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Two new jumping spider species of the *Habronattus clypeatus* group (Araneae, Salticidae, Harmochirina)

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Abstract

Two species of the *Habronattus clypeatus* species group are described, *Habronattus arcalorus* **sp. n.** from Texas and Colorado, and *Habronattus gilaensis* **sp. n.** from New Mexico. *Habronattus arcalorus* males have extravagant ornamentation: a green first leg with an unusually dense lateral fringe of orange and white hairs, and a large grey triangular patella on the third leg with blue-white scales nearby. *Habronattus gilaensis* males are considerably more muted, lacking ornamentation on the third leg's patella and tibia. Photographs of living specimens are given, as well as notes on habitat.

Keywords

Araneae, Salticidae, Salticinae, Plexippini, Harmochirina, jumping spider

Introduction

The genus *Habronattus* F.O. Pickard-Cambridge, 1901 includes jumping spiders whose males have remarkably complex courtship displays, especially the members of a large clade with modified first and third legs (Peckham and Peckham 1890; Griswold 1987; Elias et al. 2003, 2012). Within this clade, the first leg of most males is laterally fringed with modified setae, and the third femur, patella and tibia have several modifications

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of apophyses, swellings, tufts, and colours. This clade contains 38 described species (Griswold 1987) divided into three species groups, of which the *clypeatus* species group is restricted to the southwestern United States and Mexico. We here describe two new species of the *clypeatus* group, one of which has some of the most exaggerated courtship ornamentation known in *Habronattus*. These two species were referred to in molecular phylogenetic and chromosome studies by the names "*Habronattus* sp. (CNCTY)" and "*Habronattus* cf. *dossenus*" (Maddison and Hedin 2003; Maddison and Leduc-Robert 2013), and are the current focus of continuing behavioural and molecular studies.

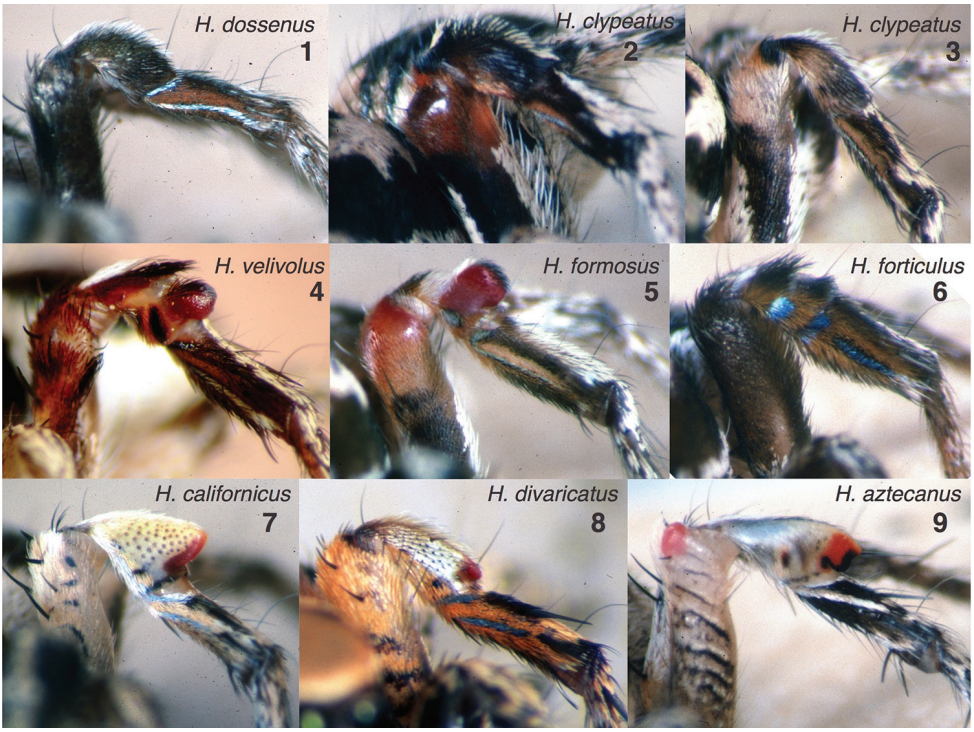
Methods

Preserved specimens were examined under both dissecting microscopes and a compound microscope with reflected light. Drawings were made with a drawing tube on a Nikon ME600L compound microscope. All specimens are deposited in the Spencer Entomological Museum of the University of British Columbia (UBC-SEM), except for paratypes deposited (as noted) in the AMNH (American Museum of Natural History), MCZ (Museum of Comparative Zoology, Harvard University) or OSAC (Oregon State Arthropod Collection, Oregon State University, Corvallis).

Terms are used in standard fashion for Araneae. The descriptions were written with primary reference to the focal specimen indicated, which was used for measurements and carefully checked for details, but they apply as far as known to the other specimens examined. All measurements are given in millimeters. Carapace length was measured from the base of the anterior median eyes (AME) not including the lenses to the rear margin of the carapace medially; abdomen length to the end of the anal tubercle. Positions on the bulb of the male palp (left, ventral view) are described using hours of an analog clock's face. The following abbreviations are used: **ALE**, anterior lateral eyes; **PLE**, posterior lateral eyes; **PME**, posterior median eyes (the "small eyes").

The *Habronattus clypeatus* species group

The eight described species of this group can be distinguished from one another by their male third legs (Figs 1–9) as well as other features. The group is distinctive for having the male clypeus with vertical-oblique dark bands descending from the AME, the basal white band of the abdomen divided by one or two longitudinal medial dark bands, and (usually) a medial longitudinal dark band on the underside of the abdomen. The retrolateral tibial apophysis appears as a hook in ventral view. In addition, in living males of the *clypeatus* group a pattern of dark spots is visible when the observer looks into the AMEs, consisting of an array of dark pigment patches (see, for example, this video: https://www.youtube.com/watch?v=Dq5ky7vjPYo). It is possible that all *Habronattus* have such a pigmentation pattern in the eyes, but that it has been observed only in the *clypeatus* group because the broad depigmented areas on the thorax let in light.



Figures 1–9. Third legs of males of described species in the *Habronattus clypeatus* species group. Left legs shown, except Figs 1, 2, 7, 9 which show digitally-flipped images of right legs. **1** *H. dossenus* Griswold, 1987, from U.S.A.: Arizona: Cochise Co.: Lower Turkey Creek, Chiricahua Mts, 109.42°W, 31.85°N, **2** *H. clypeatus* (Banks, 1895) (montane form), from U.S.A.: Arizona: Jack's Canyon near Flagstaff **3** *H. clypeatus* (desert form), from U.S.A.: Arizona: Pima Co.: Santa Catalina Mts., Molino Basin, Prison Camp road **4** *H. velivolus* Griswold, 1987, from México: Jalisco: Laguna Sayula, 19.9963°N, 103.5522°W **5** *H. formosus* (Banks, 1906), from U.S.A.: California: Inyo Co.: Bishp **6** *H. forticulus* (Gertsch & Mulaik, 1936), from U.S.A.: Texas: Travis Co., Austin, 30.25°N, 97.71°W **7** *H. californicus* (Banks, 1904), from U.S.A.: California: San Diego Co.: Oceanside, 33.27°N, 117.44°W **8** *H. divaricatus* (Banks, 1898), from México: Baja California Sur: near La Laguna trailhead, San Juan del Aserradero **9** *H. aztecanus* (Banks, 1898), from México: Nayarit: San Blas. All photos credit W. P. Maddison.

Species boundaries in the group are not entirely clear. *Habronattus clypeatus* (Banks, 1895) has two forms, a montane form in most of its range, dark and with the third femur swollen and orange terminally (Fig. 2), and a smaller paler form from the deserts of southern Arizona whose third femur is less modified (Fig. 3). The desert form appears to intergrade with *H. formosus* (Banks, 1906) (Fig. 5), with the third leg's patella becoming more red and swollen moving west from Phoenix to Yuma. Most recognized species are parapatric or allopatric, but there is at least one case of sympatry: *H. dossenus* Griswold, 1987 and *H. clypeatus* in southern Arizona, where they can co-occur in the same small habitat. In such situations, apparent hybrids – male specimens that blend the ornaments of both species – can be found occasionally. The two species described here are distinctive, without any known intergradation with other forms.

Results

Habronattus arcalorus **Maddison & Maddison, sp. n.**
http://zoobank.org/479C8BE5-6F5D-470E-9ECC-2F63175C6159
Figs 10–21

Holotype. Male in UBC-SEM, with labels: "TEXAS: Jeff Davis Co.: Davis Mountains State Park 103.939°W, 30.593°N, 22-23 April 1997 Maddison/Hedin/Hebets WPM#97-001" and "♂W167".

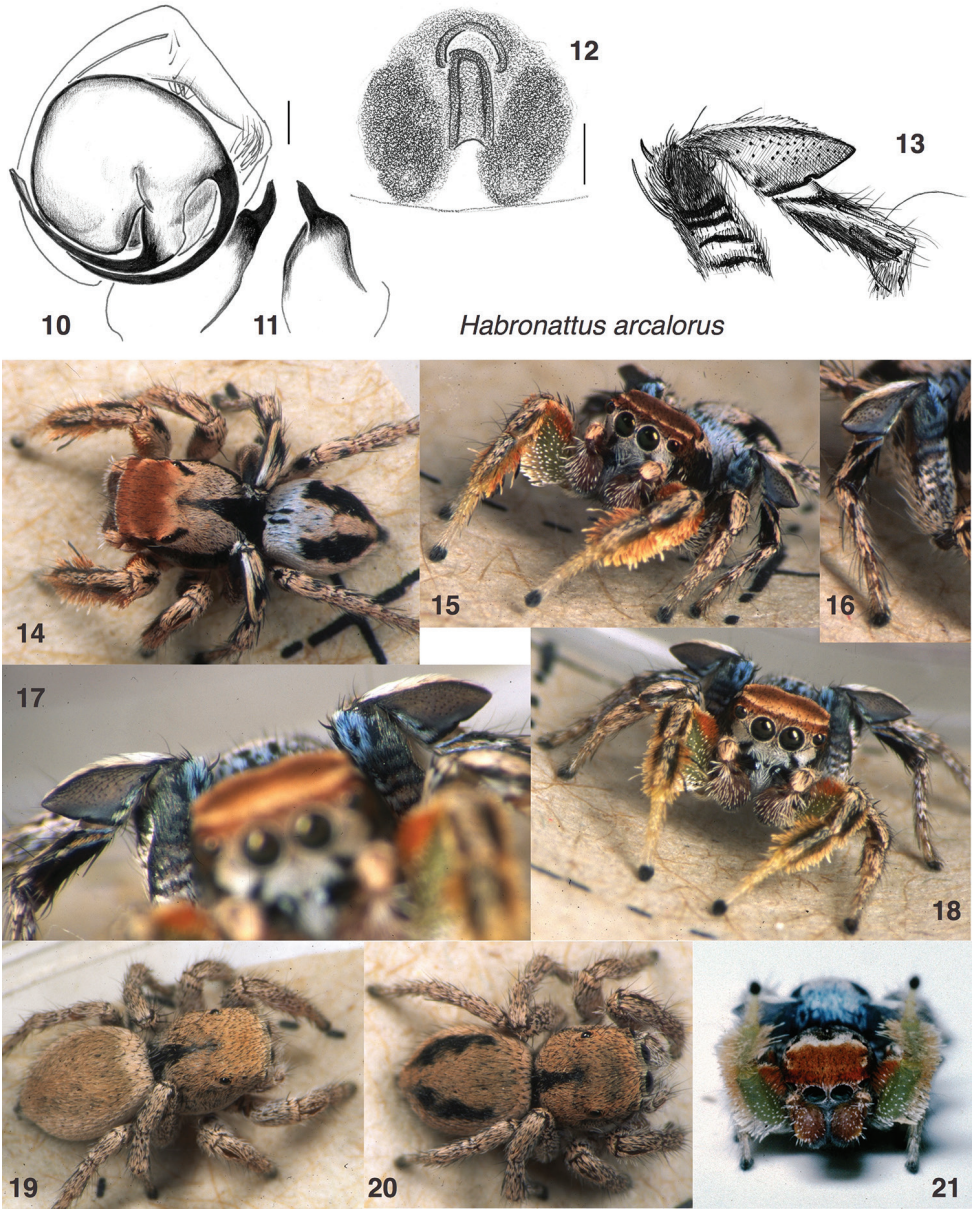
Paratypes (5♂♂ 3♀♀). 1♂ (specimen W328) 1♀ (specimen W332) in UBC-SEM; 1♂ 1♀ in AMNH; 1♂ 1♀ in MCZ; 2♂ in OSAC; all with same data as holotype.

Etymology. The name is an arbitrary combination of letters, including the syllable "arc", included to evoke the rainbow of colors in the male ornaments.

Diagnosis. The large triangular patella of the third leg of males is distinctive in salticids. *Habronattus viridipes* (Hentz,1846) has a triangular patella also (Griswold 1987: figure 99), but it is different from that of *H. arcalorus* in colour (orange-yellow with a black spot, rather than grey) and in having a terminal apophysis. *Habronattus californicus* (Banks, 1904), also of the *clypeatus* group, has a triangular patella (Griswold 1987: figure 107), but the patella is smaller and yellow and red. *H. arcalorus* also differs from *H. californicus* in having green first legs with a much denser fringe. Females of *H. arcalorus* are distinctive from most specimens of other *clypeatus* group species in having a distinct black triangle on the thorax (Figs 19-20).

Note. This species was referred to by Maddison and Hedin (2003) as *H.* sp. (CNCTY) (and possibly also *H.* sp. (CHIH); see note below on Chihuahuan form). Molecular data suggest that *H. arcalorus* and *H. forticulus* (Gertsch & Mulaik, 1936) are outside the clade containing the bulk of the *clypeatus* group species – *H. aztecanus* (Banks, 1898), *H. californicus* (Banks, 1904), *H. clypeatus*, *H. divaricatus* (Banks, 1898), *H. dossenus*, *H. formosus*, and *H. velivolus* Griswold, 1987. *H. arcalorus* and *H. forticulus* are the easternmost representatives of the *clypeatus* group.

