergite 1.0–1.1 times as long as its apical width. Vein 1 cu-a postfurcal. An-
	tennae 18–20–segmented. Body length 1.0–1.5 mm. Cape Verde Islands,
	Greece, Turkey, Iran
_	First flagellar segment 3.0 times as long as its maximum width. Middle flagel-
	lar segments about 2.0 times as long as their width. First metasomal tergite 0.8
	times as long as its apical width. Vein 1 cu-a interstitial. Antennae more than
	14-segmented (missing apically). Body length 1.3 mm. Turkmenistan
6(3)	First flagellar segment 2.5 times as long as its width. Middle flagellar seg-
	ments about 1.5 times as long as their width. – Maxillary palpi as long as head
	height. Hind femur 4.5 times as long as its maximum width. Vein 1 cu-a
	almost interstitial. Antennae 19-segmented. Body length 1.7 mm. India
	<i>I. humillimus</i> Fischer (°)
_	First flagellar segment 3.0 times as long as its width. Middle flagellar seg-
	ments about twice as long as their width7
7(6)	Maxillary palpi 0.7 times as long as head height. First metasomal tergite 1.2
	times as long as its apical width. Head in dorsal view 1.7 times as wide as
	its median length. Antenna 18-19-segmented. Body length 1.3-1.4 mm.
	China
_	Maxillary palpi as long as head height. First metasomal tergite as long as its
	apical width. Head in dorsal view 1.9 times as wide as its median length. An-
	tenna 18-segmented. Body length 1.1 mm. Turkey
	<i>I. yilmazae</i> Fischer & Beyarslan (\bigcirc)

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References

Achterberg van C (1988) Revision of the subfamily Blacinae Foerster (Hymenoptera, Braconidae). Zoologische Verhandelingen Leiden 249: 1–324.

- Achterberg van C (1993) Illustrated keys to the subfamilies of the Braconidae (Hymenoptera: Ichneumonoidea). Zoologische Verhandelingen Leiden 283: 1–189.
- Fischer M (1971) Index of entomophagous insects, Hym., Braconidae, World Opiinae. Le François, Paris, France.
- Fischer M (1972) Hymenoptera: Braconidae (Opiinae I) Das Tiereich 91: 1-620.
- Fischer M (1977) Hymenoptera: Braconidae (Opiinae II-Amerika). Das Tiereich 91: 1–1001.
- Fischer M (1983) Ein neuer *Indiopius* von Kreta (Hymenoptera, Braconidae, Opiinae). Zeitschrift der Arbeitsgemeinschaft Österreichischer Entomologen 35: 1–2.
- Fischer M (1987) Hymenoptera: Braconidae (Opiinae III-Äthiopische, orientalische, autralische und oceanische Region. Das Tierreich 104: 1–734.
- Li X, Achterberg van C, Tan J (2013) Revision of the subfamily Opiinae (Hymenoptera, Braconidae) from Hunan (China), including thirty-six new species and two new genera. ZooKeys 268: 1–186. doi: 10.3897/zookeys.268.4071
- Shaw MR, Huddleston T (1991) Classification and biology of Braconidae wasps (Hymenoptera: Braconidae). Handbooks for the Identification of British Insects 7 (11): 1–126.
- Wharton RA (1999) A review of the Old World genus *Fopius* Wharton (Hymenoptera: Braconidae: Opiinae), with description of two new species reared from fruit-infesting Tephritidae (Diptera). Journal of Hymenoptera Research 8: 48–64.
- Yu DSK, Achterberg van C, Horstmann K (2012) Taxapad 2012, Ichneumonoidea 2011. Ottawa, Ontario, Canada. Database on Flash-drive. www.taxapad.com

RESEARCH ARTICLE



Distribution and nests of paper wasps of Polistes (Polistella) in northeastern Vietnam, with description of a new species (Hymenoptera, Vespidae, Polistinae)

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Abstract

Seven species of the subgenus *Polistella* Ashmead of the genus *Polistes* Latreille including a new species, *P. brunetus* Nguyen & Kojima, **sp. n.** described here, are recognized to occur in northeastern Vietnam, the easternmost part of the eastern slope of the Himalayas. A key to these species is provided. Their distributional records are remarked. Nests of *P. delhiensis* Das & Gupta, *P. mandarinus* de Saussure and *P. brunetus* are also described.

Keywords

Hymenoptera, Vespidae, Polistinae, Polistes, Polistella, new species, nest, northeastern Vietnam

Introduction

Of the four subgenera in the cosmopolitan paper wasp genus *Polistes, Polistella*, with some 85 extant species, is the largest in terms of the number of species among the three subgenera endemic to Old World (*Gyrostoma* Kirby & Spence, *Polistella* Ashmead, and *Polistes* Latreille). The subgenus *Polistella* is known to show a high species diversity in the northern part of Indochina, the area on the eastern slope of the Himalayas. This is especially the case, together with strong endemism, for the *Polistella* species that are characterized by a basally strongly swollen second metasomal sternum. These species may form a monophyletic group and show the distribution pattern of so–called "Himalayan Corridor origin", namely they occur in the zone from the southern slopes of the Himalayas, through the eastern slope of the Himalayas and eastern coastal areas of continental Asia and Taiwan, to Ussuri and eastern Siberia in Russia and Hokkaido in Japan (Nguyen et al. 2011). Locating in the easternmost part of the eastern slope of the Himalayas, the *Polistella* fauna in the northern parts of Vietnam would be a key to understanding the process of forming the current distribution pattern of these *Polistella* wasps.

While the *Polistella* fauna of northwestern Vietnam has been more or less well studied (Nguyen et al. 2011), that in northeastern Vietnam has been little known. The present study has recognized seven species of *Polistes (Polistella)* including a new species described herein to occur in northeastern Vietnam. Their distribution records are remarked. Nests of three species (*P. delhiensis* Das & Gupta, *P. mandarinus* de Saussure and *P. brunetus* Nguyen & Kojima, sp. n.) are also described.

Materials and methods

Based on the geographical and climatic features, "northeastern Vietnam" is used in the present paper for the area consisting of the following provinces: Ha Giang, Cao Bang, Tuyen Quang, Bac Kan, Thai Nguyen, Lang Son, Bac Giang and Quang Ninh (Fig. 1). The specimens examined in the present study are unless otherwise mentioned deposited in the Institute of Ecology and Biological Resources in Hanoi; they were mainly collected by ourselves during a research trip to Cao Bang, Bac Kan and Bac Giang made in 2012.

The adult morphological and color characters except for male terminal sterna and genitalia were observed on pinned–and–dried specimens under a stereomicroscope. Apical parts of male metasomata were dissected for the terminal sterna and genitalia. They were put in lactic acid for several hours, washed in distilled water, and observed in glycerin under a stereomicroscope. The terminology of male genitalia follows Kojima (1999). Drawings were made with the aid of a drawing tube. Photos were taken with Panasonic Lumix DMC–FX 100 and Leica EZ4HD 3.0 MegaPixel Digital Stereo Microscope, using LAS exclusive microscopy software (LAS EZ 2.0.0).

In the descriptions of morphology, the following abbreviations are used: POD, distance between the inner margins of the posterior ocelli; OOD, distance between



Figure 1. Map of Vietnam showing the provinces in the northeastern part (green) and those in which the specimens examined were collected (light violet).

the outer margin of the posterior ocellus and the inner margin of the eye at vertex; Od, transverse diameter of the posterior ocellus.

The parts measured for the morphometric are defined as follows: body length, the lengths of head, mesosoma and first two metasomal segments combined; clypeus width, the distance between the uppermost points where clypeus touches the eyes; clypeus height, the distance from the bottom of the dorsal emergination to the apex; distance between inner eye margins at vertex and at clypeus, respectively the distance between the inner eye margins at the level of anterior ocellus in frontal view of head and at the level where inner eye margins approached each other most closely; interantennal and antennocular distances, the distance between the inner margins of antennal sockets and between the outer margin of antennal socket and inner eye margin at the level of middle of antennal socket, respectively; antennal socket width, the transverse diameter; eye and gena width, the maximum width for each in strictly lateral view of the head; metasomal tergum I length, the distance in lateral view from the posterior end of the tergum; metasomal tergum II, length, the distance in lateral view from the bottom of the basal depression or "neck" to the posterodorsal end of the tergum; metasomal tergum I and II width, the maximum width for each in dorsal view.

Taxonomy and distribution

Polistes (Polistella) dawnae Dover & Rao, 1922 http://species-id.net/wiki/Polistes_dawnae

Polistes dawnae Dover & Rao, 1922: 248, female, Dawna Hills, Burma [Myanmar], [holotype in the Zoological Survey of India, Calcutta].

Material examined. Northeastern provinces: **Bac Kan:** 2 females, Kim Hy NP, Na Ri, 22°14'N, 106°05'E, alt. ca 300 m, 3.VIII.2012, L.T.P. Nguyen et al. Other province: **Son La:** 1 female, Nong Truong, Moc Chau, 20°50'N, 104°40.283'E, alt. ca 950 m, 2.VII.2013, D.D. Nguyen.

Remarks on distribution. *Polistes dawnae*, one of the two species characterized by a basally strongly swollen second metasomal sternum and recorded in northeastern Vietnam (the other is *P. mandarinus*), was originally described from Dawna Hills [16°50'N, 98°15'E], northern Myanmar, and has been recently recorded in northeastern part of Laos (Gusenleitner 2013) and northern Vietnam, such as in the provinces of Lai Chau, Dien Bien, Hoa Binh (Nguyen et al. 2011), Bac Kan and Son La (present study). This species may be restricted in its distribution to the areas on the eastern slope of the Himalaya.

Polistes (Polistella) mandarinus de Saussure, 1853

http://species-id.net/wiki/Polistes_mandarinus

Polistes mandarinus de Saussure, 1853 in de Saussure 1853–1858: 58, female, "Le norde de la Chine" [lectotype in The Natural History Museum, London].

Material examined. Northeastern provinces: Cao Bang: 12 males, 4 females, Phi Oac Nature Reserve, Thanh Cong, Nguyen Binh, 22°32.5'N, 105°53'E, alt. ca

1000 m, L.T.P. Nguyen et al. [11 males, 3 females, Nest#VN-NE2012-P-07, 8.VIII.2012; 1 male & 1 female, 9.VIII.2012]; 2 females, Phi Oac Nature Reserve, 22°35.567'N, 105°51.417'E, alt. ca 1035 m, 7-10.V.2013, T.V. Hoang. **Bac Kan:** 7 females, Kim Hy NP, Na Ri, alt. ca 600-700 m, 22°19'N, 105°54'E, Nest#VN-NE2012-P-03, 4.VIII.2012, L.T.P. Nguyen et al. Other province: **Hai Phong:** 1 female, Cat Ba NP, Cat Hai, 20°47'N, 106°59'E, 26.VII.2013, L.T.P. Nguyen & D.D. Nguyen.

Remarks on distribution records. The distribution records of *P. mandarinus* reported may need confirmation as several species were erroneously identified as "*P. mandarinus*" (see Kojima 1997). In Vietnam, this species has been known from the provinces of Quang Tri (Nguyen and Ta 2008), Phu Tho, Vinh Phuc, Thua Thien Hue (Nguyen et al. 2011), Cao Bang, Bac Kan, Hai Phong (present study); this species may occur in the areas north of the Hai Van Pass, but its occurrence in nothwestern Vietnam needs further researches. The species has also been recorded from eastern China and Tibet (Hou et al. 2012) and Korea (Carpenter 1996), however its occurrence in Korea may need confirmation (J.K. Kim & J. Kojima, unpublished data).

Polistes (Polistella) delhiensis Das & Gupta, 1989

http://species-id.net/wiki/Polistes_delhiensis

Polistes delhiensis Das & Gupta, 1989: 63, female, Delhi [India], [holotype in Zoological Survey of India, Calcutta].

Material examined. Northeastern provinces: **Ha Giang:** 1 female, Cao Bo, Vi Xuyen, 22°44'N, 104°54'E, alt. ca 532 m, 21–24.IV.2000, L.T.P. Nguyen; **Bac Kan:** Kim Hy NP, Na Ri, 22°14'N, 106°05'E, alt. ca 600–700 m, 4.VIII.2012, L.T.P. Nguyen et al. [1 female, 3 females of Nest# VN–NE2012–P–04]; 2 females, Kim Hy NP, Vu Muon, Bach Thong, alt. ca 550 m, 22°12.5'N, 105°58'E, 5.VIII.2012, L.T.P. Nguyen et al. Other provinces: **Phu Tho:** 2 females, Xuan Son NP, 21°10'N, 104°58'E, alt. ca 400 m, 11–12.VI.2004, L.T.P. Nguyen; **Hoa Binh:** Pa Co, Mai Chau, 20°44'N, 104°55'E [1 female, alt. ca 1000 m, 27.VI.2001; 2 females, alt. ca 1000 m, 22–23. IV.2002, T.V. Hoang; 1 female, alt. ca 1350 m, 28.VIII.2006, L.T.P. Nguyen, F. Saito & J. Kojima]; **Vinh Phuc:** 1 female, Tam Dao NP, 21°32'N, 105°37'E, alt. ca 800 m, 2.VII.2003, L.T.P. Nguyen.

Remarks on distribution. This species could be placed in the "Stenopolistes" group and has been recorded from Delhi in India and North Vietnam [Son La (Nguyen and Pham 2011), Ha Giang, Bac Kan, Phu Tho, Hoa Binh, Vinh Phuc (present study)]. The other two species of the "Stenopolistes" group occurring in Vietnam, *P. nigritarsis* Cameron and *P. khasianus* Cameron, similarly have such the disjunct distribution records, which are probably due to lack of intensive field works in the areas in the southern slope and western part of the eastern slope of the Himalaya.

Polistes (Polistella) japonicus de Saussure, 1858

http://species-id.net/wiki/Polistes_japonicus

Polistes japonicus de Saussure, 1858: 260, female, "le Japon" [lectotype in the Museum d'Histoire Naturelle, Géneve].

Material examined. Northeastern provinces: Bac Kan: 4 females, Kim Hy NP, Na Ri, 21°15'N, 106°06'E, alt. ca 270 m, 3-4.VIII.2012, J. Kojima, H. Nugroho et al.; Bac Giang: 2 females, Thanh Son, Son Dong, 21°13'N, 106°45'E, alt. ca 300 m, 1.VII.2010, P.H. Pham; Tay Yen Tu NP, Son Dong, 21°21'N, 106°11'E, P.H. Pham [2 females, alt. ca 200-300 m, 3.VII.2010; 1 female, alt. 150 m, 2.VII.2010]; 1 female, Khe Ro, Son Dong, 17.V.2013, D.D. Tran. Other provinces: Son La: 1 female, 20°50'37"N, 104°40'17"E, alt. ca 950m, Nong Truong, Moc Chau, 2.VII.2013, D.D. Nguyen; Lang Son: 1 female, Bac Son, 21°54'N, 106°19'E, 1.VII.2003, L.X. Truong. Other provinces: Phu Tho:1 male, 6 females, Xuan Son NP, 21°10'N, 104°58'E, alt. ca 200-600 m, 13-16.VI.2004, L.T.P.Nguyen; 1 female, Xuan Dai, Tan Son, 20.V.2011, P.H. Pham; Ninh Binh: 1 male, 3 females, Cuc Phuong NP, 20°19'N, 105°37'E, 7-9.V.2002, T. V. Hoang; Thanh Hoa: 1 male, 2 females, Lung Cao, Ba Thuoc, 20°28'N, 105°10'E, alt. ca 500 m, 12.VI.2003; 1 female, Hon Can, Van Xuan, Thuong Xuan, 23-24.VIII.2012, L.T.P. Nguyen & T.V. Hoang; Nghe An: Mon Son, Con Cuong 18°56'N, 104°56'E [2 males, 2 females, 22-24.VII.2004, L.T.P. Nguyen; 1 female, 9.VIII.2002; 3 females, 11.VIII.2002; 2 females, 13.IX.2005]; 1 female, Pu Mat NP, 19°6'N, 104°44'E, 26.VII.2004, L.T.P. Nguyen; 1 female, Chau Cuong, Quy Hop, 19°21'N, 105°6'E, 14-19.VII.2004, H.X. Le; 1 female, Chau Thanh, Quy Hop, 19°23'N, 105°2'E, 16.VII.2004, H.X. Le; 1 female, Co Phat, Con Cuong, 18°53'N, 104°52'E, ca 200 m, 22.VII.2006, ISD-c; 1 female, Tuong Duong, Con Cuong, 19°20'N, 104°34'E, 12.VII.2006; Ha Tinh: 4 males, 1 female, Son Tay, Huong Son, 18°27'N, 105°20'E, 19-27.V.2004, L.T.P. Nguyen; 1 female, Rao An, Huong Son, 18°34'N, 105°10'E, 20.IV.1998, L.D. Khuat.

Remarks on distribution. In Vietnam, this species has been recorded in the provinces of Ha Giang, Lai Chau, Hoa Binh, Ha Noi, Thua Thien Hue (Nguyen and Khuat 2003), Phu Tho, Hai Phong (Nguyen et al. 2005), Quang Binh, Quang Tri, Thua Thien Hue, Quang Nam (Nguyen and Ta 2008), Son La (Nguyen and Pham 2011), Bac Kan, Lang Son, Bac Giang, Ninh Binh, Thanh Hoa, Nghe An, Ha Tinh (present study), showing that the species is widely distributed in Vietnam except for southern provinces. This species could occur widely in eastern parts of subtropical and temperate Asia, from Vietnam, through eastern parts of continental China, to Korea and Honshu Island of Japan; its closely related species, *P. formosanus* Sonan may co-occur with this species in Taiwan and only *P. formosanus* is known to occur in the Nansei Islands (Saito et al. 2007).

Polistes (Polistella) sagittarius de Saussure, 1853

http://species-id.net/wiki/Polistes_sagittarius

Polistes sagittarius de Saussure, 1853: 56, female, "Les Indes-Orientales, la Chine" [syntypes in the Museum d'Histoire Naturelle, Genève, and The Natural History Museum, London].

Material examined. Northeastern provinces Ha Giang: 1 male, Tung Ba, Vi Xuyen, 24.VI.2013, T.V. Nguyen; Bac Kan: 2 females, Kim Hy NP, Na Ri, alt. ca 600–700 m, 22°19'N, 105°54'E, 4.VIII.2012, L.T.P. Nguyen et al.; Bac Giang: 1 female, Thanh Son, Son Dong, 21°13'N, 106°45'E, 7.VII.2010, D.D. Tran. Other provinces: Hai Phong: 1 female, Cat Ba NP, Cat Hai, 20°43'N, 107°04'E, alt. ca. 30 m, 26.VII.2013, L.T.P. Nguyen & D.D. Nguyen; Other provinces: Lao Cai: 1 female, Ta Chai, Bac Ha, 22°31'N, 104°17'E, 26.VI.2008, L.T.P. Nguyen & P.H. Pham; Nghe An: 1 female, Mon Son, Con Cuong, 18°56'N, 104°56'E, 27.VII.2004, L.T.P. Nguyen; 1 female, Khe Bo, Con Cuong, 19°03'N, 104°43'E, alt. ca 120 m, 25–28. IV.1998, J.M. Carpenter; Gia Lai: 3 females, Ia Pal, Chu Se, 13°39'N, 108°08'E, alt. ca 370 m, 20–21.VII.2012, L.T.P. Nguyen; Binh Duong: 2 females, Binh Hoa, Thuan An, 10°54'N, 106°43'E, 20.VII.2002, L.D. Khuat.

Remarks on distribution. This species is widely distributed in southern Asia with subtropical and tropical climates, from northwestern India in the west, through continental southeast Asia, to Palawan in the Phillippines, Sulawesi and Flores in the Lesser Sunda Islands in the east. Recorded widely in the provinces of Ha Giang, Lai Chau, Vinh Phuc, Ha Tay (Nguyen and Khuat 2003), Son La, Hoa Binh (Nguyen and Pham 2011), Lao Cai, Bac Kan, Bac Giang, Hai Phong, Nghe An, Gia Lai, Dak Lak, Binh Duong (present study), this species may occur throughout Vietnam.

Polistes (Polistella) strigosus Bequaert, 1940

http://species-id.net/wiki/Polistes_strigosus

Polistes strigosus Bequaert, 1940: 269, female, male "Wong-Sa-Shui, South Kwangsi, China" [holotype female in the Museum of Comparative Zoology, Cambridge, USA].

Material examined. Northeastern provinces: **Ha Giang:** 1male, 6 females, Cao Bo, Vi Xuyen, 22°44'N, 104°54'E, 21.X.2006, L.D. Khuat; **Cao Bang:** 1 female, Phi Oac NR, Thanh Cong, Nguyen Binh, 22°35'34"N, 105°51'25"E, alt. ca 1035 m, 7–10.V.2013, T.V. Hoang; **Lang Son:** 1 female, Nong truong Thai Binh, Dinh Lap, 16.V.2013, D.D. Tran; **Bac Giang:** Son Dong, P.H. Pham [1 male, 2 females, Thanh Lam, 21°20'N, 106°19'E, alt. ca 120 m, 4.VII.2010; 1 female, Tay Yen Tu NP, 21°24'N, 106°56'E, alt. ca 150m, 2.VII.2010]. Other provinces: **Vinh Phuc:** 1 female, Tam Dao NP, 21°27'N, 105°39'E, alt. ca 1200 m, 2.VII.2003, L.T.P. Nguyen; **Ha**

Noi: 1 female, Khat Thuong, Ba Vi, 21°5'N, 105°22'E, alt. ca >100 m, 16.VIII.2006, ISD-c; 1 female, Yen Bai, Van Hoa, Ba Vi, 21°1'N, 105°27'E, 15.VIII.2006, ISD-c; **Nghe An:** 3 females, Chau Cuong, Quy Hop, 19°21'N, 105°6'E, 14–19.VII.2004, H.X. Le; 1 female, Pha Lay, Mon Son, Con Cuong, 18°56'N, 104°56'E, 9.VIII.2002, ISD-c; **Ha Tinh:** 1 male, 1 female, Son Tay, Huong Son, 18°27'N, 105°21'E, 19–27.V.2004, L.T.P. Nguyen; Ta Rut, Dakrong, Quang Tri 16°25'N, 106°59'E [4 females, 17.VII.2004; 9 females, alt. ca 400–500 m, 17.VII.2004], ISD-c.

Remarks on distribution. The following three subspecies are currently recognized in *P. strigosus*: the nominotypoical subspecies known to occur in Laos, China and Taiwan; *minimus* Bequaert, 1940 distributed in Nepal, Malaysia (Sabah) and the Philippines; and *atratus* Das and Gupta, 1984 in India. The color form from Vietnam agrees with non of the above-mentioned subspecies. It has the head reddish brown, mesosoma dark yellowish brown with metanotum and propodeum dark yellow, metasomal terga I–III dark yellow, and the other metasomal terga brownish black (in some specimens, all metasomal terga dark yellow). This species is widely recorded from the provinces of Hai Phong (Nguyen et al. 2005), Quang Binh, Quang Tri, Thua Thien Hue (Nguyen and Ta 2008), Hoa Binh (Nguyen and Pham 2011), Ha Giang, Cao Bang, Lang Son, Vinh Phuc, Bac Giang, Ha Noi, Nghe An, Ha Tinh (present study), and may occurs in eastern parts of Vietnam north of the Hai Van Pass.

Polistes (Polistella) brunetus Nguyen & Kojima, sp. n. http://zoobank.org/CFB91293-9882-43F9-97EB-85472BB3FCB4 http://species-id.net/wiki/Polistes_brunetus

Type-locality. Vietnam, Bac Kan: Kim Hi National Park, Lang San, Na Ri, 22°19'N, 105°54'E, 600–700 m.

Type specimens. Holotype, female, pinned (deposited in the Institute of Ecology and Biological Resources, Hanoi). Original label: "VIETNAM, Kim Hy NP, Lang San, Na Ri, Bac Kan, 22°19'N, 105°54'E, 600–700 m, Nest#VN–NE2012–P–02, 4.viii.2012, J.Kojima, L.T.P. Nguyen & IED–c". Paratypes: **Bac Kan:** 2 females & 1 male, 1 female (Natural History Collection at Ibaraki University, Mito), same data as holotype.