Description. *Male* (focal specimen: holotype, specimen W167). Carapace length 2.3; abdomen length 2.3. Structure of chelicerae, legs, and body typical for *Habronattus* and the *clypeatus* group. Palp (Figs 10, 11) standard for the *clypeatus* group (Griswold 1987 figures 180, 181, 203), with the embolus arising at about 3:00 and the basal portion of the terminal apophysis pointing to 6:00. The tibial apophysis is hooked from ventral view (Fig. 10); in retrolateral view it narrows abruptly before its tip leans ventrally (Fig. 11). First legs (Figs 14, 15, 18, 21): Fringed laterally on the femur, patella, and tibia. Femur prolaterally and ventrally covered with spoon-shaped setae, swollen at the tips. Tibia with two large spatulate macrosetae prolaterally. Third legs (Figs 13, 16, 17): Patella swollen into a large triangle. Colour in life (Figs 14–18, 21): Chelicerae dark, with white setae medially. Palp femur and patella covered with beige scales above; cymbium with scattered white erect setae, especially retrolaterally. First leg dark above, but with light green integument below, and with dense fringes of erect setae prolaterally and retrolaterally on the femur, patella and tibia. These fringes are orange on the femur prolaterally, grading to white elsewhere on femur, patella,



Figures 10–21. *Habronattus arcalorus* sp. n. All specimens from Davis Mountains, Texas except Fig. 12, from Cañon City, Colorado. **(10–11** paratype male W328) **10** left palp, ventral view **11** left palp, retrolateral view. **12** epigynum, ventral view, paratype female W332. Scale bar = 0.1 mm. 4 third leg femur, patella and tibia **13–18** living male **19–20** living females. **21** male in courtship pose. All photos credit W. P. Maddison.

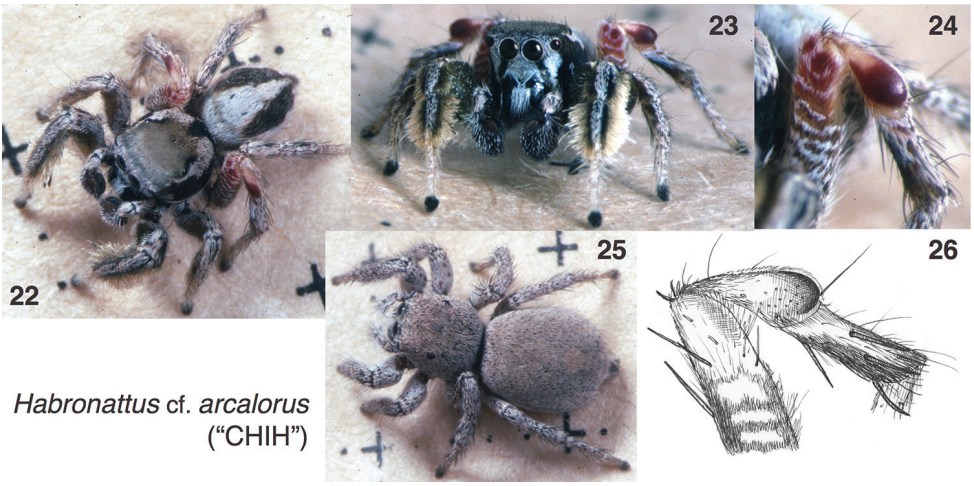
and tibia. Spoon-shaped setae on underside of first femur are white at tips. Femur of third leg with transverse white and dark bands (Figs. 13, 16) in basal half, blue-white scales in distal half that match similar blue-white scales at front of abdomen (Figs.

14-18). Third patella steel-grey with a white fringe above; tibia black with pale band rising obliquely toward the distal and dorsal (Figs 13, 17). Clypeus covered in white setae except for a dark band descending obliquely from each AME. Ocular area rust-coloured, with thin tan band arching between PMEs. Pale thoracic bands wide anteriorly, narrowing to a point at back margin. Abdomen with generous basal band of blue-white scales, broken at the front by two small black lines (Fig. 14). Longitudinal medial band of pale scales on dorsum of abdomen triangular, much wider at front than back. Colour in alcohol: more or less as in life, but with first leg integument yellow-orange, no longer green, and bluish cast of third femur and abdominal basal band not visible.

Female (focal specimen: paratype, specimen W332). Carapace length 2.7; abdomen length 2.8. Structure typical for *Habronattus* and the *clypeatus* group. Epigynum (Fig. 12) with long narrow central pocket in front of which is a small semicircular atrium, as is usual for the species group. Colour (Figs 19-20): Uniform beige to tan, with black triangular patch on posterior thorax. Some specimens show two longitudinal dark bands on abdomen (Fig. 20). The clypeus is covered with white scales, but a hint of the dark oblique bands of the male can be seen beneath the white scales. Likewise, there is a hint of the transverse bands on the third femur.

Additional material examined. USA: TEXAS: Jeff Davis Co.: Davis Mountains State Park 103.939°W, 30.593°N, 22–23 April 1997 Maddison/Hedin/Hebets WPM#97-001 (11 ♂♂, 2 ♀♀, 6 subadult ♂♂, 16 other juveniles). TEXAS: Jeff Davis Co.: Davis Mountains, roadside, highway 118 104.098°W, 30.704°N, 23 April 1997 Maddison/Hedin/Hebets WPM#97-003 (2 ♂♂ 1 ♀). COLORADO: Fremont Co., Royal View Campground. ~9 mi W of Cañon City on HWY 50. N38.495° W105.354°. 24 May 1982 D. & W. Maddison WPM#82-108 (10 ♂♂, 6 ♀♀).

Chihuahuan form. Specimens of a form that may be either a geographical variant of *H. arcalorus*, or a distinct species, were found in the Tomochic area of Chihuahua, Mexico (Figs 22-26). This is the form referred to by Maddison and Hedin (2003) as "*H.* sp. (CHIH)". The markings, robust body, and dense fringes on the male first leg match those of *H. arcalorus*, but the third patella is different (Figs. 24, 26): smaller, rounded, dark red in the distal half. The tip of the femur is dark red with white bands, not blue-white as in *H. arcalorus*. The front of the abdomen is not bluish, nor does the basal band have two clear dark stripes. *Habronattus arcalorus* and the Chihuahuan form do not group together by mitochondrial data in Maddison and Hedin's (2003) gene tree, but Maddison and Hedin suggest there is introgression from the *coecatus* group into the Chihuahuan form, and nuclear data are lacking. It seems likely that a diversity of geographic forms exists in satellite ranges of the Sierra Madre Occidental and Sierra Madre Oriental in Chihuahua and Coahuila, yet to be discovered. Until more detailed studies are done, we will avoid giving a new taxon name to the Chihuahan form, and call it *H.* cf. *arcalorus* ("CHIH"). The material examined of the Chihuahuan form (all in UBC-SEM) is: MEXICO: CHIHUAHUA: 1 mi E of Tomochic on HWY 16. 14 Mar 1996 Susan Masta (6 ♂♂, 5 ♀♀). CHIHUAHUA: 2.7 mi E of Tomochic on HWY 16. 14 Mar 1996 Susan Masta (2 ♀♀). CHIHUAHUA: 10.1 mi



Figures 22–26. *Habronattus* cf. *arcalorus* ("CHIH"). All specimens from near Tomochic, Chihuahua. **22–23** living male. **24** third leg, living male **25** female **26** third leg in alcohol. All photos credit W. P. Maddison.

E of Tomochic on HWY 16. 14 Mar 1996 Susan Masta (4 ♂♂, 4 ♀♀). CHIHUAHUA: 15 mi N of Madera road to 40 Casas archaeological zone. 15 Mar 1996 Susan Masta (2 ♂♂, 1 ♀).

Natural history. *Habronattus arcalorus* was found abundantly in both areas in which it was collected. At the Cañon City locality it was found on rocks on a dry hillside with sparse vegetation and pinyon pines (Fig. 33). In the Davis Mountains of Texas it was found on oak leaf litter mixed with grass and rocks in a stream valley, and on rocks mixed with grass in a juniper-pinyon-oak woodland. Fig. 21 shows a male in courtship pose. Maddison and Leduc-Robert (2013) report the karyotype of *H. arcalorus* as 26 acrocentric autosomes plus 2 acrocentric X chromosomes in males, as is typical for salticids.

Habronattus gilaensis **Maddison & Maddison, sp. n.**
http://zoobank.org/B708C045-57ED-4286-806E-E96615C6ADF3
Figs 27–32

Holotype. male in UBC-SEM, with labels "USA: New Mexico: Grant Co., Bill Evans Lake, 1400 m. 32.865°N, 108.5784°W, 10 April 1996 D.R. Maddison DRM96.015" and "W123".

Paratypes (5♂♂ 2♀♀). New Mexico: Grant Co., Gila River, Billings Vista, 32.8137°N, 108.6011°W, 11 August 2005 D.R. Maddison DRM 05.042 (1♂ in each of AMNH, MCZ, OSAC; 1♀ in UBC-SEM); New Mexico: Grant Co., Bill Evans Lake, 1400 m. 32.865°N, 108.5784°W, 10 April 1996 D.R. Maddison DRM96.015

(1♂ in UBC-SEM); New Mexico: Grant Co., route 90 S of Tyrone, 1755 m. 32.702°N, 108.3043°W, 10 April 1996 D.R. Maddison DRM96.016 (1♂ in UBC-SEM); New Mexico: Walnut Creek Road NW of Silver City, 32.8309°N, 108.3326°W, 6 April 2012, C. Wu (1♀ in UBC-SEM, "female 434" of D. Elias laboratory);

Etymology. The name refers to the known distribution of this species, in the region of the Gila River drainage of New Mexico.

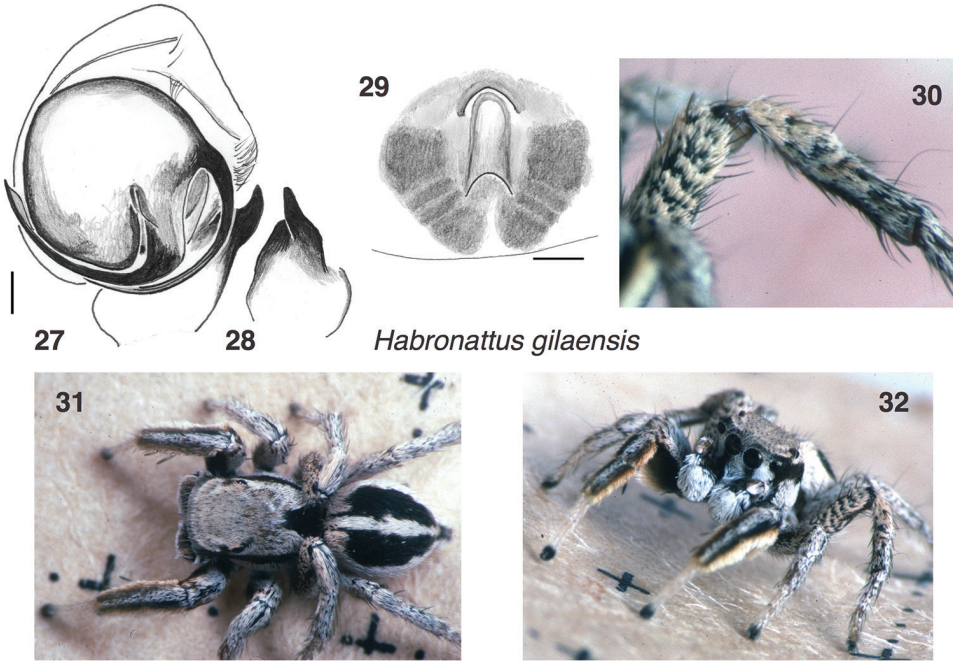
Diagnosis. The third leg has an unornamented patella and tibia (Fig. 30), unlike all other members of the *H. clypeatus* group (Figs 1-9). The only other species of the group with green first legs in the male are *H. dossenus* and *H. arcalorus*. From *H. arcalorus*, *H. gilaensis* differs in many details of ornamentation, especially the third leg. *H. gilaensis* is most similar to *H. dossenus*, but differs (in males) in almost complete lack of ornamentation on the third leg, in the paler ocular area, denser and longer fringes on the first leg, denser covering of white setae on the cymbium, and in having the white scales on the side of the carapace broken by a black band extending posteriorly.

Note. This species was referred to as *H.* cf. *dossenus* by Maddison and Hedin (2003).

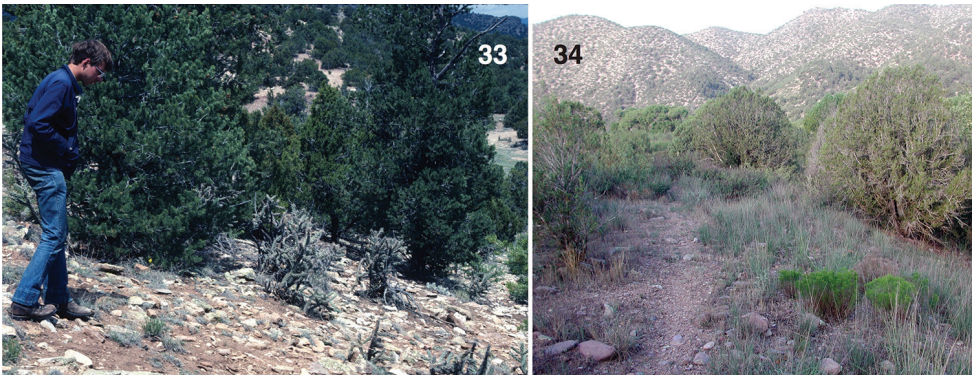
Description. *Male* (focal specimen: holotype, specimen W123). Carapace length 2.2; abdomen length 2.0. Structure of chelicerae, legs, and body typical for *Habronattus* and the *clypeatus* group. Palp (Figs 27–28) standard for the *clypeatus* group, very similar to that of *H. arcalorus*. First legs (Figs 31, 32): Dense fringes of erect setae retrolaterally on the femur, and prolaterally and retrolaterally on the patella and tibia. The setae of these fringes are simple, not swollen as in *H. arcalorus*. Tibia with two large spatulate macrosetae prolaterally. Third legs (Figs 30, 32): Almost as in female, little modified. Colour in life (Figs 31, 32): Chelicerae dark, with white setae medially. Palp femur and patella covered with beige scales above; cymbium fairly densely covered with white setae. First leg dark above, but with green integument below, and fringes tan to white. Femur of third leg with indistinct transverse bands (Figs 30, 32). Clypeus covered in white setae except for a dark band descending obliquely from each AME. Ocular area light brown to tan, with thin tan bands arching between PMEs. Pale thoracic bands wide anteriorly, narrowing to a point at back margin. Abdomen with basal band of white scales broken at the front by two small black lines (Fig. 31). Longitudinal medial band of pale scales on dorsum of abdomen narrow. Medial longitudinal dark band on underside of abdomen. The dark clypeal bands, pale arching band in the ocular area, and broken basal band are also typical for the *clypeatus* group. Colour in alcohol: more or less as in life, but with first leg integument yellow-orange, no longer green.

Female (focal specimen: paratype, "female 434"). Carapace length 2.6; abdomen length 3.2. Structure typical for *Habronattus* and the *clypeatus* group. Epigynum (Fig. 29) with long narrow central pocket in front of which is a small semicircular atrium, as is usual for the species group. Colour: uniform beige to tan scales covering body. Clypeus covered with white scales. Third femur with a hint of the transverse bands seen in the male.

Additional material examined. USA: New Mexico: Grant Co., route 90 S of Tyrone, 1755 m. 32.702°N, 108.3043°W. 10 April 1996. D.R. Maddison. DRM96-016 (3 ♂♂). New Mexico: Grant Co., Big Burro Mountains, road from Lordsburg



Figures 27–32. *Habronattus gilaensis*, sp. n. **27–28** male holotype left palp **29** Paratype female epigynum, ventral view **30–32** living male from south of Tyrone, New Mexico (32.702°N 108.3043°W) **30** is anterior lateral view of third leg. All photos credit W. P. Maddison.



Figures 33–34. Habitats of *H. arcalorus* and *H. gilaensis*. **33** *H. arcalorus* habitat with second author searching for specimens, USA: Colorado: ~9 mi W of Cañon City, 38.495°N, 105.354°W, 24 May 1982 WPM#82–109 **34** *H. gilaensis* habitat (on ground in foreground), USA: New Mexico: Grant Co., Gila River, Billings Vista, 32.8137°N, 108.6011°W, 11 August 2005 DRM 05.042.

to Silver City. 32.505°N, 108.464°W. 9 August 1997. W. Maddison & M. Hedin. WPM#97-028 (1 ♂).

Natural history. Found on open dry ground, on rocks and leaf litter (Fig. 34).

Acknowledgments

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References

Elias DO, Maddison WP, Peckmezian C, Girard MB, Mason AC (2012) Orchestrating the score: complex multimodal courtship in the *H. coecatus* group of *Habronattus* jumping spiders (Araneae: Salticidae). Biological Journal of the Linnaean Society 105: 522–547. doi: 10.1111/j.1095-8312.2011.01817.x

Elias DO, Mason AC, Maddison WP, Hoy RR (2003) Seismic signals in a courting male jumping spider (Araneae: Salticidae). Journal of Experimental Biology 206: 4029–4039. doi: 10.1242/jeb.00634

Griswold CE (1987) A revision of the jumping spider genus *Habronattus* F.O.P.-Cambridge (Araneae; Salticidae), with phenetic and cladistic analyses. The University of California Publications in Entomology 107: 1–344.

Maddison WP, Hedin MC (2003) Phylogeny of *Habronattus* jumping spiders (Araneae: Salticidae), with consideration of genital and courtship evolution. Systematic Entomology 28: 1–21. doi: 10.1046/j.1365-3113.2003.00195.x

Maddison WP, Leduc-Robert G (2013) Multiple origins of sex chromosome fusions correlated with chiasma localization in *Habronattus* jumping spiders (Araneae: Salticidae). Evolution 67: 2258–2272. doi: 10.1111/evo.12109

Peckham GW, Peckham EG (1890) Additional observations on sexual selection in spiders of the family Attidae, with some remarks on Mr Wallace's theory of sexual ornamentation. Occasional Papers of the Wisconsin Natural History Society 1: 117–151.

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DATA PAPER

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A geographical distribution database of the genus *Dysdera* in the Canary Islands (Araneae, Dysderidae)

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Abstract

The ground-dweller spider genus *Dysdera* shows very high species richness on the oceanic archipelago of the Canary Islands, providing one of the most outstanding examples of island radiation among spiders, only paralleled by *Tetragnatha* spiders on the Hawaiian archipelago. A georeferenced database of the 48 *Dysdera* species occurring in the Canary Islands was assembled to facilitate ongoing and future research on this remarkable lineage. All species are endemic to the archipelago except for the cosmopolitan *Dysdera crocata*. The dataset consists of 794 distributional records documented from 1971 to 2015, each locality being represented only once per species. Distribution maps are provided for each species, along with basic diversity and distribution information. The database and geographical maps included in this article stand for the most updated, accurate and complete information on the distribution of the spider genus *Dysdera* in the Canary Islands.

Keywords

Canary Islands, dataset, distribution maps, species richness, spiders

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Introduction

Because of their high level of endemism and conservation challenges, the Macaronesian archipelagos, located in the eastern Mid-Atlantic ocean, are listed among Earth's biodiversity hot spots (Myers et al. 2000). The Canary Islands are one of the most diverse and better studied archipelagos in Macaronesia. The islands are of volcanic origin and were never connected to the continent, which lays merely 100 km off the northwest coast of Africa (Fig. 1). The islands are roughly ordered in a straight line. Fuerteventura is the oldest (22–23 My) and closest island to the North African coast. Lanzarote (15 My) is the second oldest and geologically related to Fuerteventura. The rest of the islands become progressively younger towards the west: Gran Canaria (14.5–15 My), Tenerife (12 My), La Gomera (11 My), La Palma (1.7–2 My) and El Hierro (1.1–1.2 My) (Carracedo et al. 1998; van den Bogaard 2013). The major climatic, geological, and ecological differences between Fuerteventura and Lanzarote and the remaining islands are due to the higher erosion and aridification of the former, which have reduced habitat diversity in comparison to the younger central-western islands. Geological and geographic features of the islands have influenced biodiversity patterns in the archipelago. Along the altitudinal gradient, different bioclimatic and vegetation communities characterize the northern and southern slopes of each island. Six main ecological zones can be observed according to altitude, although their limits can differ substantially between windward and leeward slopes, and some zones may even be absent from the leeward slope: (1) the zone from the seashore up to 250 m (700 m on leeward) is characterised by xerophytic shrub communities (lowland xerophytic shrub); (2) from 250 to 600 m the vegetation is dominated by thermosclerophyllous woodlands, almost absent on the lee side; (3) from 600 to 1000 m by subtropical mesic to humid laurel forest influenced by the cloud belt, very scarce on leeward slopes, where it is replaced by native pine forest; (4) from 1000 to 2000 m (700 to 2200 on the leeward slope) by a mesic to dry endemic *Pinus canariensis* forest; (5) from 2000–2200 m to 3250 by dry subalpine scrub, and (6) from 3000–3250 m to the top (3718 m on Tenerife), an extremely scarce vegetation is found, with a limited permanent invertebrate community.

Arthropods are by far the most diverse assemblage of terrestrial Canarian organisms, with nearly 7000 species, 465 of which are spiders, most of them endemisms (64%) (Macías-Hernández 2010). The genus *Dysdera* is the richest spider genus in the Canaries. It includes medium size, nocturnal wandering hunters that mostly inhabit humid places, although some species are adapted to mesic and even dry habitats (Fig. 2). During daytime they find shelter in silk cocoons under rocks, trunks and tree barks (Roberts 1995). The genus is distributed along the circum-Mediterranean region, including the Macaronesian archipelagoes (Deeleman-Reinhold and Deeleman 1988), and presently includes approximately 250 species (World Spider Catalog 2016). To date, 47 endemic species have been documented in the Canary Islands (Arnedo et al. 1996, Arnedo and Ribera 1997, Arnedo and Ribera 1999b, Arnedo et al. 2000a, Arnedo et al. 2007, Macías-Hernández et al. 2010). Most of the species are

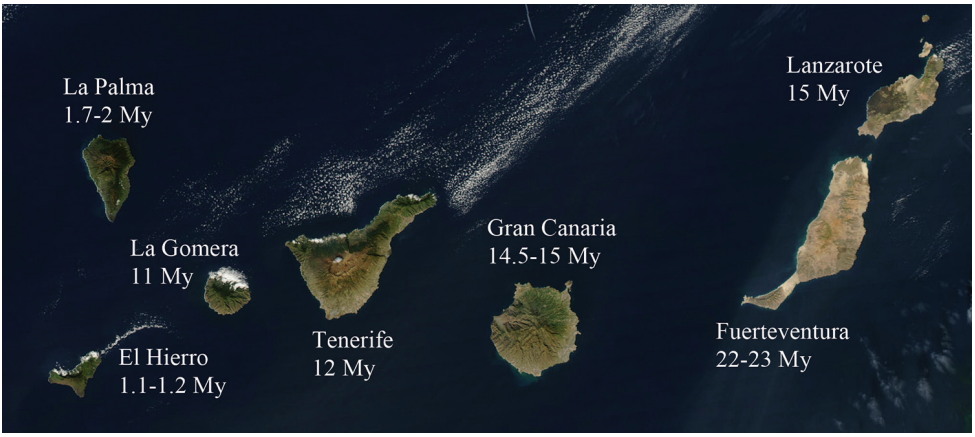

Figure 1. Map of the Canary Islands showing their geological age according to Carracedo et al., (1998) and van den Bogaard (2013).

single island endemics, but a few are present in two or three islands. The synanthropic *D. crocata* Koch, 1839 has also been reported on most of the islands.

During the last 20 years several systematic studies conducted on Canarian *Dysdera* have resulted in a large amount of specimen records and information on geographic distribution that is not always easily accessible. Part of this information is scattered among several publications, and many records remain unpublished. Moreover, many records of Canarian *Dysdera* only included information on locality names, sometimes difficult to identify on a map. The conversion of the locality names into georeferenced points facilitates the correct assignment and visualization of species distribution (Chapman and Wieczorek 2006). The creation of an up to date, fully georeferenced distribution database of *Dysdera* in the Canary Islands based on all available records, is a necessary step to summarize and further improve our current knowledge on this remarkable genus, and will offer an invaluable tool for future systematic, evolutionary, biogeographic, and ecological studies.

Methods

Sampling

Specimens referred in this database were collected during the last 45 years, mainly by members of the **GIET** (Grupo de Investigaciones Entomológicas de Tenerife) from the University of La Laguna (henceforth **ULL**), and by members of the University of Barcelona (**UB**), as well as by some external collaborators. The material used to assemble the database comes from different sources, most of the records corresponding to collection trips conducted during PhD and master thesis fieldwork, and by other research projects (see details below):

Figure 2. Habitus of some *Dysdera* species: **a** *D. calderensis* **b** *D. longa* **c** *D. verneaui* **d** *D. unguimmanis* **e** *D. minutissima* **f** *D. arabisenen* **g** *D. sibyllina* **h** *D. silvatica.* Photographs by P. Oromí.

1. Specimens from PhD and master dissertations carried out at the University of La Laguna and University of Barcelona (A.L. Medina 1991, J.L. Martín 1992, M.A. Arnedo 1996, N. Macías-Hernández 2010, M. Roca-Cusachs 2016) (70%, approx. 3,200 records).
2. Specimens collected during research projects and other unpublished studies conducted by i) GIET at the University of La Laguna: LIFE-Nature Project in Teide National Park (Oromí et al. 2002), Archipiélago Chinijo (Macías et al. 2004), NetBiome Project (unpublished data); ii) National Museum of Natural History at Madrid: 1999 and 2000 in Caldera de Taburiente National Park (Domingo-Quero et al. 2003) (30%, approx. 1,380 records).

Most of the material used in the dataset is deposited in the collections of the Department of Animal Biology, Edaphology and Geology, University of La Laguna (**DZUL**), and the *Centre de Recerca de Biodiversitat Animal*, University of Barcelona (**CRBA: UB**).

Although the sampling procedure depended on each research project, it was mainly conducted by direct searching under stones and logs, beneath tree bark, scraping soil and rocky embankments, or in volcanic caves. A large amount of specimens were also collected by using pitfall traps both on the surface and in the mesovoid shallow substratum (MSS) (López and Oromí 2010). The specimens collected were later preserved in individual vials either in 70% or in 95% ethanol. Labels for each specimen included information on locality, date, and collectors, as well as the taxonomic identification. This information was digitized following the criteria and standards of the Global Biodiversity Information Facility (GBIF http://www.gbif.org), and the specimens stored at the ULL are kept in the DZUL collection, which includes type material of 29 Macaronesian species of *Dysdera* (Reboleira et al. 2012).

Geographic distribution of *Dysdera*

A complete raw dataset of specimen-based records identified to species level by the authors was firstly assembled. All available information (i.e. number of specimens, species identification, identifier's name, sex, locality, geographic coordinates, altitude, type of habitat, date, collector and observations) was digitized in a Microsoft Excel 2011 spreadsheet (data not shown). A second species distribution table (Suppl. material 1) was constructed using the previous raw dataset, including all localities where each species was found, the type of habitat and the altitude of each location.