Other material examined. Northeastern provinces: Ha Giang: 1 female, Tung Ba, Vi Xuyen, 3.VII.2013, T.V. Nguyen; Lang Son: 1female, Bac Son, 21°54'N, 106°19'E, 1.VII.2003, L.X. Truong; Bac Giang: Son Dong, P.H. Pham [2 females, Tay Yen Tu NP, 21°24'N, 106°54'E, alt. ca 200–300 m, 3.VII.2010; 1 male, 1 female, Thanh Lam, 21°19'N, 106°20'E, alt. ca 120 m, 4.VII.2010]; 1 female, Khe Ro, Son Dong, 17.V.2013, D.D. Nguyen. Other provinces: Hai Phong: 1 male, Cat Ba NP, 20°42'N, 107°4'E, 15–18.VIII.2003, L.T.P. Nguyen; Phu Tho: Xuan Son NP, 21°8'N, 104°59'E, alt. ca 400–600 m [2 females, 11.VI.2004; 1 female, 13.VI.2004; 1 female, 16.VI.2004], L.T.P. Nguyen; Hoa Binh: 1 male, Lac Thinh, Yen Thuy, 20°24'N, 105°33'E, 30.IV.2002, L.D. Khuat; 1 female, Da Phuc, Yen Thuy, 20°24'N, 105°33'E, 27.VII.2000, L.X. Truong; Ninh Binh: 1 male, 2 females, Cuc Phuong NP,

20°18'N, 105°37'E, 7.V.2002, T. V. Hoang; **Thanh Hoa:** 1 female, Bat Mot, Thuong Xuan, Xuan Lien NP, 19°59'N, 104°59'E, ca 705 m, 23.IV.2013, L.T.P. Nguyen; **Nghe An:** 1 female, Chau Cuong, Quy Hop, 19°21'N, 105°6'E, 14–19.VII.2004, H.X. Le; **Thua Thien Hue:** 1 female, Phu Loc, Bach Ma NP, 30.V.2001, L.D. Khuat.

Diagnosis. This species can be distinguished from the other *Polistes (Polistella)* species by the following combination of characters: pronotum with dense and coarse punctures, their edges forming reticulation; metasomal sternum II in lateral view swollen ventrally in anterior half; sternum IV with two long parallel longitudinal ridges medially (this character also occurs in *P. japonicus*); proximal margin of the penis valve of the male genitalia in lateral view produced ventrally at proximoventral to form a small tooth.

Description. Female. Body length 15–16.5 mm (holotype: about 16 mm); fore wing length 16–17 mm (holotype: about 16.5 mm).

Head in frontal view about 1.1 times as wide as high (Fig. 2); in dorsal view weakly swollen laterally behind eyes, then narrowed posteriorly, with posterior margin shallowly and broadly emarginate. Vertex slightly raised in area among ocelli, slightly sloped down behind posterior ocelli towards occipital carina; POD:OOD = about 1:1.7; POD about 1.2 times Od (Fig. 3). Gena, in lateral view about 0.8 times as wide as eye (Fig. 4); occipital carina fine, evanescent in ventral one third of gena. Inner eye margins weakly convergent ventrally, in frontal view about 1.1 times further apart from each other at clypeus than at vertex (Fig. 2). Antennal sockets closer to inner eye margin than to each other; anterior tentorial pit slightly further apart from antennal socket than from inner eye margin; interantennal space weakly raised. Clypeus in frontal view as wide as high, produced ventrally into blunt angle; in lateral view weakly swollen anteriorly (Fig. 3); length of lateral margin of clypeus lying along inner eye margin longer than diameter of antennal socket and about as long as the length of malar space. Antenna (Fig. 5): scape more than 3 times as long as its maximum width; flagellomere I about 3 times as long as its maximum width, about 1.2 times as long as the length of flagellomeres II and III combined; flagellomere II and III longer than wide; terminal flagellomere bullet-shaped, about 1.4 times as long as its basal width.

Pronotal carina sharply raised, produced dorsally into thin lamella in dorsal part, slightly sinuate backward on lateral side, reaching ventral corner of pronotum. Mesocutum weakly convex, about 0.9 times as long as wide between tegulae; anterior margin broadly rounded. Scutellum convex, slightly concave medially. Metanotum weakly convex, disc nearly flat but strongly depressed anterior margin. Propodeum short; posterior face widely (about half the maximum width of propodeum) and shallowly excavated medially, more or less smoothly passing into lateral faces; propodeal orifice elongate, about 1.8 times as long as wide (measured at widest part), somewhat narrowed in dorsal half. Wings hyaline, jugal lobe of hind wing rounded (Fig. 7).

Metasomal tergum I short and thick, about 0.8 times as long as its apical width, in lateral view abruptly swollen dorsally just behind basal slit for reception of propodeal suspensory ligament; corner between anterior and dorsal faces bluntly angled (Fig. 8). Sternum II in lateral view swollen ventrally in smoothly curved line in anterior half, then ventral margin nearly straight line parallel to ventral margin of the tergum.



Figures 2–9. *Polistes (Polistella) brunetus* sp. n., female. 2–5 Head 2 Frontal view 3 Vertex in dorsal view 4 Lateral view 5 Right antenna 6 Pronotum in lateral view 7 Jugal lobe of left hind wing 8 Metasomal segments I and II, lateral view 9 Metasomal segment II–VI. Scale 1 mm.

Clypeus with scattered large punctures, each bearing sharply pointed golden bristle; tomentum on clypeus medially restricted in dorsal one-fourth of clypeus, laterally extending ventrally. Mandible with several small and shallow punctures at base and deep punctures at anterior margin. Frons covered with deep punctures. Vertex and gena with sparse small and shallow punctures; area around ocelli smooth; ventral one third of gena with coarse punctures. Pronotum with dense, coarse punctures, their edges forming reticulation (Fig. 6). Mesocutum densely with coarse flat-bottomed punctures; punctures on scutellum and metanotum dense coarser but smaller than those on mesoscutum. Mesepisternum with dense coarse well-defined punctures in posterodorsal part (punctures in dorsal margin similar to those on pronotum), scattered punctured in anteroventral part; border between posterodorsal and anteroventral parts indistinct. Dorsal metapleuron with striae and shallow large punctures; ventral metapleuron with sparse strong punctures. Propodeum with strong transverse striae; lateral face with sparse ill-defined punctures. Metasomal segements covered with minute punctures in addition to scattered small punctures (stronger and larger on sterna) except sternum IV with two long medial parallel longitudinal ridges along sternum and several shorter ridges on each side of the long one ended by large shallow punctures (Fig. 9), area between paired longitudinal ridges smooth; sternum II-IV each with a stuff of long hair at apical margin, sternum V and VI entirely covered with long hairs.

Dark brown; following parts yellow to orange-yellow: clypeus except apical black margin, mandible except a black spot at base and apical margin, and narrow band along inner eye margin extending from bottom of frons to middle of eye emargination; following parts black: area around ocelli, apical margin and a longitudinal line along lateral faces and at the middle of propodeum, spot on valvula, mid and hind coxae and trochanters beneath.

Male. Body length about 13.5–15.5 mm; fore wing length about 15.5–16.5 mm.

Like female, but differing from the latter as follows: head about 1.2 times as wide as high in frontal view (Fig. 10); eye strongly swollen laterally; inner eye margins about as long from each other at vertex as at clypeus; gena in lateral view about half as wide as eye (Fig. 11), with weakly raised blunt ridge running along posterior margin of eye; clypeus in frontal view about as wide as high (Fig. 10), only slightly produced ventrally, evenly and slightly convex apically, in lateral view weakly convex in dorsal part (Fig. 11). Antenna (Fig. 12) slenderer than in female; scape short, about 2.8 times as long as its maximum width; flagellomere I longer than length of flagellomeres II and III combined; flagellomeres II and III each longer than wide; terminal flagellomere elongate, slightly curved, about 2.5 times as long as its basal width. Metasomal sternum VII depressed medially (Fig. 13), without tubercle (Fig. 14).

Body surface sculpture as in female, but clypeus without large punctures and densely covered with long golden hairs and with a faint longitudinal ridge medially.

Genitalia in general as that of *Polistes* species, with the following specific characters: digitus in inner aspect of paramere (Fig. 15) about 3.2 times as long as wide (measured at widest part), distinctly swollen near base, gradually narrowed apically to midlength, then slightly swollen towards the rounded apex; aedeagal penis valves (Figs 16–17) slightly longer than aedeagal basal apodeme, in ventral view narrowest near mid-length, weakly swollen proximally from mid-length then strongly swollen and distinctly produced laterally near proximal margins, in lateral view slightly thickened



Figures 10–17. *Polistes (Polistella) brunetus* sp. n., male. 10–11 Head 10 Frontal view 11 Lateral view 12 Right antenna 13–14 Terminal sterna 13 Ventral view 14 Apical part, lateral view 15 Inner aspect of paremere with digitus and volsella 16–17 Aedeagus 16 Ventral view 17 Lateral view. Scale 1 mm.

in proximal one fourth and with dorsal margin weakly and smoothly sinuate, with proximoventral corner produced into abuse angle (Fig. 17); ventral margins of penis valves finely serrated along entire length.

Color and marking pattern similar to female, but more extensively marked with yellow as follows: clypeus except a broad longitudinal median band, narrow long band on gena along posterodorsal margin of eye, antennal flagellomeres beneath, narrow band along pronotal lamella, narrow band on basal metanotum, paired longitudinal lines on lateral face of propodeum, valvulae, narrow band at apical margin of tergum I–III and sternum II and III; more extensively black marked as follow: two longitudi-

nal bands at lateral margin on mesoscutum, a wide band on basal margin of tergum I and II (sometimes on tergum III); spot at upper corner of meseposternum (closed to dorsal part of metapleuron); propodeum and legs more black.

Etymology. The specific name, *brunetus*, is a Latin adjective, referring to the brown body color.

Distribution. Known only from localities in northern Vietnam listed above.

Nests

Polistes (Polistella) mandarinus de Saussure, 1853

Hou et al. (2012) described the nest of this species based on the nests observed in Tibet, with light ferruginous brown (juggling from the figures). A nest (#VN-NE2012-P-02) (Fig. 18) that we collected, together with 3 females and 11 males, at Phi Oac NR, Cao Bang Province has similar features of that described by Hou et al. (2012) although it differs in coloration. Our nest has 19 cells and had produced more than ten adult wasps. Its structural and color characters are as follows: Comb "paper"-like in texture, made mainly of long fine plant fibers and wasp adult oral secretion, more or less uniformly dark greysish-brown in cell walls, suboval (about 30 mm × 20 mm) in view from side of cell opening, expanded excentrically from the single terminal petiole, with surface corresponding to cell bottom weakly convex; Petiole single, terminal, attached to the border between bottoms of the first two cells, 2.5 mm long and 1.2 mm × 1.5 mm thick at the mid-length, with thin central core of plant fibers, enlarged strictly with adult oral secretion, blakish brown and lustrous, secretion coat widely expanded on comb back around the petiole and on substrate in thin film holding the fern vain; **Cells** generally arranged in regular rows, pentagonal at open end when surrounded by other cells, with free margins rounded, each cell weakly expanded towards open end, 5.3 mm × 5.6 mm (range 5.0 mm × 5.4 mm – 5.8 mm × 5.9 mm; n=17) wide at open end, 3.4 mm (range 3.1 - 3.8 mm; n=11) wide at bottom and 19 mm (range 15-22.5 mm; n=13) deep in cells containing pupae or having produced adult, cell wall about 1.12 mm thick; Cocoon cap white, produced beyond rim of cell by 0.5-4.5 mm, slightly domed.

Polistes (Polistella) brunetus Nguyen & Kojima, sp. n.

A pre-emergence stage (before any adult wasps' emergence) nest (#VN-NE2012-P-02) (Fig. 19) was collected, together with 4 adult females, at Kim Hy NP, Bac Kan Province. The nest was attached to a rattan shoot, at about 2.5 m above the ground, and has 26 cells, with the fifth (=last) instar larvae as the oldest immature (for immature composition, see Fig. 19). The fifth instar larvae were artificially fed with fresh hornet (*Vespa*) eggs, and one of them successfully spun the cocoon. The structural and



Figures 18-20. Nests. 18 Polistes mandarinus 19 P. brunetus sp. n. 20 P. delhiensis.

color characters are as follows: **Comb** "paper"–like in texture, made mainly of long fine plant fibers, usually with 2–3 mm wide horizontal stripes of different colors (pale gray to gray and pale brownish–gray) in cell walls, subcircular (about 30 mm × 25 mm) in view from side of cell opening, expanded concentrically from the single petiole, with surface corresponding to cell bottom weakly convex; **Petiole** single, central, attached to the border between bottoms of the first two cells, 3.8 mm long and 1.2 mm × 1.2 mm thick at the mid–length, with thin central core of plant fibers, enlarged strictly with oral secretion of adults, brown and lustrous, secretion coat widely expanded on comb back around the petiole and on substrate in about 8 mm × 9 mm subcircular

thin film; **Cells** generally arranged in regular rows, pentagonal at open end when surrounded by other cells, with free margins rounded, each cell weakly expanded towards open end, $6.3 \text{ mm} \times 6.7 \text{ mm}$ (range $6.0 \text{ mm} \times 6.1 \text{ mm} - 7.1 \text{ mm} \times 7.2 \text{ mm}$; n=5) wide at open end, 5.1 mm (range 4.9-5.4 mm; n=5) wide at bottom and 18.5 mm (range 17-19.5 mm; n=5) deep in cells containing mature larvae, cell wall about 0.09 mm thick; **Cocoon cap** white, slightly domed.

Polistes (Polistella) delhiensis Das & Gupta, 1989

A pre-emargence stage (before any adult wasps' emergence) nest (# VN-NE2012-P-04) (Fig. 20) examined was collected, together with 3 females, at Kim Hy NP, Bac Kan province. The nest was attached to a broad leaf, at about 1 m above the ground, and has 19 cells, which contained the pupae as the oldest immatures. The structural and morphological characters are as follows: Comb "paper"-like in texture, made mainly of long fine plant fibers mixed with adult oral secretion, pale brown to brown in cell walls, subcircular (about 19 mm × 17 mm) in view from side of cell opening, expanded concentrically from the single petiole, with surface corresponding to cell bottom slightly convex; Petiole single, central, attached to the border between bottoms of the first two cells, 2.5 mm long and 0.5 mm × 0.7 mm thick at the mid-length, with thin central core of plant fibers, enlarged strictly with adult oral secretion, dark brown and lustrous, secretion coat widely expanded on comb back around the petiole and on substrate in about 2.5 mm × 5 mm thin film; Cells generally arranged in regular rows, pentagonal at open end when surrounded by other cells, with free margins rounded, each cell weakly expanded towards open end, 4.4 mm × 4.5 mm (range 4.3 mm × 4.4 mm – 4.5 mm × 4.5 mm; n=4) wide at open end, 3.2 mm (range 3.0 -3.3 mm; n=4) wide at bottom and 14 mm (range 13.5–14.5 mm; n=4) deep in cocooned, cell wall about 0.06 mm thick; **Cocoon cap** pale greenish-yellow, prominently produced beyond rim of cell by 5-6 mm, slightly domed.

Key to species of Polistes (Polistella) of northeastern Vietnam

The characters given in the key are applicable to both sexes unless when specified.



Figures 21–28. *Polistes* species characters. 21, 23, 25 *P. mandarinus*: 21 Metasomal segment I and II, lateral view 23 Head, lateral view 25 Mesosoma, lateral view 22, 24 *P. dawnae*: 22 Head, lateral view 24 Mesosoma, lateral view 26 P. sagittarius, pronotum, lateral view 27–28 *P. strigosus*: 27 Pronotum, lateral view 28 Metasomal sternum VII, ventral view. Scale 1 mm.

_	Clypeus in lateral view distincly convex (Fig. 23). Disc of scutellum convex (Fig. 25). Male clypeus about 1.1 times as wide as high
	P. mandarinus de Saussure
3	Medium-sized wasps; fore wing length 10.5-14.5 mm. Jugal lobe of fore
	wing much reduced. Marginal cell of fore wing with dark spot at apex
	<i>P. delhiensis</i> Das & Goupta
-	Large-sized wasps; fore wing length 15.5-18 mm. Jugal lobe of fore wing
	large, rounded. Marginal cell of fore wing without dark spot4
4	Pronotum with strong striation (Figs 26, 27) Female metasomal sterna with- out longitudinal ridges
_	Pronotum with weak striation weak or absent. Female metasomal sternum IV
	medially with paired longitudinal ridges (Fig. 9)6
5	Pronotal striation somewhat irregular (Fig. 26), spaces between striae densely
	and distincly punctured. Dorsal surface of pronotum smoothly curved down
	to the lateral surface. Male metasomal sternum VII without tubercle. Cl-
	ypeus, mesoscutum, metasomal segments III-VI entirely black
	<i>P. sagittarius</i> de Saussure
_	Pronotal striation regular and very strong (Fig. 27). Border between dorsal
	and lateral surfaces of pronotum distinctly angled. Male metasomal sternum
	VII with weak tubercle (Fig. 28). Clypeus, mesoscutum, metasomal segments
	II-VI etirtely brown
6	Pronotum with dense, coarse punctures, their edges forming reticulation (Fig. 6).
	Disc of scutellum convex. Metasomal sternum II in lateral view convex ventrally
	in anterior half. Anterior margin of male clypeus nearly straight (Fig. 10). Meta-
	somal terga brown, with some black marks. Wings hyaline
	<i>P. brunetus</i> Nguyen & Kojima, sp. n.
_	Pronotum with sparse, small punctures. Disc of scutellum hardly convex. Meta-
	somal sternum II in lateral view weakly convex ventrally in the anterior two
	thirds. Anterior margin of male clypeus rounded. Metasomal terga yellow with
	dark brown and/or black bands. Wings infuscate P. japonicus de Saussure

Conclusion

Compared with the *Polistella* fauna in mountainous areas of northern (mainly northwestern) Vietnam, where 14 *Polistella* species have been recognized (Nguyen et al. 2011), *Polistella* fauna in northeastern Vietnam, with only seven species, is poorer even though the environmental, especially climatic conditions, in northeastern Vietnam are expected to be more diverse and hence to harbor richer fauna in terms of number of species than in mountainous areas of northern Vietnam. On the other hand, however, in contrast to the fact that all the 14 *Polistella* species occurring in mountainous areas in northern Vietnam may belong to a possible monophyletic species group that is characterized by a basally strongly swollen second metasomal

sternum and shows ditribution pattern of so-called "Himalayan Corridor origin", seven species recognized in northeastern Vietnam are comprised of at least three species groups, thus they are more diverse phylogenetically than those of mountainous areas of norther Vietnam. Namely, other than *P. dawnae* and *P. mandarinus* in the species group characterized by a basally strongly swollen second metasomal sternum, *P. delhiensis* belongs to so-called "*Stenopolistes*" group, the species belonging to which are distributed in tropical and subtropical continental Asia, so-called Sunda Land (Malay Peninsular, Sumatra and Borneo) and also in Papuan Region, including Pacific Islands. The last group, including the four species recognized in northeastern Vietnam, *P. japonicus, P. sagittarius, P. strigosus* and *P. brunetus*, is the *P. sagittarius* group of Carpenter (1996), which is rather ill-defined and known to be widely distributed in Oriental Region and East Asia.

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References

- Bequaert JC (1940) Note on Oriental *Polistes* wasps (Hymenoptera: Vespidae). Transaction of the American Entomology Society 66: 265–272.
- Carpenter JM (1996) Distributional checklist of species of the genus *Polistes* (Hymenoptera: Vespidae; Polistinae, Polistini). American Museum Novitates 3188: 1–39.
- Das BP, Guputa VK (1984) A catalogue of the families Stenogastridae and Vespidae from the Indian subregion (Hymenoptera: Vespidae). Oriental Insects 17: 395–464. doi: 10.1080/00305316.1983.10433698
- Dover C, Rao S (1922) A note on the diplopterous wasps in the collection of the Indian Museum. Journal and Proceedings of the Asiatic Society of Bengal 18(4): 236–249.
- Gusenleitner J (2011) Eine Aufsammlung von Faltenwespen aus Laos im Biologiezentrum Linz (Hymenoptera: Vespidae: Vespinae, Stenogastrinae, Polistinae, Eumeninae). Linzer Biologische Beitrage 43 (2): 1351–1368.
- Gusenleitner J (2013) Bemerkenswerte Faltenwespen-Funde aus der orientalischen Region Teil 7 (Hymenoptera: Vespidae, Eumeninae, Polistinae). Linzer Biologische Beitrage 45 (1): 121–132.
- Hou QB, Meng FM, Ma SJ, Liang XC (2012) Nesting habits of *Polistes mandarinus* (Hymenoptera: Vespidae) in Bomi, Tibet. Acta Entomologica Sinica 55: 255–258.
- Kojima J (1997) Taxonomic notes on the social wasps in the Kanto Plain, central Japan (Insecta: Hymenoptera: Vespidae). Natural History Bulletin of Ibaraki University 1: 17–44.

- Kojima J (1999) Male genitalia and antennae on an Old World paper wasp genus *Ropalidia* Guérin–Méneville, 1831 (Insecta: Hymenoptera; Vespidae, Polistinae). Natural History Bulletin of Ibaraki University 3: 51–68.
- Nguyen LTP, Khuat LD (2003) A survey of social wasps (Hymenoptera: Vespidae) in Ba Vi and Tam Dao National Parks. Proceedings of the 2nd National Conference on Life Sciences, Hue, July 2003: 658–661. [In Vietnamese with English summary]
- Nguyen LTP, Kojima J, Saito F (2005) Survey of social wasps (Hymenoptera: Vespidae) in Xuan Son and Cat Ba National Parks. Proceedings of the National Conference on Life Sciences, Hanoi Medical University, November 2005: 218–220. [In Vietnamese with English summary]
- Nguyen LTP, Kojima J, Saito F (2011) *Polistes (Polistella)* wasps (Hymenoptera: Vespidae: Polistinae) from mountainous areas of northern Vietnam, with description of five new species. Zootaxa 3060: 1–30.
- Nguyen LTP, Pham PH (2011) Study on social wasps (Hymenoptera: Vespidae) from some natural reserves in northwestern Vietnam. Proceeding of the 7th Vietnam National Conference on Entomology, Hanoi, April 2011: 848–851. [In Vietnamese with English summary]
- Nguyen LTP, Ta TH (2008) Survey of social wasps (Hymenoptera: Vespidae) in Truong Son range, which belong to Quang Binh, Quang Tri, Thua Thien Hue and Quang Nam Provinces. Proceeding of the 6th Vietnam National Conference on Entomology, Hanoi, April 2008: 964–968. [In Vietnamese with English summary]
- Saito F, Kojima J, Nguyen LTP, Kanuka M (2007) Polistes formosanus Sonan, 1927 (Hymenoptera: Vespidae), a good species supported by both morphological and molecular phylogenetic analyses, and a key social wasp in understanding the historical biogeography of the Nansei Islands. Zoological Science 24: 927–939. doi: 10.2108/zsj.24.927
- Saussure H de (1853–1858) Études sur la Famille des Véspides. 2. Monographie des Guêpes Sociales, ou de la Tribe de Vespiens, ouvrage faisant à la Monographies des Guêpes Solitaires. V. Masson, Paris & J. Kessmann, Genéve, i–cxliv + 1–256 pp. + 37 pls. (1853) 1–96 + pls. 2–8, 13; (1854) 97–256 + pls. 9–12, 14–18, 20–24, 27–33; (1855) pl. 1; (1857) i–xlviii + pls. 35–37; (1858) xlix–cxliv + pls. 19 bis, 30 bis.
- Saussure H de (1858) Note sur la famille des Vespides. Revue et Magasin de Zoologie Serie 2, 10: 63–66.

RESEARCH ARTICLE



Temnothorax pilagens sp. n. – a new slave-making species of the tribe Formicoxenini from North America (Hymenoptera, Formicidae)

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Abstract

A new species of the ant genus *Temnothorax* Forel, 1890 – *Temnothorax pilagens* sp. n. is described from eastern North America. *T. pilagens* sp. n. is an obligate slave-making ant with two known hosts: *T. long-ispinosus* (Roger, 1863) and *T. ambiguus* (Emery, 1895). A differential diagnosis against *Temnothorax duloticus* (Wesson, 1937), the other dulotic congener from the Nearctic, is presented and a biological characteristics of the new species is given.

Keywords

Temnothorax, Nearctic region, dulosis, slave-raiding behavior, morphometrics

Introduction

Three slave-making species of the *Temnothorax* genus group (Bolton 2003) of the ant tribe Formicoxenini are known from North America. Each of the three represents an unmistakable combination of phenotypic characters. They all use species of the genus Temnothorax Forel, 1890 as hosts and cluster genetically with species of this genus (Beibl et al. 2005). The first species and outgroup to all the others (Beibl et al. 2005), Protomognathus americanus (Emery, 1895), is characterized by an elongated, semi-rectangular head capsule with extremely long antennal scobes that fully accommodate the short and flattened scape when it is folded back. These characters are a convergence to the Holarctic genus Harpagoxenus that belongs to the distantly related Leptothorax genus group. The second one, Temnothorax duloticus (Wesson, 1937), shows an acute, frontoventrally directed dent on the postpetiolar sternite, a high petiole with a bulky, truncate node that slopes down to the caudal cylinder with a distinct step. This particular petiolar shape and the reduction of antennal segments to 11 resemble the situation in the subgenus Mychothorax Ruzsky, 1904 of the genus Leptothorax Mayr, 1855. However, *T. duloticus* differs from the latter by the absence of a curved transverse carina on the stipes of the maxillae. On the third species, as yet taxonomically undescribed, only little information exists to date (Herbers and Foitzik 2002, Beibl et al. 2005). Its phenotype is an unmistakable combination of an acute, frontoventrally directed dent on postpetiolar sternite, a stout, hump-backed mesosoma, small scape length, a high petiole that is in lateral aspect roughly triangular, a wide petiole and reduced mandibular dentition. Robin Stuart was the first who recognized the new species (Herbers and Foitzik 2002). We follow his proposal to name this slave-making species Temnothorax *pilagens* sp. n. and provide here the formal taxonomic description and differential diagnosis plus a short comparative life history.