GPS coordinates were converted to decimal degrees with the online coordinate converter available at http://www.asturnatura.com/sinflac/calculadora-conversiones-coordenadas.php. Old locality records without available geographic coordinates were identified and assigned by the IDECanarias online platform *Sistema de Información Territorial de Canarias* of the Canary Islands Government (http://visor.grafcan.es/visorweb/). Information about doubtful localities was requested from the collectors when possible. Records that were not georeferenced (1% of the total) were excluded from the database.

Table 1. Presence of each *Dysdera* species per island, indicating the corresponding number of endemic species. The troglobitic species are marked on grey. H: El Hierro, P: La Palma, G: La Gomera, T: Tenerife, C: Gran Canaria, F: Fuerteventura, L: Lanzarote.

Species	H	P	G	T	C	F	L
Dysdera alegranzaensis Wunderlich, 1992							X
Dysdera ambulotenta Ribera, Ferrández & Blasco, 1985				X			
Dysdera andamanae Arnedo & Ribera, 1997					X		
Dysdera arabisenen Arnedo & Ribera, 1997					X		
Dysdera bandamae Schmidt, 1973					X		
Dysdera brevisetae Wunderlich, 1992				X			
Dysdera brevispina Wunderlich, 1992				X			
Dysdera calderensis Wunderlich, 1987		X	X				
Dysdera chioensis Wunderlich, 1992				X			
Dysdera cribellata Simon, 1883				X			
Dysdera crocata Koch, 1838	X	X	X	X	X		
Dysdera curvisetae Wunderlich, 1992				X			
Dysdera enghoffi Arnedo, Oromí & Ribera, 1997			X				
Dysdera esquiveli Ribera & Blasco, 1986				X			
Dysdera gibbifera Wunderlich, 1992				X			
Dysdera gollumi Ribera & Arnedo, 1994				X			
Dysdera gomerensis Strand, 1911	X		X				
Dysdera guayota Arnedo & Ribera, 1999			X	X			
Dysdera hernandezi Arnedo & Ribera, 1999				X			
Dysdera hirguan Arnedo, Oromí & Ribera, 1997			X				
Dysdera iguanensis Wunderlich, 1987				X	X		
Dysdera insulana Simon, 1883				X	X		
Dysdera labradaensis Wunderlich, 1992				X			
Dysdera lancerotensis Simon, 1907						X	X
Dysdera levipes Wunderlich, 1987			X	X	X		
Dysdera liostethus Simon, 1907					X		
Dysdera longa Wunderlich, 1992						X	
Dysdera macra Simon, 1883				X			
Dysdera madai Arnedo, 2007				X			
Dysdera mahan Macías-Hernández & Arnedo, 2010						X	X
Dysdera minutissima Wunderlich, 1992				X			
Dysdera montanetensis Wunderlich, 1992				X			
Dysdera nesiotes Simon, 1907							X
Dysdera orahan Arnedo, Oromí & Ribera, 1997	X		X				
Dysdera paucispinosa Wunderlich, 1992					X		
Dysdera ramblae Arnedo, Oromí & Ribera, 1997			X				
Dysdera ratonensis Wunderlich, 1992		X					
Dysdera rugichelis Simon, 1907					X		
Dysdera sanborondon Arnedo, Oromí & Ribera, 2000						X	

Species	H	P	G	T	C	F	L
Dysdera sibyllina Arnedo, 2007				X			
Dysdera silvatica Schmidt, 1981	X	X	X				
Dysdera simbeque Macías-Hernández & Arnedo, 2010							X
Dysdera spinidorsum Wunderlich, 1992						X	
Dysdera tilosensis Wunderlich, 1992					X		
Dysdera unguimmanis Ribera, Ferrández & Blasco, 1985				X			
Dysdera verneaui Simon, 1883				X			
Dysdera volcania Ribera, Ferrández & Blasco, 1985				X			
Dysdera yguanirae Arnedo & Ribera, 1997					X		
Total single-island endemic species	-	1	3	19	8	3	3
Total Canarian endemic species	3	3	9	23	11	5	5

Following data entry, a data checking procedure to minimize likely data-entry errors was conducted. This included an assessment of records with the same localities for spelling errors, double-checking uncertain records (species identification, geographical coordinates, etc). Accurate spelling of scientific names and taxonomic synonyms was revised according to Arnedo (2003).

A georeferenced distribution map of each *Dysdera* species was generated using the free open source Geographic Information System program QGIS 2.12.3 (QGIS 2016).

Results

Database summary

The original raw database, from which all locality records were extracted, consisted of 4,595 individual records identified to species level (data not shown). All georeferenced localities where each species was collected are shown in Suppl. material 1.

Species distribution

The distribution of each *Dysdera* species per island is summarized in Table 1. This table includes the corrected and updated species distribution regarding the last published checklist of terrestrial species from the Canary Islands (Macías-Hernández, 2010), as well as many new citations not included in the public database *Banco de Datos de Biodiversidad de Canarias* (http://www.biodiversidadcanarias.es/atlantis/common/index.jsf).

The proportion of endemic *Dysdera* species per island and the species shared between islands is shown graphically in Fig. 3. All distributional maps of the species are presented in the Suppl. material 2.

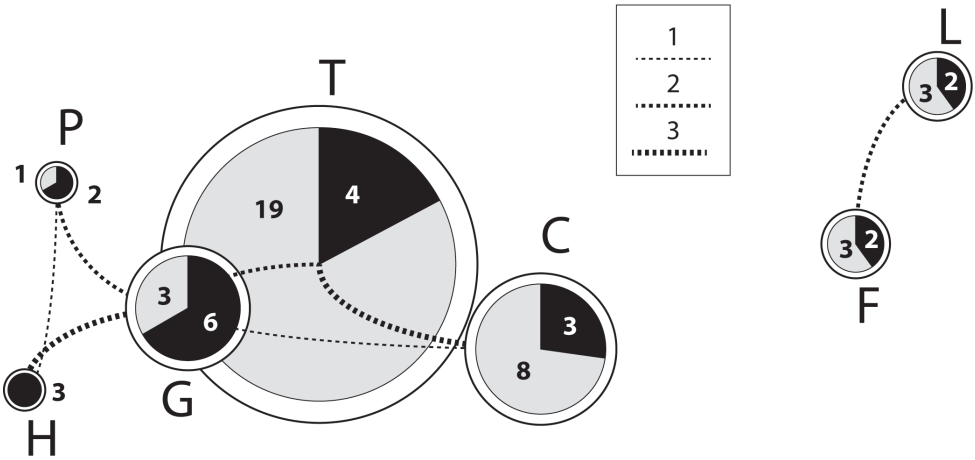

Figure 3. Graphical representation of the island endemisms and the species shared between islands. Pie sizes are proportional to the number of species on each island. Black sectors: number of species shared with other islands; grey sectors: proportion of local endemisms. Lines connecting pies: number of shared species between the corresponding islands, the width of the lines being proportional to the number of shared species. The disposition of pies reflects the geographical arrangement of the islands: P: La Palma, H: El Hierro, G: La Gomera, T: Tenerife, C: Gran Canaria, F: Fuerteventura, L: Lanzarote.

Discussion

The species composition of oceanic islands is the joint result of colonization from nearby continental regions, and local speciation and extinction processes (MacArthur and Wilson 1967). Molecular phylogenetic analyses of Canarian *Dysdera* suggest that the present day diversity of the genus on the island traces back to two or three colonization events (Arnedo et al. 2001, Arnedo et al. 2007). The species on the western Canaries and the species on the eastern Canaries form distinct clades, the relationships of which remain unresolved. The last colonization event corresponds to the species *D. lancerotensis*, which has its closest relatives in Morocco and colonized the eastern Canaries more recently.

Species richness in Canarian *Dysdera* is positively correlated with the area, the elevation and the habitat diversity of the islands (Arnedo and Ribera 1999a, Arnedo et al. 2000a, Cardoso et al. 2010). It shows the typical humpback relationship with age also described for other organisms inhabiting the Canary Islands (Fig. 3) (Emerson and Oromí 2005, Cardoso et al. 2010): the central islands, of intermediate age (La Gomera, Tenerife and Gran Canaria) harbour the highest number of both species (9, 23, and 11 respectively) and single island endemics. Both the youngest (La Palma and El Hierro) and the oldest (Fuerteventura and Lanzarote) islands have less species, but the proportion of island endemics is higher in the oldest islands. This pattern is interpreted as the result of high local diversification and low extinction in the central islands, high extinction rates on the oldest ones due to the habitat lost driven by ero-

sion (Arnedo et al. 2000b, Macías-Hernández et al. 2008) and the dominant role of immigration on the youngest islands.

Dysdera spiders have colonized all types of terrestrial habitats in the Canaries, from the intertidal (Macías-Hernández et al. 2010) to the highest altitudes (Macías-Hernández et al. 2013). *Dysdera* seems to be especially diverse in the laurel forest. The laurel forest of Anaga and Teno in Tenerife harbour nine species each. Similarly, the laurel forest of La Gomera has seven species, and the small remnants of such forest in Gran Canaria, five species. *Dysdera* have also colonized the underground environment. The Canary Islands harbour the highest number of cave-dwelling species in the whole genus (10 out of the 16 species reported so far in the whole genus). Furthermore, *Dysdera* is the spider genus with the highest number of troglobitic species in the archipelago (Oromí et al. 2001, Oromí 2004). To date, nine troglobitic species have been found in Tenerife and one in La Palma (highlighted in grey in Table 1) (Arnedo et al. 2007). Cave-dwelling species are not restricted to lava tubes and recent prospection of the mesovoid shallow environment (MSS) in El Hierro, Tenerife and Gran Canaria have shown evidence of taxa with morphological adaptations to subterranean environments.

A striking pattern of *Dysdera* in the Canaries is the frequent co-occurrence of species in the same locality. With very few, if any, exceptions, all the species overlap distributions with at least one other species. This observation raises the question of what are the factors that promote species coexistence in Canarian *Dysdera*. Co-occurring species tend to differ in size and cheliceral shape, which in *Dysdera* has been shown to be a proxy for diet specialization: some species seem to be generalist predators while other are oniscophagous specialists (ie. they feed preferably on woodlice) (Řezáč et al. 2008). A study combining phylogenetic information with distribution ranges in cave-dwelling *Dysdera* suggested that character displacement in phenotypic characters related to prey capture following secondary contact may explain the high levels of species range overlapping (Arnedo et al. 2007).

The conservation status of the genus *Dysdera* in the Canary Islands has not been adequately addressed yet. Some of the richest localities in terms of number of endemic *Dysdera* are protected areas included in the *Red Canaria de Espacios Naturales Protegidos* and the Natura 2000 Network. One of the main threats on the species survival is the loss or degradation of suitable habitats. This is especially true for species with very restricted distributions, or low abundances, and the very specialized species inhabiting the fragile underground environment. Previous studies conducted in several caves of the Canarian archipelago (Oromí et al. 2001), revealed high levels of severe soil pollution, sometimes favoured by the illegal use of lava tubes as house sewages. The introduction of exotic species represents an additional thread, due to the competition for resources with the endemic species. In the Macaronesian archipelagos the cosmopolitan *Dysdera crocata* has been catalogued as an invasive species (Cardoso et al. 2008, Boletín Oficial del Estado, 2013) due to its negative impact on the ecosystems, and in the Azores it has been considered as the main cause of extinction for native *Dysdera* (Cardoso et al. 2010). In the Canary Islands *D. crocata* is widespread, and can also be found in natural habitats (Macías-Hernández and de la Cruz in prep.), but further studies are required to determine the real impact on the native fauna. Several Canar-

ian *Dysdera* species are currently being assessed for the IUCN (Macías-Hernández in prep.) for eventual redlisting and to guarantee the protection of declining or endangered species. Future studies focused on the conservation of the Canarian biota are needed to ensure the survival of these fragile island ecosystems.

The database and geographical maps presented in this article stand for the most updated and extensive information on the distribution of the spider genus *Dysdera* in the Canary Islands. One of the most powerful applications of this database is its use as a data-exploration tool for further ecological, biogeographical, taxonomic and conservation studies. It will facilitate the visualization of widespread and narrowly distributed species, the patterns of species coexistence, and the distribution gaps. Furthermore, the combination of georeferenced distribution data with environmental information (habitat use, vegetation, projected climate, etc.) can be used for ecological niche modelling studies (Graham et al. 2004).

Data resources

Data package title: A geographical distribution database of the genus *Dysdera* in the Canary Islands (Araneae, Dysderidae)
Provisional doi: 10.5061/dryad.t63mn
Data files: Macías-Hernandez et al. (2016), Zookeys, Species Distribution Table
Macías-Hernandez et al. (2016), Zookeys, Species Distribution Maps

Acknowledgements

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References

Arnedo MA (2003) Lost and found: rediscovery of type material of some endemic species of the spider genus *Dysdera* (Araneae, Dysderidae) from the Canary Islands, and its nomenclatural and taxonomic implications. Revista Ibérica de Aracnología 7: 141–148.

Arnedo MA, Oromí P, Múrria C, Macías-Hernández N, Ribera C (2007) The dark side of an island radiation: systematics and evolution of troglobitic spiders of the genus *Dysdera* Latreille (Araneae: Dysderidae) in the Canary Islands. Invertebrate Systematics 21: 623–660. doi: 10.1071/IS07015

Arnedo MA, Oromí P, Ribera C (1996) Radiation of the genus *Dysdera* (Araneae, Haplogynae, Dysderidae) in the Canary Islands: the Western Islands. Zoologica Scripta 25: 241–274. doi: 10.1111/j.1463-6409.1996.tb00165.x

Arnedo MA, Oromí P, Ribera C (2001) Radiation of the spider genus *Dysdera* (Araneae, Dysderidae) in the Canary Islands: Cladistic assessment based on multiple data sets. Cladistics 17: 313–353. doi: 10.1111/j.1096-0031.2001.tb00129.x

Arnedo MA, Oromí P, Ribera C (2000a) Systematics of the genus *Dysdera* (Araneae, Dysderidae) in the Eastern Canaries. Journal of Arachnology 28: 261–292. doi: 10.1636/0161-8202(2000)028[0261:SOTGDA]2.0.CO;2

Arnedo MA, Oromí P, Ribera C (2000b) Systematics of the genus *Dysdera* (Araneae, Dysderidae) in the Eastern Canary Islands. Journal of Arachnology 28: 261–292. doi: 10.1636/0161-8202(2000)028[0261:SOTGDA]2.0.CO;2

Arnedo MA, Ribera C (1999a) Radiation of the genus *Dysdera* (Araneae, Dysderidae) in the Canary Islands: The island of Tenerife. Journal of Arachnology 27: 604–662.

Arnedo MA, Ribera C (1997) Radiation of the genus *Dysdera* (Araneae, Haplogynae, Dysderidae) in the Canary Islands: The island of Gran Canaria. Zoologica Scripta 26: 205–243. doi: 10.1111/j.1463-6409.1997.tb00413.x

Arnedo MA, Ribera C (1999b) Radiation of the spider genus *Dysdera* (Araneae, Dysderidae) in the Canary Islands: the island of Tenerife. Journal of Arachnology 27: 604–662.

Boletín Oficial del Estado (2013) Real Decreto 630/2013, de 2 de agosto, por el que se regula el Catálogo español de especies exóticas invasoras. 185: 56764–56786.

Cardoso P, Arnedo MA, Triantis K, Borges PAV (2010) Drivers of diversity in Macaronesian spiders and the role of species extinctions. Journal of Biogeography 37: 1034–1046. doi: 10.1111/j.1365-2699.2009.02264.x

Cardoso P, Borges PAV, Macías-Hernández N (2008) *Dysdera crocata* C.L. Koch, 1838. In: Silva L, Ojeda EL, Rodríguez-Luengo JL (Eds) Invasive terrestrial flora and fauna of Macaronesia. TOP 100 in Azores, Madeira and Canaries ARENA, Ponta Delgada, 415–417.

Chapman AD, Wieczorek J (2006) Guide to best practices for georeferencing. Global Biodiversity Information Facility, Copenhagen, 90 pp.

Deeleman-Reinhold CL, Deeleman PR (1988) Révision des Dysderinae. Tijdschrift voor Entomologie 131: 141–269.

Domingo-Quero T, Alonso-Zarazaga MÁ, Sánchez-Ruiz A, Araujo R, Navas A, Sánchez Moreno S, García Becerra R, Nebreda M, Sánchez Ruiz M, Fontal-Cazalla F (2003) Inventariando la biodiversidad en el Parque Nacional de la Caldera de Taburiente (La Palma, Islas Canarias, España): novedades científicas. Graellsia 59: 45–68. doi: 10.3989/graellsia.2003.v59.i2-3.235

Emerson BC, Oromí P (2005) Diversification of the forest beetle genus *Tarphius* on the Canary Islands, and the evolutionary origins of island endemics. Evolution 59: 586–598. doi: 10.1111/j.0014-3820.2005.tb01018.x

Graham CH, Ferrier S, Huettman F, Moritz C, Peterson AT (2004) New developments in museum-based informatics and applications in biodiversity analysis. Trends in Ecology & Evolution 19: 497–503. doi: 10.1016/j.tree.2004.07.006

López H, Oromí P (2010) A type of trap for sampling the mesovoid shallow substratum (MSS) fauna. Speleobiology Notes 2: 7–11.

MacArthur RH, Wilson EO (1967) The theory of island biogeography. Princeton University Press, Princeton.

Macías N, Pérez A, López H, Oromí P (2004) Fauna de artrópodos de Montaña Clara (Islas Canarias) III: arácnidos, miriápodos y crustáceos terrestres. Revista de la Academia Canaria de Ciencias 15: 53–68.

Macías-Hernández N (2010) *Araneae*. Lista de especies silvestres de Canarias (hongos, plantas y animales terrestres). In: Arechavaleta M, Rodríguez S, Zurita N, García A (Eds) Consejería de Medio Ambiente y Ordenación Territorial, Gobierno de Canarias. S/C Tenerife, 202–212.

Macías-Hernández N, Bidegaray-Batista L, Emerson BC, Oromí P, Arnedo M (2013) The imprint of geologic history on within-island diversification of woodlouse-hunter spiders (Araneae, Dysderidae) in the Canary Islands. Journal of Heredity 104: 341–356. doi: 10.1093/jhered/est008

Macías-Hernández N, Oromí P, Arnedo Ma (2010) Integrative taxonomy uncovers hidden species diversity in woodlouse hunter spiders (Araneae, Dysderidae) endemic to the Macaronesian archipelagos. Systematics and Biodiversity 8: 531–553. doi: 10.1080/14772000.2010.535865

Macías-Hernández N, Oromí P, Arnedo MA (2008) Patterns of diversification on old volcanic islands as revealed by the woodlouse-hunter spider genus *Dysdera* (Araneae, Dysderidae) in the eastern Canary Islands. Biological Journal of the Linnean Society 94: 589–615. doi: 10.1111/j.1095-8312.2008.01007.x

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403: 853–858. doi: 10.1038/35002501

Oromí P (2004) Biospeleology in Macaronesia. Association for Mexican Cave Studies Bulletin 19: 98–104.

Oromí P, Zurita N, Arechavaleta M, Camacho A (2002) Fauna de invertebrados del Parque Nacional del Teide. Ministerio de Medio Ambiente, Madrid.

Oromí P, Zurita N, Muñoz E, de la Cruz S (2001) Conservación de la fauna invertebrada cavernícola de las islas de Tenerife, El Hierro y La Palma, 394 pp.

QGIS DT (2016) QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org

Reboleira ASP, Pérez AJ, López H, Macías Hernández N, de la Cruz S, Oromí P (2012) Catalogue of the type material in the entomological collection of the University of La Laguna (Canary Islands, Spain). I. Arachnida. Zootaxa 3556: 61–79.

Řezáč M, Pekár S, Lubin Y (2008) How oniscophagous spiders overcome woodlouse armour. Journal of Zoology 275: 64–71. doi: 10.1111/j.1469-7998.2007.00408.x

Roberts MJ (1995) Spiders of Britain & Northern Europe. Harper Collins Publisher, London.

World Spider Catalog (2016) World Spider Catalog. Natural History Museum Bern. http://wsc.nmbe.ch [version 17.0, accessed on March 2016]

Supplementary material 1

Supplementary Table 1

Authors: Nuria Macías-Hernández, Salvador de la Cruz López, Marcos Roca-Cusachs, Pedro Oromí, Miquel A. Arnedo

Data type: distribution data

Explanation note: Distribution of each *Dysdera* species, indicating species name, locality, district, island, type of habitat, geographic coordinates, and altitude (m).

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Supplementary material 2

Supplementary Figure 1

Authors: Nuria Macías-Hernández, Salvador de la Cruz López, Marcos Roca-Cusachs, Pedro Oromí, Miquel A. Arnedo

Data type: distribution data

Explanation note: Distributional map of each species of *Dysdera* (alphabetically ordered).

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Phylogeography of a good Caribbean disperser: *Argiope argentata* (Araneae, Araneidae) and a new 'cryptic' species from Cuba

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Abstract

The Caribbean islands harbor rich biodiversity with high levels of single island endemism. Stretches of ocean between islands represent significant barriers to gene-flow. Yet some native species are widespread, indicating dispersal across oceans, even in wingless organisms like spiders. *Argiope argentata* (Fabricius, 1775) is a large, charismatic, and widespread species of orb-weaving spider ranging from the United States to Argentina and is well known to balloon. Here we explore the phylogeography of *A. argentata* in the Caribbean as a part of the multi-lineage CarBio project, through mtDNA haplotype and multi-locus phylogenetic analyses. The history of the *Argiope argentata* lineage in the Caribbean goes back 3-5 million years and is characterized by multiple dispersal events and isolation-by-distance. We find a highly genetically distinct lineage on Cuba which we describe as *Argiope butchko* **sp. n.** While the *argentata* lineage seems to readily balloon shorter distances, stretches of ocean still act as filters for among-island gene-flow as evidenced by distinct haplotypes on the more isolated islands, high F^{ST} values, and strong correlation between intraspecific (but not interspecific) genetic and geographic distances. The new species described here is clearly genetically diagnosable, but morphologically cryptic, at least with reference to the genitalia that typically diagnose spider species. Our results are consistent with the intermediate dispersal model suggesting that good dispersers, such as our study species, limit the effect of oceanic barriers and thus diversification and endemism.

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Keywords
Biogeography, CarBio, dispersal, diversification, GAARlandia, Intermediate dispersal model, Isolation by distance model

Introduction

The Caribbean diversity hotspot has been colonized by a number of lineages via varying routes over millions of years. As is typical of other old oceanic islands, the archipelago's isolation helped form numerous single-island endemic species (Agnarsson and Kuntner 2012; Gillespie and Roderick 2002; Ricklefs and Bermingham 2008; Warren et al. 2015). The Caribbean islands are diverse in origin. Some are Darwinian volcanic islands that have been colonized exclusively by overwater dispersal – airborne or across the ocean, e.g. via vegetation rafts. Others are Wallacean fragment islands whose periodic connection to the mainland may have facilitated colonization over land bridges such as GAARlandia (Iturralde-Vinent and MacPhee 1999; Ricklefs and Bermingham 2008). Regardless, all the Greater Antilles islands and most of the minor Antilles have been isolated for the last several million years (Ali 2012; Heinicke et al. 2007; Iturralde-Vinent and MacPhee 1999; Iturralde-Vinent 2006). Thus the processes of divergence and diversification among islands due to lack of gene-flow can be expected to be ongoing in all but the best dispersing organisms for which stretches of ocean do not present formidable barriers—one prediction of the intermediate dispersal model (IDM) (Agnarsson et al. 2014; Claramunt et al. 2012; Weeks and Claramunt 2014). Such organisms are typically flying animals, or plants with salt-tolerant floating seeds, that are widespread but species depauperate (Weeks and Claramunt 2014).

Being wingless, a relatively small proportion of arachnid lineages tend to colonize ocean islands. Single-island endemism is common in successfully colonizing lineages (Arnedo and Gillespie 2006; Arnedo et al. 2007; Gillespie 2005; Gillespie et al. 2008; Zhang and Maddison 2012), a pattern consistent across taxa, islands and archipelagos including the Caribbean (Alayon 2006; Cosgrove et al. 2016; Crews and Gillespie 2010; Dziki et al. 2015; Esposito et al. 2015; McHugh et al. 2014; Zhang and Maddison 2012). This pattern is also found in many other invertebrates, and in vertebrates and plants (Ricklefs and Birmingham 2008). However, this pattern is by no means universal and different lineages often show contrasting patterns, such as in certain species of the spider genus *Selenops* (Crews et al. 2010). Indeed, some spiders can readily disperse overwater by 'ballooning'—becoming airborne on silk threads anchored to their spinnerets (Bell et al. 2005). For ballooning spiders stretches of ocean could be only partial barriers (filters) leading to predictions of limited diversification among islands. Our study subject here, *Argiope* spiders (Bell et al. 2005; Levi 1983), is potentially one such lineage.

Species of the genus *Argiope* are large, sexually dimorphic, charismatic spiders with brightly colored abdomens (Cheng and Kuntner 2014, 2015) that were noted

by early taxonomists and among the first spiders to be described (Catalog 2015; Clerck 1757). Despite their large adult size, *Argiope* spiders are thought to be excellent dispersers because they occupy open tree-less habitats and have been documented to balloon (Bell et al. 2005). *Argiope argentata* (Fabricius, 1775) is a species ranging from the United States to the Caribbean islands and as far south as Argentina (Levi 1983). It occurs on practically every Caribbean island and is thus an interesting subject for phylogeographical studies on relatively good dispersers.

Here, we present mtDNA and morphological data on *Argiope argentata* collected throughout the Caribbean to reveal phylogeographical patterns within the Caribbean, to test the degree of genetic structure within and among islands, and to measure divergence in cases where genetic patterns reflect geography. We verify relationships among species with a multi-locus phylogenetic approach, and we also describe a new species, *Argiope butchko* sp. n., previously thought to represent Cuban populations of *A. argentata*.

Methods

Specimens of *Argiope argentata* s. l. were collected diurnally using standard aerial searching and beating methods from 2011-2015 across the Caribbean and in SE USA (Fig. 1, Suppl. material 4), including at four sites in Cuba: Siboney in Santiago, Alejandro in Guantanamo, Sierra de Camaguey in Camaguey, and Viñales, Sierra de los Órganos, Pinar del Rio. Specimens were preserved in 95% ethanol in the field and stored at -20° C until DNA extraction. Two sequences of mainland American *A. argentata* and seven sequences of outgroups, downloaded from Bold and GenBank, were included in the analyses (Suppl. material 4). As outgroups we included eight *Argiope* species, including the closest relatives of *A. argentata* based on a recent molecular phylogeny (Cheng and Kuntner 2014) (see Suppl. material 4).

DNA was isolated from 85 *A. argentata* s.l. and 13 other *Argiope* species with the QIAGEN DNeasy Tissue Kit (Qiagen, Inc., Valencia, CA), or using phenol extraction (Suppl. material 4). We sequenced a fragment of the mitochondrial 'DNA barcode' Cytochrome c oxidase subunit 1-COI, a useful marker at low taxonomic levels in spiders, to establish boundaries among species (Čandek and Kuntner 2015; Hebert et al. 2003). To amplify COI we used the primers LCO 1490 and HCO 2198 (Folmer et al. 1994). PCR conditions and sequencing protocols were described previously (Bloom et al. 2014; McHugh et al. 2014). Sequences were submitted to GenBank (see Suppl. material 4 for accession numbers). Sequences were assembled using Phred and Phrap (Green 2009; Green and Ewing 2002) via Chromaseq (Maddison and Maddison 2011a) in Mesquite 3.03 (Maddison and Maddison 2011b) with default parameters. The sequences were proofread and then aligned using the online EMBL-EBI MAFFT (Katoh 2013). COI nucleotide sequences were translated to amino acids to check for stop codons and to detect interspecifically consistent amino acid differences.