Material

Type material

Holotype worker labelled "USA:44.7560°N, 86.0711°W, Michigan: Sleeping Bear National Lakeshore, 180 m, 2013.05.27 – M509" and "Holotype *Temnothorax pilagens* Seifert et al."; 3 paratype workers from the holotype nest and the same collecting data; 1 paratype gyne labelled "USA:44.7560°N, 86.0711°W, Michigan: Sleeping Bear National Lakeshore, 180 m, 2013.05.27 – M502"; 4 paratype workers, each on a separate pin, labelled "USA: 44.8435°N, 86.0612°W, Michigan: North Bar Lake Dunes, 185 m, 2013.05.31 – Q534.3", "USA: 44.8435°N, 86.0612°W, Michigan: North Bar Lake Dunes, 185 m, 2013.05.31 – Q534.1", "USA: 44.8435°N, 86.0612°W, Michigan: North Bar Lake Dunes, 185 m, 2013.05.31 – Q534.2", "USA: 44.8435°N, 86.0612°W, Michigan: North Bar Lake Dunes, 185 m, 2013.05.30 – Q520"; 1 paratype gyne labelled "USA:44.7560°N, 86.0711°W, Michigan: Sleeping Bear National Lakeshore, 180 m, 2013.05.27 – M502". Different codes after the date sequence refer to different nests. All material is stored in the Senckenberg Museum of Natural History in Goerlitz.

Comparative material of *Temnothorax duloticus* (Wesson, 1937) consisted of five workers and one gyne labelled "USA: 39.9927°N, 83.2575°W, Ohio: Prairie Oaks Metro Park, 270 m, 2013.06.04 – Z698" and three workers labelled "USA: 40.1469°N, 83.0381°W, Ohio: Highbanks Metro Park, Olentangy River, 239 m, 2013.06.07 - G827".

Methods

Recording of morphological data

Twenty morphometric characters currently being used in taxonomy of Palaearctic Temnothorax (Seifert 2006, Csösz et al. 2013) were investigated. In bilaterally recorded characters, arithmetic means of both body sides were calculated. All measurements were made on mounted and dried specimens using a pin-holding stage, permitting full rotations around X, Y, and Z axes. A Leica M165C high-performance stereomicroscope equipped with a 2.0 planapochromatic objective (resolution 1050 lines/mm) was used at magnifications of ×120-384. The mean relative measuring error over all magnifications was 0.3%. A Schott KL 1500 cold-light source equipped with two flexible, focally mounted light-cables, providing 30°-inclined light from variable directions, allowed sufficient illumination over the full magnification range and a clear visualization of silhouette lines. A Schott KL 2500 LCD cold-light source in combination with a Leica coaxial polarized-light illuminator provided optimal resolution of tiny structures and microsculpture at highest magnifications. Simultaneous or alternative use of the cold-light sources depending upon the required illumination regime was quickly provided by regulating voltage up and down. A Leica cross-scaled ocular micrometer with 120 graduation marks ranging over 52 % of the visual field was used. To avoid the parallax error, its measuring line was constantly kept vertical within the visual field. Measurements of body parts always refer to real cuticular surface and not to the diffuse pubescence surface.

Z-stack photographs were made with a Leica Z6 APO photomicroscope equipped with 2.0× planapochromatic objective and the automontage software Leica application suite version 3.

- **CL** maximum cephalic length in median line; the head must be carefully tilted to the position with the true maximum. Excavations of occiput and/or clypeus reduce CL.
- **CS** cephalic size; the arithmetic mean of CL and CW, used as a less variable indicator of body size.
- **CW** maximum cephalic width; the maximum is found in *Temnothorax* and *Lep-tothorax* usually across and including the eyes.

- **EYE** eye-size index: the arithmetic mean of the large (EL) and small diameter (EW) of the elliptic compound eye is divided by CS, i.e. EYE=(EL+EW)/ (CL+CW). All structurally visible ommatidia are considered.
- **FCDV** tangens of divergence angle of frontal carinae measured along a 50 μm section from FRS level caudad. A cross-scaled ocular micrometer and full magnification is used.
- **FRS** distance of the frontal carinae immediately caudal of the posterior intersection points between frontal carinae and the lamellae dorsal of the torulus. If these dorsal lamellae do not laterally surpass the frontal carinae, the deepest point of scape corner pits may be taken as reference line. These pits take up the inner corner of scape base when the scape is fully switched caudad and produce a dark triangular shadow in the lateral frontal lobes immediately posterior of the dorsal lamellae of scape joint capsule (fig. 1 in Seifert 2006).
- **MGr** Depth of metanotal groove or depression, measured from the tangent connecting the dorsalmost points of promesonotum and propodeum; here given as per cent ratio of CS.
- MH in workers: with mesosoma in lateral view and measured orthogonal to "longitudinal mesosomal axis", MH is the longest measurable *section* line of mesosoma at mesopleural level (not height above all). "Longitudinal mesosomal axis" in lateral view is defined as straight line from the centre of propodeal lobe to the border point between anterior pronotal shield and propleuron. In gynes it is the longest section line directed perpendicular to the straight dorsal profile line of mesosoma (formed by mesonotum and scutellum). The lower reference point is usually lowest part of mesopleuron.
- **MW** maximum mesosoma width (worker); maximum mesosoma width anteriorly of the tegulae (gynes).
- ML in workers: mesosoma length from caudalmost point of propodeal lobe to transition point between anterior pronotal slope and anterior propodeal shield (preferentially measured in lateral view; if the transition point is not well defined, use dorsal view and take the centre of the dark-shaded border-line between pronotal slope and pronotal shield as anterior reference point). In gynes: length from caudalmost point of propodeal lobe to the most distant point of steep anterior pronotal face.
- **PEH** maximum petiole height. The straight section of ventral petiolar profile at node level is the reference line perpendicular to which the maximum height of petiole node is measured.
- **PEL** Diagonal petiolar length in lateral view; measured from anterior corner of subpetiolar process to dorsocaudal corner of caudal cylinder.
- **PEW** maximum width of petiole.
- **PoOc** postocular distance. Use a cross-scaled ocular micrometer and adjust the head to the measuring position of CL. Caudal measuring point: median occipital margin; frontal measuring point: median head at the level of the

posterior eye margin. Note that many heads are asymmetric and average the left and right postocular distance (fig. 2 in Seifert 2006).

- **PPW** maximum width of postpetiole.
- **SL** maximum straight line scape length excluding the articular condyle as arithmetic mean of both scapes.
- SP maximum length of propodeal spines; measured in dorsofrontal view along the long axis of the spine, from spine tip to a line, orthogonal to the long axis, that touches the bottom of the interspinal meniscus (fig. 3 in Seifert 2006). Left and right SP are averaged. This mode of measuring less ambiguous than other methods but results in some spine length in species with reduced spines.
- **SPBA** the smallest distance of the lateral margins of the spines at their base. This should be measured in dorsofrontal view, since the wider parts of the ventral propodeum do not interfere with the measurement in this position. If the lateral margins of spines diverge continuously from the tip to the base, a smallest distance at base is not defined. In this case, SPBA is measured at the level of the bottom of the interspinal meniscus.
- **SPST** distance between the centre of propodeal stigma and spine tip. The stigma centre refers to the midpoint defined by the outer cuticular ring but not to the centre of real stigma opening that may be positioned eccentrically.
- **SPTI** the distance of spine tips in dorsal view; if spine tips are rounded or thick take the centres of spine tips as reference points.
- $\label{eq:TrScuC} \begin{array}{l} \text{density of transverse microsculpture elements in centromedian vertex. Count} \\ \text{the transverse elements crossing a median line of \pm 120 μm at a central place} \\ \text{with most such elements. Unit: number of elements / mm.} \end{array}$

Results

Temnothorax pilagens sp. n.

http://zoobank.org/816821D0-8B10-4BEE-889D-CA45A80ABFDD http://species-id.net/wiki/Temnothorax_pilagens

Etymology. The species epithet refers to the slave raiding behaviour of the new ant species (from Latin: pilare, English: to pluck, plunder, pillage).

Description and differential diagnosis. The differential diagnosis is done in relation to the congeneric slave-making species *Temnothorax duloticus*. Measurements and indices in the text of description are the arithmetic means of the whole samples (for full data see Table 1).

Worker (Figs 1, 3 and 5, Table1): Body size close to the genus average of *Temnothorax*, mean CS 645 μ m. Head relatively broader [CL/CW 1.048 but 1.078 in *duloticus*], in dorsal aspect with strongly convex postocular sides and nearly linear, converging genae. Postocular distance smaller [PoOc/CL 0.364 but 0.394 in *duloticus*]. Antennae with 11 segments only, scape strikingly shorter [SL/CS 0.721 but 0.801



Figure 1. Temnothorax pilagens sp. n., worker, head of holotype in dorsal view.

in *duloticus*]. Vertex finely longitudinally rugulose, distance between rugulae on central vertex 12 μ m. The rugulae are connected by very delicate transverse anastomosae, which have on central vertex a mean distance of 12–14 μ m. Clypeus finely longitudinally carinulate and in full-face view with straight or feebly emarginated anteromedian margin. Only the apical and subapical dent of the masticatory margin of the mandibles are fully developed and acute, the following dents are reduced to an undulating line of 3–6 shallow waves [in *duloticus* at least the first three dents are fully developed and the whole dentition is more similar to the normal *Temnothorax* situation]. Genae each with 2–6 semi-erect to erect setae [these are absent in *duloticus*]. Mesosoma massive, in lateral view with strongly convex dorsal profile, appearing hump-backed – i.e., much more compact and shorter than in *duloticus* [ML/CS 1.174 but 1.272 in *duloticus*].



Figure 2. Temnothorax duloticus, worker, head in dorsal view.

Spines significantly shorter and thicker [SPST/CS 0.364 and SP/CS 0.300 but 0.425 and 0.361 respectively in *duloticus*]; spines in lateral view semi-erect, deviating from longitudinal axis of the mesosoma by 27–35°; in dorsal view diverging by 36–39° and with a larger basal distance [SPBA/CS 0.382, but 0.317 in *duloticus*]. The entire mesosoma exhibits a rugulose-microreticulate sculpture. Petiolar node in lateral view with a straight or weakly concave frontal profile forming with the short dorsal plane an angle of 81–91°; caudal petiolar profile steeply but linearly sloping down to junction with postpetiole [in *duloticus* there is a distinct step in the caudal slope caused by a prolongation of the caudal cylindric part of petiole]. Petiole clearly shorter [PEL/CS 0.452 but 0.517 in *duloticus*]. Postpetiolar sternite in lateral view with a strongly developed, triangular dent, directed anteroventrad, comparable to situation in *duloticus*. Dorsum of petiole node in dorsal view 1.7–2.0 fold wider than long, postpetiole in dorsal view roughly trapezoidal and much wider than in any independent *Temnothorax*



Figure 3. Temnothorax pilagens sp. n., worker, holotype in lateral view.

species, PPW/CS 0.491. Whole surface of petiolar and postpetiolar nodes coarsely microreticulate. Surface of 1st gaster tergite smooth and shining, but with a very delicate (sculpture lines only 0.5 μ m thick), patchily missing microreticulum [in *duloticus* there is nowhere a connected microreticulum – it is reduced to isolated, scattered structures in the form of an "X" or of a matchstick man]. All dorsal body surfaces with setae of medium length. Dorsal head dark brown. Mesosoma, waist and appendages yellowish, propodeum, meso- and metapleuron sometimes darker brownish. Gaster tergites yellowish, often with small brown bands at posterior margin; the first tergite usually shows big brown patches on each side that may fuse medially in some specimens, then covering 70% of total surface.