For Bayesian analyses, the GTR+I+G model was selected as the appropriate substitution model by the AIC criterion (Posada and Buckley 2004) in jModeltest 2.1.4

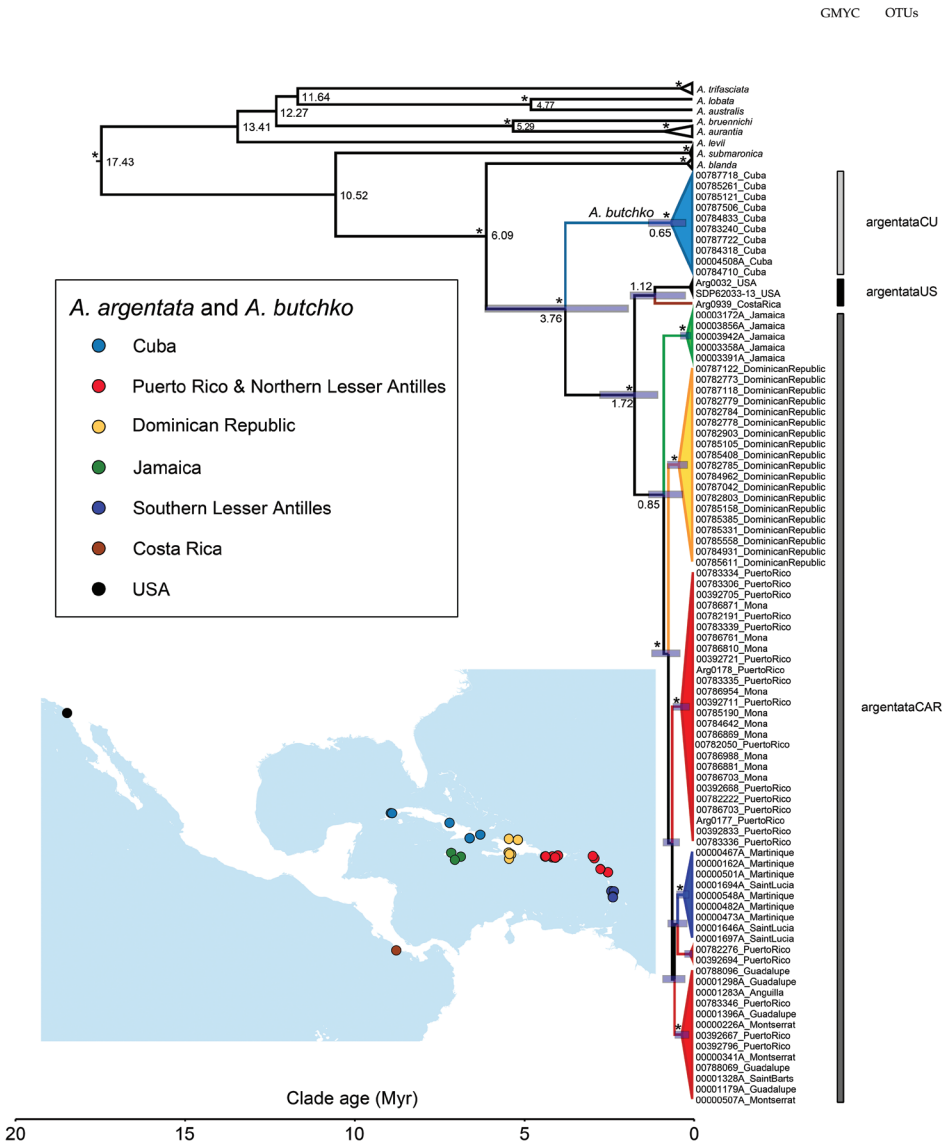

Figure 1. A dated phylogeny of *Argiope argentata* in the Caribbean, and other *Argiope* relatives. Shown are the results of tree based species delimitation analyses (GMYC method) on a BEAST phylogeny (node ages in million years) and the location of spiders used in this study (inset picture). Asterisk denotes posterior probability support >95%. The OTUs (operational taxonomic units) correspond to a cryptic species, *Argiope butchko* sp. n., from Cuba (argentataCU) and populations from other Caribbean islands (argentataCAR) plus mainland (argentataUS) treated as conspecific (*A. argentata*).

(Darriba et al. 2012). We employed a Bayesian approach to phylogenetic reconstruction implemented in MrBayes 3.1.2 (Ronquist et al. 2012). Two independent runs, each with four Markov chain Monte Carlo (MCMC) chains, were performed simulta-

neously with random starting trees, and the MCMC process was run for 10,000,000 generations, with a sampling frequency of 100 and a burn-in of the first 25% generations. We then ran BEAST (Drummond and Rambaut 2007; Drummond et al. 2012) for dating analyses of the mtDNA data. The BEAST run comprised 40,000,000 generations, using a lognormal relaxed clock with fixed estimated substitution rate (mean = 0.0112, SD = 0.001) (Bidegaray-Batista and Arnedo 2011), assuming a birth-death speciation model for the tree prior, with the best fit substitution models, and default options for all other prior and operator settings. The final consensus tree was produced in TreeAnnotator v1.8.0, with 25% burn-in.

To test the phylogenetic relationships from COI data, we also ran Bayesian analysis with a multi-locus dataset with two nuclear markers (28S and Histone 3) and 1–2 exemplars per species. The PCR reactions of 28S and Histone 3 followed established protocols for argiopine spiders (Cheng and Kuntner, 2014). The Bayesian analysis was performed with the GTR+I+G model identified as the best fit substitution model for all loci, and using all other settings as above.

To test for cryptic species in *A. argentata*, we used a combination of tree-based species delimitation methods and genetic distances. For tree-based species delimitation method, the General Mixed Yule-Coalescent model with single threshold (GMYC) (Pons et al. 2006) was applied to the BEAST tree in R 3.0.3 (R_Core_Team 2014) with the Splits package (http://splits.r-forge.r-project.org/). We then calculated the genetic distance among potential OTUs as well as within and among the two species (with Cuban populations defined as putative species), and among all individual specimens. In all cases we used Kimura 2-parameter (K2P) (Kimura 1980) in Mega 6.06 (Tamura et al. 2013). (Table 1). Genetic distances were then correlated with geographic distances, the latter estimated (in m) from latitude and longitude data using the Geographic Distance Matrix Generator (Ersts 2016). Regression analyses between genetic and geographic distances were done in JMP Pro 11 and scatter plots produced in Excel and then modified in Illustrator. In addition to analyses including all ingroup individuals, regression analyses were run separately for intraspecific and interspecific comparisons to test the taxonomic hypothesis of *A. argentata* s. l. containing a cryptic Cuban species. The prediction here is that a correlation between genetic and geographic distances would hold within (e.g., Hamilton and Eckert 2007; Eckert et al. 2008), but not between, species as these should have non-geographic barriers to gene flow.

Fst and Kxy indexes were calculated in DNAsp v5 (Librado and Rozas 2009).

Haplotype networks were constructed using median-joining networks (Bandelt et al. 1999) in PopART (http://popart.otago.ac.nz/index.shtml) with default settings. Networks were exported as graphs and then edited in Adobe Illustrator.

Adult males and females were imaged using a Visionary Digital BK Plus digital imaging system. Specimens arranged in hand sanitizer and covered in 95% ethanol were photographed at dorsal, ventral, and lateral angles. Taxonomic measurements were derived from photographs in Adobe Photoshop. Genitalia observations and illustrations were made from photographs and by dissecting out the epigyna, digested in potassium hydroxide solution to remove soft tissue to make internal structures visible.

Table 1. Descriptive statistics for K2P (Kimura 2-parameter) distances within and between the molecular operational taxonomic units (OTUs), which were identified by molecular species delimitation methods.

Within OTUs			
OTU	**N**	**K2P**	
		Mean	**Std. Err**
argentataCAR	74	0.009	0.002
argentataUS	3	0.018	0.004
argentataCU	10	0.006	0.001
Between OUTs			
OTU 1	**OTU 2**	**K2P**	
		Mean	**Std. Err**
argentataCAR	argentataUS	0.029	0.006
argentataCAR	argentataCU	0.061	0.010
argentataUS	argentataCU	0.064	0.010

Results

A fragment of COI (659 bp) was obtained for all individuals, and with added data from Genbank, making up a total of 107 sequences, including outgroups and 87 individuals morphologically identified as *A. argentata*. 540 base pairs overlapped for all individuals and missing data was 5.1%. Bayesian analyses of this dataset produced a topology that, with some internal node exceptions, was well supported (Figs 1, Suppl. material 1). This tree suggests that *A. argentata* s.l., being sister to *A. blanda*, contains a clade from Cuba and a clade that contains all other sampled populations. The phylogenetic structure within the latter suggests a grade of North American, Costa Rican, and the island Caribbean clades (not Cuba), respectively. Only Hispaniola and Jamaica have monophyletic island populations, and Martinique + St. Lucia together form a clade, other island populations do not emerge as monophyletic. To test the relationships between the major lineages suggested by mitochondrial-only results, we ran phylogenetic analyses of a subset of terminals with only nuclear data. The concatenated matrix consisted of 12 sequences (7 outgroups and 3 OTUs of *A. argentata*) and 1172 base pairs (28S - 829 bp and Histone 3 - 343 bp), with 1.3% missing data. These results (Suppl. material 2) confirm the core relationships among the Caribbean, North American, and Cuban populations of *A. argentata* s. l., respectively. Thus, both nuclear only and mitochondrial only phylogenies recover the sister relationship of Cuba with a clade that contains North American mainland plus other Caribbean island representatives. BEAST analyses, likewise, confirm these relationships (Suppl. material 3), the only significant difference being that Costa Rican and mainland American populations are monophyletic. Estimated node ages from BEAST, summarized in Fig. 1, date the mrca of *A. blanda* and *A. argentata* s.l. at roughly 6 Ma, and date the split between the Cuban clade and the remainder of *A. argentata* s.l. to about 3.8 Ma. The split between the mainland and Caribbean island populations of *A. argentata* s.s. is estimated at 1.7 Ma, and the Caribbean island 'diversification' is less than 1 Ma.

The GMYC analysis split the COI data into 11 OTUs, including eight correctly identified outgroup species. GMYC model provided a significantly better fit to the data than the null hypothesis of no structure (likelihood ratio: 27.06, $P < 0.001$), thus identifying 3 OTUs within *A. argentata* s. l. (Fig. 1): individuals from mainland C. and N. America (argentataUS), individuals from throughout most of the Caribbean (argentataCAR), and individuals from Cuba (argentataCU). The genetic distance test revealed very low K2P values within the OTUs (Table 1). In contrast, K2P values between the OTUs were all above 3%, but were particularly high between the Cuban OTU and the others (Table 1): While the Caribbean plus mainland OTUs comfortably fall within the intraspecific range typical for spiders, the average genetic distances between the Cuban OTU and the remaining two (average around 6%) were higher than the typical interspecific boundary in spiders (Čandek and Kuntner 2015).

Other measures of nucleotide differences (Kxy) and gene flow (F^{ST}) likewise indicate particularly high distinction and genetic isolation of these lineages (Suppl. material 5, 6). Genetic and geographic distances were significantly correlated across the ingroup specimens (Fig. 2, R^2=0.14, P<0.01). However, this correlation was entirely due to comparisons among specimens within each of the two species defined here (Fig. 2, R^2=0.69, p<<0.01), whereas there was no correlation between genetic and geographic distances in comparison of specimens across species (Fig. 2, R^2=0.0005, p>0.05). Thus, phylogenetic, population genetic, and species delimitation analyses all agree that the Cuban population is heterospecific with the broadly distributed *A. argentata*.

Finally, haplotype analyses indicate clear phylogeographic structuring of COI haplotypes despite generally shallow divergences among islands (Fig. 3), as also indicated by the correlation between genetic and geographic distances (Fig. 2). More isolated islands have unique haplotypes, while haplotypes are shared among islands like Puerto Rico, Mona, and the northern Lesser Antilles. The Cuban population is a clear outlier in the haplotype network (Fig. 3), separated from other haplotypes by numerous mutations. Thus, based on all phylogenetic topologies and population genetic results we refer to the Cuban clade of *A. argentata* s. l. as *A. butchko* sp. n. and its sister clade as *A. argentata* s.s. (see Taxonomy for formal justification).

Discussion

Archipelagos such as the Caribbean provide opportunities for colonization followed by isolation, restriction of gene flow, and the formation of local endemics (Ricklefs and Birmingham 2008). However, the degree of endemism will depend, in part, on the dispersal ability of the lineages in question (e.g. Claramunt et al. 2012; Diamond et al. 1976). We studied a spider lineage known to 'balloon' and thus expected to be able to cross oceanic barriers rather readily. We find that our sample of *A. argentata* s. l. from the Caribbean nevertheless represents several geographically structured populations and one highly genetically distinct lineage in Cuba (Figs 1, 3, Suppl. material 1–3).

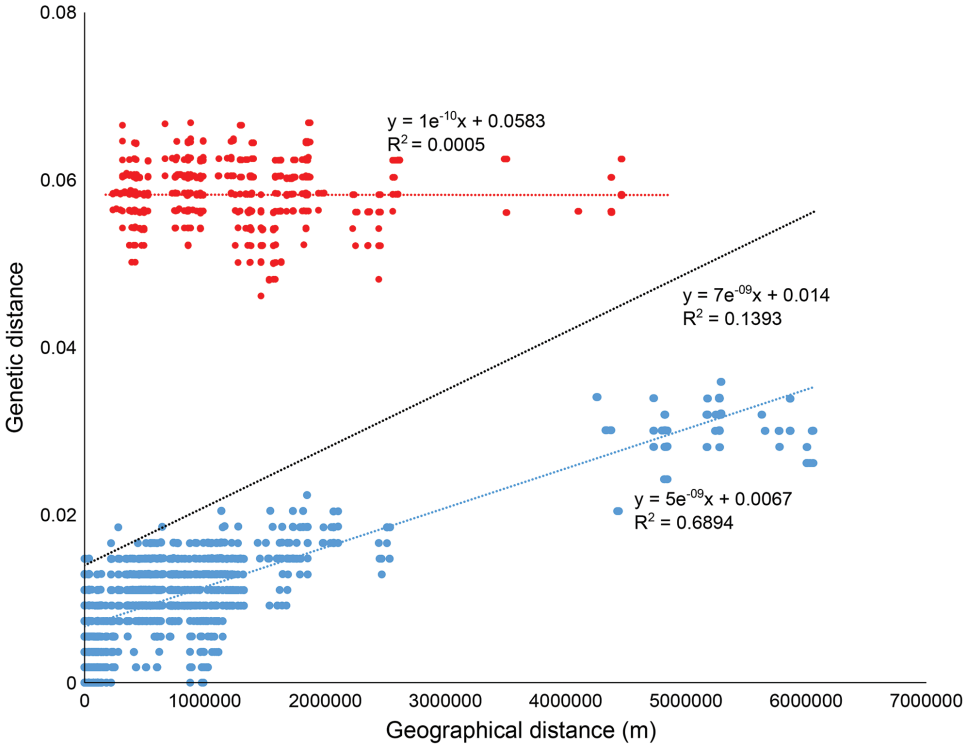

Figure 2. Regression analysis between geographic and genetic distances among all specimens of *A. argiope* s.l. included here. Blue dots and line represent within species comparisons and red dots and line those among the two species as here defined. Black line is regression across all data. Geographical distances well explain genetic distances within species, but not between the species, as expected.

The larger Caribbean islands (Cuba, Hispaniola and Jamaica), and mainland continent, have genetically isolated populations (Fig. 3) among which there is likely limited or no recent gene-flow, while there seems to be movement and gene flow among islands in the eastern Caribbean. As we discuss below, isolation-by-distance (Fig. 2) coupled with a single speciation event, may readily explain these observed patterns.

We find strong evidence for two species in our dataset, the widespread *A. argentata* and one new species, *A. butchko* sp. n. from Cuba. Both genetic distances (Table 1), phylogenies, networks, and species delimitation analyses (Figs 1, 3, and supplementary material), and analyses of genetic vs geographic distances (Fig. 2) support this conclusion. Specimens from the Caribbean apart from Cuba are monophyletic with some island-level genetic structure (Fig. 3). Representatives from both Jamaica and the Dominican Republic are respectively monophyletic, with both islands sampled from widespread localities. However, patterns in the remaining Caribbean fauna are consistent with short-distance overwater dispersal, as evidenced by shared haplotypes between Puerto Rico and several of the Lesser Antilles east and south of it (Fig. 3). The relatively young age of these Caribbean lineages (3–5 my, Figs 1, S3) furthermore suggests that

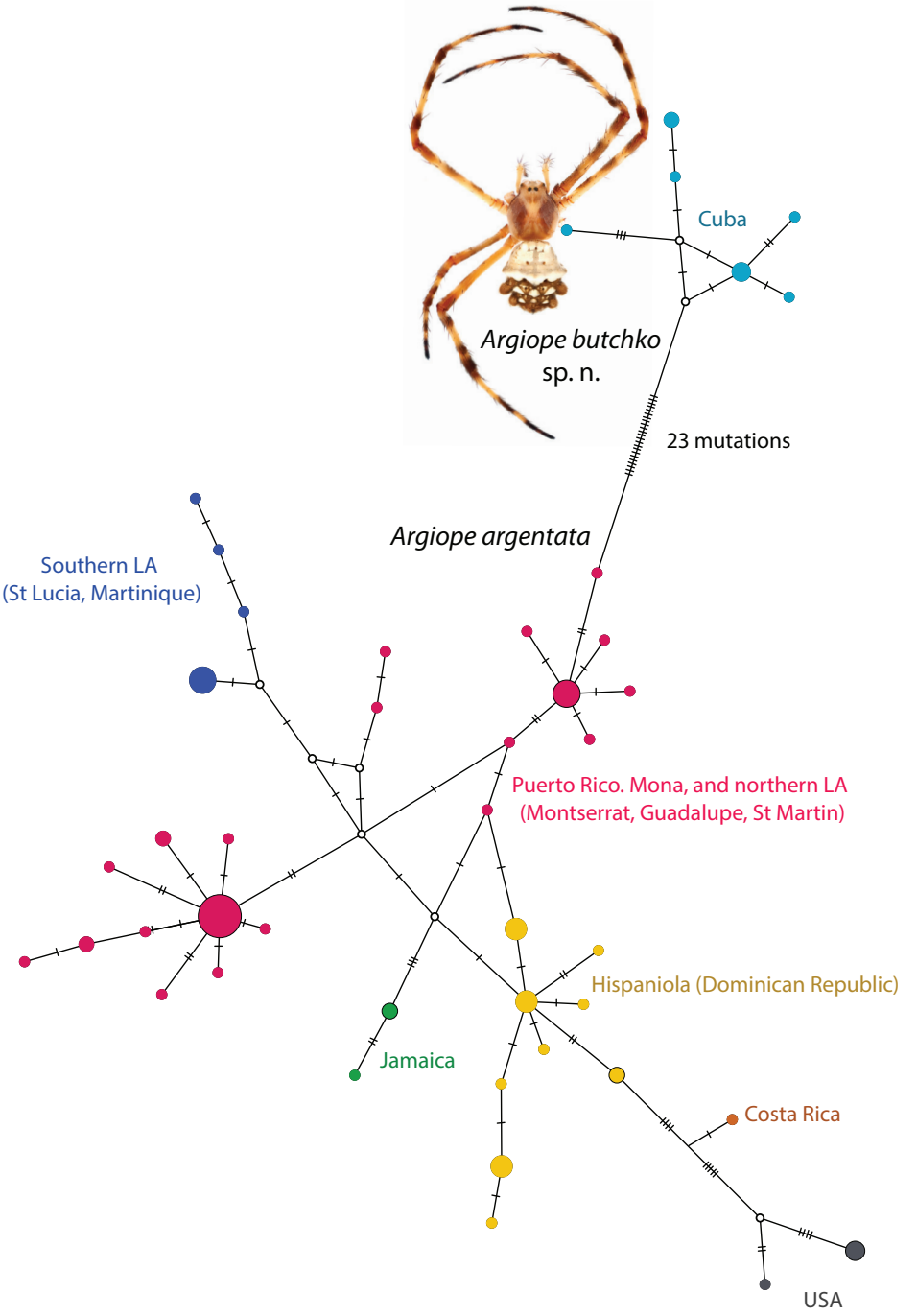

Figure 3. A haplotype network of Caribbean *A. argentata* and *A. butchko* sp. n. Haplotypes are colored by locality as indicated, circle size reflects number of individuals carrying that haplotype from 1-7 in total, open circles represent unobserved haplotypes. Hash marks indicate mutational differences among haplotypes. Inset photograph is of a female *A. butchko*.

overwater dispersal has been the mode of colonization of each of the major islands. Thus, consistent with well documented ballooning behavior in *Argiope* (Bell et al. 2005), these spiders seem to be quite capable of crossing oceanic barriers. However, they do not seem to do so frequently enough to establish panmixia across the Caribbean. Therefore, processes of ongoing diversification result in island-specific haplotypes. That open ocean represents a filter rather than a barrier to dispersal is further evidenced by the strong correlation between geographic and genetic intraspecific distances (Fig. 2). Populations on nearby islands tend to be genetically close (or identical) with the greatest genetic distances, and splits exceeding 1.5 my, found between Caribbean island vs geographically distant mainland lineages. Such isolation-by-distance patterns are not unexpected when dispersal is restricted (e.g., Hamilton and Eckert 2007; Eckert et al. 2008) and have been found in other Caribbean taxa such as pines (Jardon-Barbolla et al. 2011). However, these patterns do not hold between the populations of *A. butchko* (Cuba) and the populations of *A. argentata* (from elsewhere). This is further evidence that *A. butchko* has undergone speciation after isolation for over 3 my (Fig. 1). The intermediate dispersal model (Agnarsson et al. 2014; Agnarsson and Kuntner 2012; Claramunt et al. 2012; Diamond et al. 1976) predicts that species richness across archipelagos peaks in intermediate dispersers but is comparatively low in excellent dispersers where oceanic barriers are less effective. Our findings are consistent with this model – *Argiope* spiders are effective dispersers and unlike most arachnid lineages studied in the Caribbean where single island endemism is prominent (Cosgrove et al. 2016; Crews and Gillespie 2010; Dziki et al. 2015; Esposito et al. 2015; McHugh et al. 2014) *Argiope* have undergone little diversification in the Caribbean. A similar pattern was found, for example, in some *Selonops* lineages in the Caribbean (Crews et al. 2010) and in the nephilids of the western Indian Ocean islands. In the latter group the excellent disperser *Nephila* showed relatively shallow intraspecific divergences among some islands but the poorer dispersers *Nephilingis* and *Clitaetra* have formed single island endemics (Kuntner and Agnarsson 2011a; b). High dispersal ability in *Argiope*, therefore, may have limited diversification in the Caribbean.

Based on this finding we examined in detail the comparative morphology of *A. argentata* and the putative new species from Cuba, here described as *A. butchko* sp. n. We found no diagnostic differences in the morphology of male and female genitalia that would be consistent with the deep genetic divergence (~6.3% average sequence divergence, Table 1) and genetic isolation that has persisted for substantial time (Figs 1–2). Rather, variation seems profuse (Figs 4–5). Likewise, the Cuban species does not differ from *A. argentata* in body size measures. Hence, *A. butchko* sp. n. can be characterized as a morphologically 'cryptic' species. Further sampling and analysis of *Argiope argentata* DNA and morphology throughout its distribution range outside the Caribbean, especially in S. America, is a logical next step and may reveal additional species in this complex.

We note that our main results are based on a single mitochondrial locus and thus our phylogeographic conclusions are restricted to the picture expected from female inheritance. Nevertheless, multi-locus phylogenetic analyses support the general conclusions.

Conclusions

Consistent with predictions of the intermediate dispersal model, our analyses of *Argiope argentata* mtDNA haplotype diversity and phylogeography across the Caribbean established that geographical structuring of haplotypes of this good disperser taxon was mostly consistent with a single widespread species model. Nevertheless, specimens from Cuba were deeply divergent from the remaining areas in all analyses, which we take as evidence for discovery of a cryptic species here described as *A. butchko* sp. n. 'Hidden' taxonomic diversity in the Caribbean is being revealed in multiple lineages by the CarBio project (Cosgrove et al. 2016; Dziki et al. 2015; Esposito et al. 2015; McHugh et al. 2014) and future work aims to test taxonomic hypotheses in other 'widespread' arachnid species that range from excellent to relatively poor dispersers and thus test the intermediate dispersal model at various taxonomic levels.

Taxonomy

Family *Araneidae* Clerk, 1757
Subfamily *Argiopinae* Simon, 1890
Genus *Argiope* Audouin, 1826

***Argiope butchko* LeQuier & Agnarsson, sp. n.**
http://zoobank.org/CF438FAF-2E27-44A4-9FF3-1DADDDA942DD

Etymology. The species epithet, a noun in apposition, honors the memory of Dennis Butchko, an inspiring science teacher.

Type material. Female holotype from Siboney, Santiago de Cuba (19.9608°N, 75.7076°W), April 1, 2012, Col. Team CarBio, deposited in the Smithsonian (NMNH). Two female paratypes, one from the type location and one from Sierra de Camaguey, Camaguey, Cuba (21.5916°N, 77.7882°W). Three male paratypes, one from holotype location, one from Sierra de Camaguey, Camaguey, Cuba, and one from Viñales, Sierra de los Órganos ,Pinar del Rio, Cuba 22.6210°N, 83.7383°W. Paratypes will be deposited in the Smithsonian (NMNH).

Diagnosis. *Argiope butchko* sp. n. differs from all other *Argiope* except *A. argentata* by the presence of the embolic distal curl (Levi, 2004: fig. 43, arrow). No distinct feature of the male palp and female epigynum were found that reliably diagnose A. *butchko* sp. n. from *A. argentata*.