Gyne (only one gyne was evaluated in both *pilagens* sp. n. and *duloticus*): Head size similar to the genus average of *Temnothorax*, mean CS 673 μ m. Head very short [CL/ CW 0.982 but 1.044 in *duloticus*], in full-face view with strongly convex postocular sides, a feebly concave occipital margin and linear, converging genae. Postocular distance very short [PoOc/CL 0.341 but 0.401 in *duloticus*]. Antennae with 11 segments only, scape very short [SL/CS 0.683 but 0.748 in *duloticus*]. Vertex longitudinally rugulose, distance between rugulae on central vertex 15 μ m, the interspaces between rugulae with reticulate microsculpture. Clypeus finely longitudinally carinulate and in full-face view with feebly notched anteromedian margin. The three apical dents of the mandibular masticatory margin are fully developed and acute, the following four dents are reduced to denticles. Mesomoma very small for *Temnothorax* in general,
T. pilagens		ANOVA E p	T. duloticus	T. duloticus	
	(n=8)	momp	(n=6)	photo of paratype	
CS	645 ± 29	0.22	639 ± 22	562	
[µm]	[603,684]	n.s.	[616,678])02	
PoOc/CL	0.364 ± 0.006	91.76	0.394 ± 0.005	0.377	
	[0.354,0.371]	< 0.001	[0.388,0.400]	0.377	
SL/CS	0.721 ± 0.019	87.55	0.801 ± 0.008	0.831	
31/03	[0.684,0.742]	< 0.001	[0.785,0.807]	0.001	
ML/CS	1.174 ± 0.027	55.96	1.272 ± 0.020	1 296	
IVIL/C3	[1.142,1.229]	< 0.001	[1.242,1.300]	1.290	
DEL/CS	0.452 ± 0.011	50.18	0.517 ± 0.023	0.538	
TEL/C3	[0.438,0.466]	< 0.001	[0.487,0.538]	0.)38	
SDST/CS	0.368 ± 0.015	43.71	0.425 ± 0.017	no data	
5151/C5	[0.343,0.383]	< 0.001	[0.397,0.440]	no data	
SD/CS	0.300 ± 0.014	41.90	0.361 ± 0.022	no data	
31/03	[0.268,0.311]	< 0.001	[0.327,0.392]	no data	
SDR A/CS	0.382 ± 0.027	21.23	0.317 ± 0.021	0.291	
SPDA/CS	[0.351,0.411]	0.001	[0.296,0.354]	0.281	
	0.13 ± 0.27	14.60	0.89 ± 0.47	1.00	
MFGR/C3 [%]	[0.0,0.8]	0.002	[0.1,1.4]	1.09	
D-III/CS	0.158 ± 0.008	11.86	0.179 ± 0.014		
Phil/CS	[0.146,0.172]	0.005	[0.164,0.197]	no data	
DELL/CS	0.425 ± 0.014	9.00	0.451 ± 0.019	0.440	
PEH/CS	[0.400,0.444]	0.011	[0.425,0.476]	0.440	
	1.048 ± 0.023	7.00	1.077 ± 0.014	1.076	
CL/CW	[1.019,1.084]	0.021	[1.058,1.099]	1.0/6	
MW/CS	0.656 ± 0.014	2.77	0.644 ± 0.014	0.620	
IVI W/C3	[0.635,0.672]	n.s.	[0.630,0.671]	0.030	
DDW//CC	0.491 ± 0.018	2.06	0.503 ± 0.012	0 469	
FFW/C3	[0.452,0.506]	n.s.	[0.489,0.520]	0.408	
$T_{r}S_{rr}C[r, l, r, r]$	77.2 ± 5.4	2.02	73.5 ± 4.0	70	
	[69,85]	n.s.	[71,80]	/0	
EVELOS	0.236 ± 0.008	1.73	0.231 ± 0.005		
EYE/CS	[0.229,0.249]	n.s.	[0.225,0.237]	no data	
PEW/CS	0.297 ± 0.010	1.34	0.304 ± 0.012	0.20(
	[0.284,0.313]	n.s.	[0.284,0.318]	0.306	
SPTI/CS	0.469 ± 0.026	0.51	0.479 ± 0.030	no data	
	[0.434,0.505]	n.s.	[0.445,0.527]	no data	
EDSICS	0.389 ± 0.007	0.10	0.390 ± 0.010	0.201	
FRS/CS	[0.378,0.399]	n.s.	[0.376,0.404]	0.381	
MILLOS	0.569 ± 0.018	0.10	0.572 ± 0.026	0.577	
MH/CS	[0.550.0.600]	n.s.	[0.534,0.604]	0.5//	

Table I. Nineteen shape characters and one size character in *Temnothorax pilagens* sp. n. and *T. duloticus* including data extracted from the photo of a paratype specimen of *T. duloticus* F- and p-values of an univariate ANOVA are given and the shape variables are arranged by decreasing F.

but not smaller than in *duloticus* [ML/CS 1.484, MW/CS 0.904, MH/CS 0.868]. Spines well-developed and acute but significantly shorter and relatively thicker than in *duloticus* [SPST/CS 0.364 and SP/CS 0.265 but 0.457 and 0.360 respectively in *duloticus*]; spines in lateral view very weakly erected, deviating from longitudinal axis of mesosoma by 20°; in dorsal view with a very large basal distance and weakly diverg-



Figure 4. Temnothorax duloticus, worker, lateral view.



Figure 5. Temnothorax pilagens sp. n., worker, mesosoma of holotype in dorsal view.

ing [SPBA/CS 0.482, SPTI/CS 0.479; in *duloticus* more clearly diverging, SPBA/CS 0.434 and SPTI/CS 0.530]. Whole mesosoma with rugose-microreticulate sculpture that is on mesonotum and mesopleuron less developed. Petiolar node in lateral view very high and with a weakly concave frontal profile forming with the short dorsal plane an angle of 80°; caudal petiolar profile steeply and almost linearly sloping down to junction with postpetiole [in *duloticus* there is a distinct step in the caudal slope caused by a significant prolongation of the caudal cylindric part of petiole]. Petiole clearly shorter than in *duloticus* [PEL/CS 0.496 vs. 0.551]. Postpetiolar sternite in lateral view with a strongly developed, triangular dent, directed anteroventrad, comparable to



Figure 6. Temnothorax duloticus, worker, mesosoma in dorsal view.

situation in *duloticus*. Dorsum of petiole node in dorsal view 1.9fold wider than long, postpetiole in dorsal view broadly cordate and much wider than in any independent *Temnothorax* species, PPW/CS 0.491. Whole surface of petiolar and postpetiolar nodes strongly microreticulate. Surface of 1^{st} gaster tergite smooth and shining but with a very delicate (sculpture lines only 0.5 µm thick), patchily missing microreticulum [in *duloticus* the microreticulum more incomplete - frequently reduced to isolated, scattered structures in the form of an "X" or of a matchstick man]. All dorsal body surfaces with setae of medium length, the longest on occiput are 74 µm long. Head, mesosoma and waist brown, appendages yellowish to yellowish brown. Gaster tergites yellowish brown, a lighter yellowish patch is at the base of 1^{st} tergite.

Discussion

The original description of *T. duloticus* and the photos in antweb.org (CASENT0103163) of a paratype specimen of the type colony from Ohio: Jackson Country: White's Gulch clearly show the heterospecifity of *T. pilagens*. The conspecifity of our two *T. duloticus* samples with the paratype is indicated by agreement in mesosomal and petiolar shape characters and by the similarity of NUMOBAT data. Both spines of the paratype are broken off - excluding to assess the characters SP, SPTI and SPST. Furthermore, the spatial adjustment of the photo excluded estimating the characters EYE and PnHL. The remaining 15 characters (see Tab. 1) could be extracted from the image – albeit with some distortion. Using these characters and running the paratype in an LDA as wild-card, it is allocated with a posterior probability of p = 1.000 to the same cluster with our *T. duloticus* samples. The same clear allocation is provided by the 1st factor of a principal component analysis being -0.87 ± 0.20 [-1.07,-0.42] in eight specimens of *T. pilagens* sp. n. and 0.94 ± 0.37 [0.34,1.28] in seven specimens of *T. duloticus*, with the paratype scoring 1.28.

Several discriminatory characters allow easy separation of *T. pilagens* sp. n. and *T. duloticus* workers. Despite low sample size, there are highly significant differences in 55% of the tested characters (Tab. 1). The characters SL/CS or PoOc/CL alone should provide a safe and parsimonious numeric species delimitation. There is also no doubt that experienced observers can distinguish the two dulotic species by simple eye-inspection integrating subjective impressions on mesosomal, petiolar, cephalic and spine shape and mandibular dentition (compare Figs 1–6).

Short biological characteristics of Temnothorax pilagens sp. n.

Biology and host species. Obligate slave-making ant with two known hosts: T. longispinosus (Roger, 1863) and T. ambiguus (Emery, 1895). Mitochondrial DNA phylogeny indicates sister species relationship with T. longispinosus (Beibl et al. 2005). Geographical range. Nearctic. North-eastern parts of the United States and possibly south-eastern Canada. Habitat. Forest, woodland, parks. Preferentially wooded sites with little understory, and a high density of suitable nest sites, such as acorns, hickory nuts and sticks. Abundance. Patchy - occurrence depends on high density of suitable host populations; so far only known from three sites in the Northern US: Niquette Bay State Park, Vermont (44.3513°N, 73.1156°W; 8 colonies collected in 1986; Herbers and Foitzik 2002), E.N. Huyck Preserve, Rensselaerville, New York (42.3133°N, 74.1012°W; 7 colonies collected in 2002 and 2003; Beibl et al. 2005) and Sleeping Bear National Lakeshore, Empire, Michigan (44.7560°N, 86.0711°W, 6 and 44 colonies collected in 2011 and 2013, respectively). In all three populations, T. pilagens was enslaving T. longispinosus and T. ambiguus; many nests with slaves of both host species. In Vermont and New York, this species has not been re-collected recently, despite regular search by our group. Plotting data from 2011 in Michigan: between 0.08 and 0.02 slave-making colonies per m^2 (at an average of 4.66 host colonies per m²). Tennothorax pilagens occurs more often in sites with both host species, than in areas with T. longispinosus colonies only (Fisher Test; p=0.019). We did not sample Temnothorax communities with T. ambiguus only. T. pilagens colonies more often contained a mixed slave workforce than slaves of a single host species (Chi,²=49.59, p<0.001). Nest construction. As its hosts, T. pilagens nests occur in preformed cavities in acorns, hickory nuts or sticks. Colony demography. Strictly monogynous. Most likely polydomous at least during the summer season: 72% of the nests were queenless with queenright nests close-by and neighboring nests merged in the laboratory without aggression. Nests contain on average four slave-making workers (ranging from 0 to 16 T. pilagens workers) and 13 Temnothorax slaves (ranging from 2 to 50 workers) - but see Herbers and Foitzik (2002) for a nest with 27 T. pilagens and 55 slave workers. Colony foundation. Four colonies with no T. pilagens workers, but a founding queen were collected. All four contained T. longispinosus slaves only. Slave-raids. Obligatory slave hunter of Temnothorax longispinosus and T. ambiguus. Raids are performed either by a scout alone or via group recruitment of up to four slavemaking workers forming a raiding column headed by a scout. Slave raids resemble more those of its congener T. duloticus than that of P. americanus

(Alloway 1979). This is especially true for the frequent and effective use of the stinger in fights: well-aimed stings from a caudal direction between head and thorax cause paralysis in hosts followed by quick death. Effective sting use is likely facilitated by morphological adaptations, such as strongly developed flexor-muscles in the petiole and postpetiole allowing for easy gaster flexion. Similar behavioural strategies and morphological traits are also found in *T. duloticus*, but not in *P. americanus*. Hosts attacked by *T. pilagens* show little or only delayed flight responses. Occasionally, host workers try to drag slavemakers out of the nest, and only respond aggressively when attacked by them. The low host responsiveness towards invading *T. pilagens* indicate reduced or supressed enemy recognition. Due to their most effective stinging behaviour, *T. pilagens* can cause high rates of host casualties (ranging from 5% to 100%). As the high variation in the rate of casualties indicates, raids can be highly aggressive or relatively peaceful; the latter was often found in raids against queenless host nests. Slave-makers do not only take brood from the attacked host nests, but in 6 of the observed 11 raids, they also carry adult host workers back to their nest and integrated them into the slave workforce.

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References

- Alloway TM (1979) Raiding behavior of two species of slavemaking ants, *Harpagoxenus ameri*canus (Emery) and Leptothorax duloticus Wesson (Hymenoptera: Formicidae). Animal Behaviour 27: 202–210. doi: 10.1016/0003-3472(79)90140-4
- Beibl J, Stuart RJ, Heinze J, Foitzik S (2005) Six origins of slavery in formicoxenine ants. Insectes Sociaux 52: 291–297. doi: 10.1007/s00040-005-0808-y
- Bolton B (2003) Synopsis and classification of Formicidae. Memoirs of the American Entomological Institute 71: 1–370.
- Csösz S, Seifert B, Müller B, Trindl A, Schulz A, Heinze J (2013) Cryptic diversity in the Mediterranean *Temnothorax lichtensteini* species complex (Hymenoptera:Formicidae). Organisms, Diversity & Evolution. doi: 10.1007/s13127-013-0153-3
- Herbers JM, Foitzik S (2002) The ecology of slavemaking ants and their hosts in north temperate forests. Ecology 83 (1): 148–163. doi: 10.1890/0012-9658(2002)083[0148:TEOSA A]2.0.CO;2
- Seifert B (2006) Temnothorax saxonicus (Seifert, 1995) stat. nov., comb. nov. a parapatric, closely-related species of T. sordidulus (Müller, 1923) comb. nov., stat. nov. and description of two new closely-related species, T. schoedli sp. n. and T. artvinense sp. n. from Turkey (Hymenoptera: Formicidae). Myrmecological News 8: 1–12.

RESEARCH ARTICLE



Modern analyses on an historical data set: skull morphology of Italian red squirrel populations

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Abstract

Recent molecular evidence suggests that *Sciurus vulgaris* populations from Calabria (southern Italy) are distinct from those occurring in northern and central Italy. Here, we re-analyzed using multivariate and univariate techniques an historical dataset provided by Cavazza (1913), who documented measurements for the now extinct squirrel population from Campania. Both univariate and multivariate analyses confirmed that the sample from Calabria was homogenous and relatively distinct compared to the rest of the squirrel samples.

Keywords

Morphometrics, red squirrel, Italy, historical dataset

Introduction

The Eurasian red squirrel, *Sciurus vulgaris* Linnaeus, 1758, is characterized by great variability in fur coloration, which led to the description of more than 40 subspecies throughout its wide geographic distribution across the Eurasian continent (Corbet 1978). Currently only 17 of these subspecies are considered valid (Lurz et al. 2005), with the Italian populations being ascribed to three subspecies (Toschi 1965). These Italian subspecies are:

1) *S. vulgaris fuscoater* Altum, 1876 (European form occurring in the Alps and in the northern Apennines), characterized by relatively small size and a strong degree of coat-colour polymorphism both within and between populations;

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- 2) *S. vulgaris italicus* Bonaparte, 1838 (endemic to Central Italy), also characterized by relatively small size, albeit bigger than the previous subspecies. This subspecies shows some degree of coat colour polymorphism, with the dark brown morph dominant in mountainous forests at higher altitudes. The populations of the southern tip of the range are black (subspecies *alpinus, sensu* Costa 1839);
- 3) *S. vulgaris meridionalis* Lucifero, 1907 (endemic to the most southern Apennines), with uniform fur colour, always having black dorsal fur with grey shades on the sides, a black tail, and a contrasting white belly. It is also the largest Italian subspecies (Wauters and Martinoli 2008).

Although widespread in Italy, this species' distribution is associated with forested areas, and affected by their fragmentation (Celada et al. 1994, Wauters et al. 1994a, Wauters et al. 1994b, Wauters 1997, Hale et al. 2001). Thus, the European squirrel currently occurs in the whole of the Italian Peninsula with some distribution gaps: the species does not currently occurs in Campania, Apulia and Basilicata (cf. Wauters and Martinoli 2008). However, the squirrel was present in historical times also in the extreme northern part of Campania (i.e. Mt. Somma - Vesuvio) (Costa 1839, Trouessart 1910, Cavazza 1913), where it is now extinct (Capolongo and Caputo 1990, Maio et al. 2000).

Recent molecular data (Grill et al. 2009) revealed the presence of two main mitochondrial phylogroups: (i) a clade comprising the individuals from the region of Calabria in southern Italy belonging to the subspecies *S. v. meridionalis*, and (ii) another including the rest of the Italian populations.

Cavazza (1913) studied morphological variability of Italian populations of *Sciurus vulgaris*, and provided a useful set of skull measurements for squirrels collected throughout Italy. Among various populations, he analyzed specimens from an area where the species is now locally extinct (Campania), which is geographically closer to the populations of the subspecies *italicus* than to those of *meridionalis*. Cavazza's (1913) data are important for evaluating whether the extinct Campanian squirrels were more similar to those currently inhabiting Calabria, or to those typical of central Italian regions.

In this paper, we reanalyzed Cavazza's original dataset using modern statistical multivariate analyses with the aim to evaluate whether morphometric and genetic data agree with respect to patterns of geographic differentiation in Italian squirrel populations.

Materials and methods

We used the data reported in Cavazza (1913) for skull measurements of adults (Table 1). Cavazza (1913) divided specimens into the following groups: (a) Alps, (b) northern and central Italy including Latium and excluding Abruzzi, (c) southern Italy including Abruzzi and Campania, and (d) Calabria. The localities where Cavazza (1913) collected his specimens are reported in Figure 1. Unfortunately, we cannot re-measure specimens from Cavazza's (1913) paper because several of them have now become lost. Moreover, although it is possible that some of the specimens originarily measured by Cavazza

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(1913) are still available in private or public collections in Italy, unfortunately there is no labeling indication in Cavazza's paper for any of his specimens, and this fact impeded us from any further analysis of the vouchers.

Univariate measurements were log-transformed in order to achieve normality and then compared across groups by one-way Analysis of Variance (ANOVA). In this analysis, the same four groups as defined by Cavazza (1913) were used.

Specimens were divided into four Operational Taxonomic Units (hereby OTUs), according to their geographical provenance and corresponding to the Italian subspecies. These four OTUs followed exactly the subdivisions made by Cavazza (1913). We performed a cluster analysis in order to show dissimilarities among all of Cavazza's (1913) specimens in terms of their skull measurements. Skull measurements were log-transformed prior to analysis. Dendrograms were prepared using the single linkage as

ID	OTU	Skull	Skull	Skull	Mandible	Interorbital	Locality	0.074
	010	length	width	height	length	width	Locality	SCX
17Alpf	A	50.1	28.5	19.6	27.3	18.3	AlpiCentrali	f
8Apf	A	51.9	29.6	23.4	28.0	19.2	Lanzo	f
7Alpm	A	49.7	28.3	21.0	28.2	18.8	Lanzo	m
12Aplf	A	51.8	30.0	21.0	28.2	19.0	Porlezza	f
7Alpf	A	51.3	29.0	23.3	28.8	18.9	Lanzo	f
3Alpf	A	52.0	29.0	22.0	28.9	20.0	AlpiPiem.	f
9Alpf	A	51.2	29.1	21.3	28.9	18.4	Porlezza	f
5Alpm	A	49.6	29.0	20.0	29.0	19.2	Biellese	m
13Alpm	A	51.5	31.2	21.6	29.0	20.0	Porlezza	m
2Alpf	A	52.6	29.6	22.0	29.0	20.0	AlpiPiem.	f
4Alpf	A	51.8	29.0	21.0	29.0	19.8	Biellese	f
15Alpf	A	52.6	30.6	19.9	29.0	20.0	SopraLugano	f
18Alpf	A	51.7	29.8	21.0	29.0	18.2	AlpiCentrali	f
25Alpf	A	50.1	28.7	21.0	29.0	19.0	Cadore	f
26Alpf	A	51.2	29.6	22.0	29.0	19.4	Cadore	f
9Alpm	A	51.8	30.6	21.0	29.1	19.5	Lanzo	m
16Alpf	A	55.0	31.2	21.0	29.1	18.6	AlpiCentrali	f
1Alpf	A	53.0	30.8	22.0	29.2	21.0	AlpiPiem.	f
5Alpf	A	50.8	28.9	22.6	29.2	18.7	Lanzo	f
13Alpf	A	55.9	31.0	22.6	29.2	20.2	Buggiolo	f
21Alpf	A	53.0	30.0	21.2	29.2	19.0	AlpiCentrali	f
2Alpm	A	57.3	32.0	21.0	29.3	20.0	AlpiPiem.	m
10Alpm	A	52.2	31.0	21.0	29.3	20.0	Lanzo	m
14Alpm	A	49.9	27.8	20.8	29.3	18.3	Porlezza	m
6Alpf	A	52.0	29.8	23.0	29.3	19.1	Lanzo	f
14Alpf	A	52.6	30.3	21.8	29.3	21.0	SopraLugano	f
20Alpf	A	51.8	29.9	21.2	29.3	19.0	AlpiCentrali	f
19Alpf	A	52.0	30.0	21.3	29.4	19.5	AlpiCentrali	f
12Alpm	А	52.0	30.2	21.6	29.5	20.0	Lanzo	m

Table 1. Skull measurements (in mm). OTU = Operational Taxonomic Unit; A = Alps; B = North and Central Italy; C = Abruzzo and Campania; D = Calabria (from Cavazza 1913). For more details see text.

10	OTU	Skull	Skull	Skull	Mandible	Interorbital	T 1.	
ID	010	length	width	height	length	width	Locality	sex
3Alpm	A	50.0	29.2	20.4	29.6	19.2	AlpiPiem.	m
6Alpm	A	52.7	30.3	22.2	29.8	19.8	Lanzo	m
1Alpm	A	53.0	32.2	21.2	30.0	20.7	AlpiPiem.	m
18Alpm	A	52.8	32.0	21.0	30.0	20.0	SopraLugano	m
4Alpm	A	53.0	33.0	21.3	31.0	20.0	Biellese	m
26Alpm	В	51.5	30.3	21.0	28.9	19.3	Cadore	m
25Alpm	В	51.6	30.8	20.8	29.0	19.0	Cadore	m
1Lomm	В	52.2	29.4	22.6	29.0	18.8	Lombardia	m
2Emim	В	52.6	29.0	22.2	29.0	19.4	Emilia	m
3Emif	В	50.1	29.0	21.8	29.0	18.6	Emilia	f
9Tosf	В	53.2	30.2	22.1	29.0	18.8	Toscana	f
10Tosf	В	52.8	29.7	22.9	29.0	18.0	Toscana	f
4Emif	В	51.1	29.3	22.0	29.1	18.9	Emilia	f
5Emif	В	52.0	29.0	22.0	29.1	18.7	Emilia	f
11Tosm	В	52.0	30.0	22.0	29.2	18.6	Toscana	m
1Ligf	В	51.2	28.3	22.0	29.2	18.3	Liguria	f
6Emif	В	52.2	29.1	22.3	29.3	18.6	Emilia	f
7Emim	В	52.7	30.1	22.3	29.5	19.0	Emilia	m
3Emim	В	53.7	29.6	23.0	29.6	19.9	Emilia	m
8Emim	В	52.7	30.2	22.2	29.6	18.9	Emilia	m
10Tosm	В	53.2	30.1	22.0	29.6	19.0	Toscana	m
9mim	В	52.7	30.3	22.2	29.8	19.0	Emilia	m
1Alpm	В	52.9	30.1	21.3	30.0	19.8	AlpiCentrali	m
20Alpm	В	52.3	30.1	20.6	30.0	18.9	AlpiCentrali	m
22Alpm	В	50.0	31.0	22.0	30.0	18.8	AlpiCentrali	m
6Emim	В	53.5	34.3	22.1	30.0	18.0	Emilia	m
12Tosf	В	53.0	30.8	22.9	30.0	18.3	Toscana	f
13Tosf	В	52.3	30.3	22.8	30.0	18.7	Toscana	f
18Tosm	В	52.2	31.0	22.3	30.2	18.7	Toscana	m
21Alpm	В	53.1	32.0	21.2	30.3	19.7	AlpiCentrali	m
17Tosm	В	53.0	32.0	22.0	30.3	18.0	Toscana	m
13Tosm	В	52.0	30.0	20.8	30.6	18.2	Toscana	m
11Tosf	В	52.0	31.5	23.0	31.0	18.7	Toscana	f
12Tosm	В	55.0	31.9	21.0	31.2	19.0	Toscana	m
3Napf	С	52.3	29.7	24.1	28.9	18.6	Napoletano	f
4Napf	С	54.6	29.9	25.0	29.0	19.0	Napoletano	f
3Napm	С	52.8	28.9	22.9	29.2	19.0	Napoletano	m
2Napf	С	54.3	29.8	24.9	29.4	18.9	Napoletano	f
4Napm	С	55.0	29.8	22.8	29.5	19.6	Napoletano	m
2Napm	С	55.2	31.3	24.0	30.0	20.0	Napoletano	m
2Calf	D	56.3	33.6	22.7	31.8	19.1	Calabria	f
3Calm	D	56.0	33.9	22.4	32.2	19.0	Calabria	f
1Calm	D	56.0	33.5	22.6	33.9	20.7	Calabria	f
1Calf	D	57.2	33.4	22.8	33.9	19.2	Calabria	f
2Calm	D	54.5	32.9	22.3	34.1	20.2	Calabria	f



Figure 1. Map of Italy showing the localities where squirrels were collected according to Cavazza (1913).
I Porlezza 2 Lanzo 3 Central Alps 4 Alpi Piemontesi 5 Biellese 6 Lugano 7 Bassano del Grappa 8 Buggiolo
9 Lombardia 10 Emilia 11 Tuscany 12 Liguria 13 Neapolitan (Campania) 14 Calabria.

the algorithm, with Euclidean distances. This method was used because it provided the highest cophenetic index. In the single linkage (nearest neighbour), the clusters are joined based on the smallest distance between the two groups. Branch support was calculated with 10,000 bootstrap replicates. We also used neighbour joining clustering (Saitou and Nei 1987), which is an alternative method for hierarchical cluster analysis. In contrast with ultrametric methods (like the Unweighted Pair Group Method with Arithmetic Mean, UPGMA), two branches from the same internal node do not need to have equal branch lengths. A phylogram (unrooted dendrogram with proportional branch lengths) is given in this paper.

We studied the dispersion of specimens in multivariate space with Principal Components Analysis (PCA) using the covariance matrix (Davis 1986, Harper 1999) (PC1 scores serve as a proxy for size, while the other PCs capture shape variation).

	Mean (S.D.)	Range
Skull length	52.61 (1.70)	49.6–57.3
Skull width	30.37 (1.41)	27.8-34.3
Skull height	21.92 (1.04)	19.6–25.0
Mandible length	29.63 (1.18)	27.3-34.1
Interorbital length	19.21 (0.70)	18.0-21.0

Table 2. Mean and dispersion measures of the five skull variables analyzed in this study (original dataset from Cavazza (1913), for all sampled specimens pooled together.

Table 3. Mean and dispersion measures of the five skull variables analyzed in this study (original dataset from Cavazza (1913), with all sampled specimens divided by OTU. Symbols: A = Alps; B = North and Central Italy; C = Abruzzo and Campania; D = Calabria.

	Mean	SD
	A (n = 34)	
Skull length	52.05	1.64
Skull width	30.03	1.19
Skull height	21.42	0.88
Mandible length	29.12	0.62
Interorbital length	19.46	0.74
	B (n = 29)	
Skull length	52.37	1.02
Skull width	30.32	1.20
Skull height	21.97	0.68
Mandible length	29.67	0.63
Interorbital length	18.22	0.49
	C (n = 6)	
Skull length	54.03	1.20
Skull width	29.90	0.77
Skull height	23.95	0.94
Mandible length	29.33	0.39
Interorbital length	19.18	0.51
	D (n = 5)	
Skull length	56.00	0.97
Skull width	33.46	0.36
Skull height	22.56	0.20
Mandible length	33.18	1.08
Interorbital length	19.64	0.76

Results

The original dataset reported by Cavazza (1913) is summarized in Table 1. Mean and standard deviations for each measurement considered are reported in Table 2 with all specimens pooled, and in Table 3 with samples divided into OTUs. Using the same categories as in Cavazza (1913), there were among-group statistical differences for skull



Figure 2. PCA of skull measurements (VARIMAX rotation applied) based on Cavazza's (1913) dataset. Eigenvalues: component 1 = 2.559; component 2 = 1.099.

length (one-way ANOVA $F_{3,70} = 14.76$, P < 0.00001), skull width ($F_{3,70} = 13.50$, P < 0.00001), skull height ($F_{3,70} = 18.93$, P < 0.00001), and mandible length ($F_{3,70} = 56.83$, P < 0.00001), but not for interorbital length ($F_{3,70} = 1.92$, P < 0.133). Post-hoc Tukey HSD tests revealed that Calabria specimens differed significantly from every other group for mandible length (all P < 0.01), and for skull width (all P < 0.001). For skull length, Calabria specimens differed from Alpine and central Italian specimens (all P < 0.01) but not from Campania specimens (P = 0.088). For skull height, they differed from Campania (P = 0.024) and Alpine specimens (P = 0.018) but not from central Italian specimens (P = 0.43). Principal component scores indicated that there were significant statistical shape differences among the four populational groups (one-way ANOVA: $F_{3,70} = 30.362$, P < 0.0001), and a Tukey HSD post-hoc test revealed that (i) the Calabria population differed significantly from all the others (at least, P < 0.000154), (ii) the Campania population significantly differed, other than from Calabria specimens, also from Alpine specimens (P = 0.470).

Both sets of multivariate analyses revealed that the sample from Calabria was homogenous and relatively distinct compared to the rest of the squirrel samples (Figures 2 and 3). In the PCA (variance explained by the first two axes: 56.5%; with axis 1 explaining 28.7% and axis 2 explaining 27.8% of the total variance; see Table 4 for the loadings) there was a trend suggesting clinal variation from the Alps to Campania, with Calabria specimens, while distinct, being more similar to those of Campania than to those of northern Italy (Figure 2). The Campania group showed less variance (Levene's test; F = 6.67, P < 0.03) compared to the rest of the central and northern Italian samples in the PCA than in the neighbor joining analysis (Figure 3).

	Component 1	Component 2
Skull length	0.876	0.154
Skull width	0.882	-0.159
Skull height	0.341	0.836
Mandible length	0.842	-0.034
Interorbital length	0.432	-0.684

Table 4. Loadings of the PCA as in Figure 2.

Discussion

Both multivariate and univariate tests identified some morphometric differentiation among different squirrel populations that were previously highlighted by the molecular results of Grill et al. (2009). That is: the populations from Calabria differed from the others morphologically (this study) and genetically (D-Loop: Mean genetic distance between groups: 6%, within group: 2%; see Grill et al. 2009). Our analyses also suggest that the currently extinct population from Campania belonged to a central Italian grouping. It may be that patterns of craniometric variation in Italian red squirrels represent a clinal size trend within a formerly contiguous population once occurring from the Alps south to Campania, and, with expectations fitting Bergmann's rule (e.g., Freckleton et al. 2003; Blackburn and Hawkins 2004).

On the other hand, Calabria specimens do appear to be quite distinct from the rest of the Italian squirrels in size (Figure 2), though we note that our analyses involve guite small sample sizes (Cardini and Elton 2007). Notably, Calabria populations occur mainly at relatively high altitudes, closely linked to that of extensive high-altitude mixed forest dominated by the native Calabrian black pine *Pinus laricio* (Cagnin et al. 2000, Rima et al. 2010) and they are characterized both by large size and monomorphic color fur. Overall, our study could neither substantiate nor reject the hypothesis that Sciurus vulgaris meridionalis is a full species, as previously suggested by Gippoliti (2013). However, some morphological differentiation is certainly evident also with respect to the Campania extinct population (this study), and remarkable genetic differences are found between Calabria populations and all the remaining European populations (Grill et al. 2009). Indeed, the majority of individuals analyzed by Grill et al. (2009) formed one monophyletic clade without particular differentiation, whereas Calabrian squirrels were clearly separate. The Calabrian lineage appears to have experienced a different history from the rest of European squirrels probably due to the fact that it became isolated after glaciations and never reconnected to Central Italian populations (Grill et al. 2009). It should be stressed, however, that the sample sizes available for Campania and Calabria were too small to make any firm conclusions.

Our approach in this paper highlights the lasting value of historical publications on biodiversity, especially when they present data on populations which are now extinct. These often overlooked publications – such as Cavazza's, published in Italian in a regional journal – can be important sources of data that can be re-analysed, for renewed insight, using modern statistical tools.



Figure 3. Neighbor joining dendrogram of skull measurements (with 10,000 bootstraps) based on Cavazza's (1913) dataset.

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References

- Blackburn TM, Hawkins BA (2004) Bergmann's rule and the mammal fauna of northern North America. Ecography 27: 715–724. doi: 10.1111/j.0906-7590.2004.03999.x
- Cagnin M, Aloise G, Fiore F, Oriolo V, Wauters L (2000) Habitat use and population density of the Calabrian squirrel *Sciurus vulgaris meridionalis* Lucifero, 1907 in the Sila Grande mountain range (Calabria, Italy). Italian Journal of Zoology 67: 81–87. doi: 10.1080/11250000009356299
- Capolongo D, Caputo V (1990) Mammals of the Partenio Mountains (Campanian Apennines, southern Italy). Vie Milieu 40: 156–159.
- Cardini A, Elton S (2007) Sample size and sampling error in geometric morphometric studies of size and shape. Zoomorphology 126: 121–134. doi: 10.1007/s00435-007-0036-2
- Cavazza F (1913) Studio intorno alla variabilità dello *Sciurus vulgaris* in Italia. Atti della Regia Accademia dei Lincei, Classe Science Fisiche, Matematiche e Naturali, IX, Ser. 5: 504–593.
- Celada C, Bogliani G, Gariboldi A, Maracci A (1994) Occupancy of isolated woodlots by the red squirrel *Sciurus vulgaris* L. in Italy. Biological Conservation 69: 177–183. doi: 10.1016/0006-3207(94)90057-4
- Corbet GB (1978) The Mammals of the Palaearctic region: a taxonomic review. British Museum (Natural History), London, UK.
- Costa OG (1839) Fauna del Regno di Napoli, ossia enumerazione di tutti gli animali che abitano le diverse regioni di questo Regno e le acque le bagnano, contenente la descrizione de' nuovi o poco esattamente conosciuti. Stamperia Azzolino e Compagno, Napoli, Italy.
- Davis JC (1986) Statistics and Data Analysis in Geology. John Wiley and Sons, New York, USA.
- Freckleton RP, Harvey PH, Pagel M (2003) Bergmann's rule and body size in mammals. American Naturalist 161: 821–825. doi: 10.1086/374346
- Gippoliti S (2013) Checklist delle specie dei mammiferi italiani (esclusi Mysticeti e Odontoceti): contributo per la conservazione della biodiversità. Bolletino del Museo Civico di Storia Naturasle di Verona (Botanica e Zoologia) 37: 7–28.
- Grill A, Amori G, Aloise G, Lisi I, Tosi G, Wauters L, Randi E (2009) Molecular phylogeography of European *Sciurus vulgaris*: refuge within refugia? Molecular Ecology 18: 2687–2699. doi: 10.1111/j.1365-294X.2009.04215.x
- Hale ML, Lurz PWW, Shirley MDF, Rushton S, Fuller RM, Wolff K (2001) Impact of landscape mamagement on the genetic structure of red squirrel populations. Science 293: 2246–2248. doi: 10.1126/science.1062574
- Harper DAT (Ed) (1999) Numerical Palaeobiology. John Wiley and Sons, New York, USA.
- Lurz PWW, Gurnell J, Magris L (2005) *Sciurus vulgaris*. Mammalian Species 769: 1–10. doi: 10.1644/1545-1410(2005)769[0001:SV]2.0.CO;2

- Maio N, Aprea G, D'Amora G, Picariello O (2000) La teriofauna del Parco Nazionale del Vesuvio ed aree limitrofe. In: Picariello O, Di Fusco N, Fraissinet M (Eds) Elementi di biodiversità del Parco Nazionale del Vesuvio. Ente Parco Nazionale del Vesuvio, Napoli, Italy.
- Rima P, Cagnin M, Aloise G, Preatoni D, Wauters LA (2010) Scale-dependent environmental variables affecting red squirrel (*Sciurus vulgaris meridionalis*) distribution. Italian Journal of Zoology 77: 92–101. doi: 10.1080/11250000902766926
- Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4: 406–425.
- Toschi A (1965) Mammalia. Lagomorpha, Rodentia, Carnivora, Artiodactyla, Cetacea. Fauna d'Italia. Vol. VII. Calderini Ed., Bologna, Italy.
- Trouessart EL (1910) Faune des Mammiféres d'Europe. Friedländer & Sohn, Berlin, Germany.
- Wauters LA (1997) The ecology of red squirrel in fragmented habitats: a review. In: Gurnell J, Lurz PWW (Eds) The conservation of red squirrel, *Sciurus vulgaris* L. Peoples Trust of Endangered Species, London, UK, 5–12.
- Wauters LA, Casale P, Dhondt AA (1994a) Space use and dispersal in red squirrel in fragmented habitats. Oikos 69: 140–146. doi: 10.2307/3545294
- Wauters LA, Hutchinson Y, Parkin DT, Dhondt AA (1994b) The effects of habitat fragmentation on demography and on the loss of genetic variation in the red squirrel. Proceeedings of the Royal Society, London, B 255: 107–111.
- Wauters LA, Martinoli A (2008) Sciurus vulgaris Linnaeus, 1758. In: Amori G, Contoli L, Nappi A (Eds) Mammalia II, Erinaceomorpha, Soricomorpha, Lagomorpha, Rodentia. Fauna d'Italia XLIV: 349–360.