Argiope butchko sp. n. and *A. argentata* can be diagnosed from one another, and other related *Argiope* species, on the basis of the following unique, synapomorphic, mtDNA nucleotide substitutions at the following standard DNA barcode alignment positions in each species (following Agnarsson et al. (2015)):

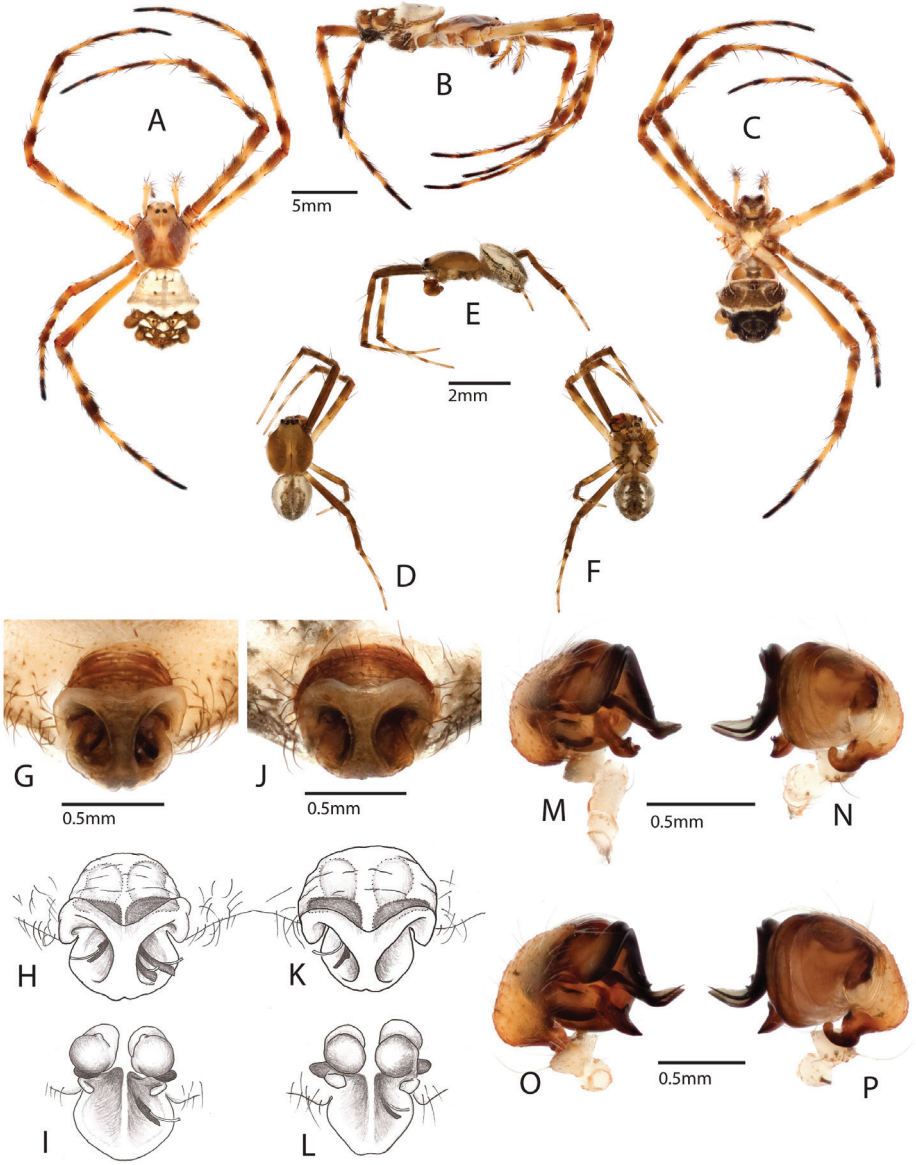

Figure 4. Female holotype *Argiope butchko* sp. n. **a** dorsal **b** lateral **c** ventral; Male paratype *Argiope butchko* sp. n. **d** dorsal **e** lateral **f** ventral **g** external epigynum **h** external epigynum illustration showing spermatheca and spiraling ducts **i** internal epigynum illustration dorsal **m** palp lateral **n** palp ventral; *Argiope argentata* **j** external epigynum **k** external epigynum illustration showing spermatheca and spiraling ducts **l** internal epigynum illustration dorsal **o** palp lateral **p** palp ventral.

A. butchko: A (127), C (133), C (157) C (178), T (190), G (208), C (226), A (293), G (316), A (379, G (502), G (508), C (607); *A. argentata*: G (49), G (211), A (508), A (511), G (643).

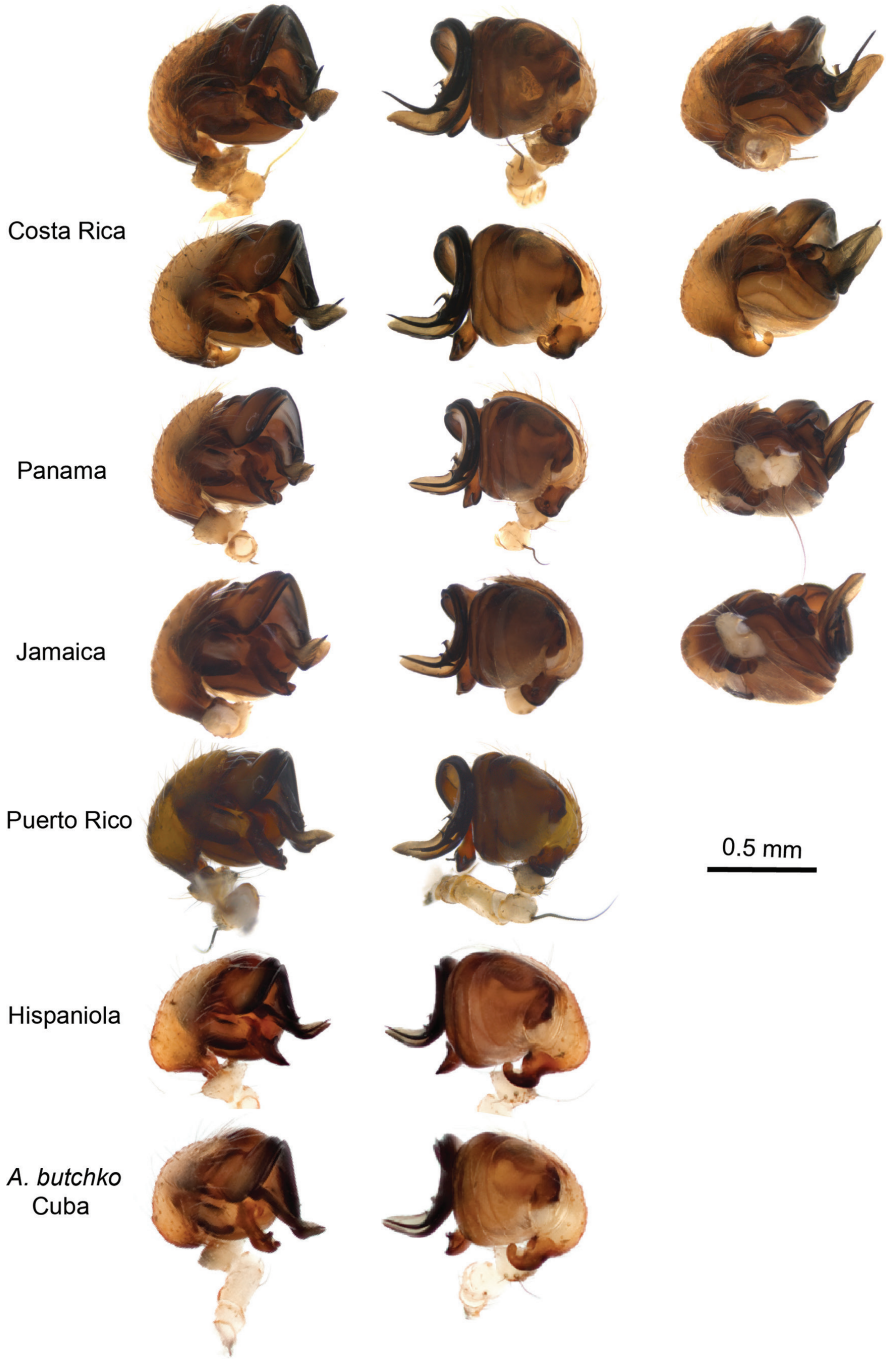

Figure 5. Comparative morphology of the male palpal organ of the widespread *A. argentata* and the new *A. butchko*. No clearly diagnostic features were identified in the new species, though slight differences in the terminal parts of the median apophysis and the embolus are observed and merit further comparative investigation.

Description. Males and females of this species closely resemble *Argiope argentata* (Fabricius 1775; Levi 1983; Levi 2004). Males have a distal curl on the embolus (Levi, 2004: fig. 43, arrow) and a median apophysis that is blunt at the tip (Figs 4m, o, 5). Females of *A. argentata* typically have a brown sternum with a median white line (Levi 1968). In *A. butchko* sp. n. the posterior half of the sternum is white or off-white and the anterior half is brown with a small median white dot on the anterior edge (Fig. 4c). However, this feature is not clearly diagnostic as variation is observed in *A. argentata*. The epigynum of *A. butchko* sp. n. has a wider posterior plate than Caribbean *A. argentata* and a smaller anterior bulge (Fig. 4G–I), but again variation in *A. argentata* outside the Caribbean suggests this feature is not diagnostic for the species.

Dimensions (mm). Holotype (female) - Total body length excluding chelicera 10.74, carapace length 5.00, carapace width 4.36. Leg I: femur length 8.28, patella and tibia length 8.63mm, metatarsus 8.00, tarsus 2.09. Leg II: patella and tibia length 8.06. Leg III: patella and tibia length 4.66. Leg IV: patella and tibia length 7.13.

Variation (mm). Female (N=4) - Total body length ranged from 10.29–10.74, carapace length 3.84–5.00, carapace width 3.16–4.36. Leg I: femur length 6.50–8.28, patella and tibia length 6.40–8.63, metatarsus 5.77–8.00, tarsus 1.80–2.09. Leg II: patella and tibia length 7.83–8.06. Leg III: patella and tibia length 3.73–4.66. Leg IV: patella and tibia length 4.66–7.13. *Male* (N=3) - Total body length ranged from 2.88–3.44, carapace length 1.70–1.88, carapace width 1.44–1.57. Leg I: femur length 1.62–2.14, patella and tibia length 1.99–2.37, metatarsus 1.74–1.88, tarsus 0.81–0.88. Leg II: patella and tibia length 1.41–1.90. Leg III: patella and tibia length N/A. Leg IV: patella and tibia length 1.40–1.63.

Distribution. The species is restricted to Cuba.

Natural history note. Three embolus tips were found embedded in the epigynum of a female *A. butchko*, one in the left opening and two in the right opening (Fig. 4g–h) This is similar to *A. argentata*, which has been known to have up to five embolic tips in one female (Jaeger 2012).

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References

Audouin JV (1826) Explications sommaires des planches d'Arachnides de l'Egypte et de la Syrie. In: Savigny JC (Ed.) Description de l'Egypte et de la Syrie. Volume 4, 121.

Agnarsson I, Cheng RC, Kuntner M (2014) A Multi-Clade Test Supports the Intermediate Dispersal Model of Biogeography. PLoS ONE 9(1): e86780. doi: 10.1371/journal.pone.0086780

Agnarsson I, Jencik BB, Veve GM, Hanitriniaina S, Agostini D, Goh SP, Pruitt J, Kuntner M (2015) Systematics of the Madagascar Anelosimus spiders: remarkable local richness and endemism, and dual colonization from the Americas. ZooKeys 509: 13–52. doi: 10.3897/zookeys.509.8897

Agnarsson I, Kuntner M (2012) The generation of a biodiversity hotspot: biogeography and phylogeography of the western Indian Ocean islands. In: Anamthawat-Jonsson K (Ed.) Current Topics in Phylogenetics and Phylogeography of Terrestrial and Aquatic Systems. Tech Publishers, Rijeka, 33–82. doi: 10.5772/38958

Alayon G (2006) Endemicidad y relaciones de las aranas (Araneae) de las Antillas Mayores. Cocuyo 16: 63 68 16: 63–68.

Ali JR (2012) Colonizing the Caribbean: is the GAARlandia land-bridge hypothesis gaining a foothold? Journal of Biogeography 39: 431–433. doi: 10.1111/j.1365-2699.2011.02674.x

Arnedo MA, Gillespie RG (2006) Species diversification patterns in the Polynesian jumping spider genus *Havaika* Proszynski, 2001 (Araneae, Salticidae). Molecular Phylogenetics and Evolution 41: 472–495. doi: 10.1016/j.ympev.2006.05.012

Arnedo MA, Oromi P, Murria C, Macias-Hernandez N, Ribera C (2007) The dark side of an island radiation: systematics and evolution of troglobitic spiders of the genus Dysdera Latreille (Araneae : Dysderidae) in the Canary Islands. Invertebrate Systematics 21: 623–660. doi: 10.1071/is07015

Bandelt HJ, Forster P, Rohl A (1999) Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16: 37–48. doi: 10.1093/oxfordjournals.molbev.a026036

Bell JR, Bohan DA, Shaw EM, Weyman GS (2005) Ballooning dispersal using silk: world fauna, phylogenies, genetics and models. Bulletin of Entomological Research 95: 69–114. doi: 10.1079/BER2004350

Bidegaray-Batista L, Arnedo MA (2011) Gone with the plate: the opening of the Western Mediterranean basin drove the diversification of ground-dweller spiders. BMC Evolutionary Biology 11: 317. doi: 10.1186/1471-2148-11-317

Bloom T, Binford G, Alayon G, Esposito L, Peterson I, Nishida A, Loubet-Senear K, Agnarsson I (2014) Discovery of two new species of eyeless spiders within a single Hispaniola cave. Journal of Arachnology 42: 148–154. doi: 10.1636/K13-84.1

Candek K, Kuntner M (2015) DNA barcoding gap: reliable species identification over morphological and geographical scales. Molecular Ecology Resources 15: 268–277. doi: 10.1111/1755-0998.12304

Catalog WS (2015) World Spider Catalog, version 16. Natural History Museum Bern, Bern.

Cheng R-C, Kuntner M (2014) Phylogeny suggests non-directional and isometric evolution of sexual size dimorphism in argiopine spiders. Evolution 68(10): 2861–2872.

Cheng R-C, Kuntner M (2015) Disentangling the size and shape components of sexual dimorphism. Evolutionary Biology 42: 223–234. doi: 10.1007/s11692-015-9313-z

Claramunt S, Derryberry EP, Remsen Jr JV, Brumfield RT (2012) High dispersal ability inhibits speciation in a continental radiation of passerine birds. Proceedings of the Royal Society B-Biological Sciences 279: 1567–1574. doi: 10.1098/rspb.2011.1922

Clerck C (1757) Aranei Suecici, descriptionibus et figuris oeneis illustrati,ad genera subalterna redacti speciebus ultra LX determinati. Svenska Spindlar,uti sina hufvud-slagter undelte samt.. [Publ. not given], Stockholmiae.

Cosgrove J, Agnarsson I, Harvey M, Binford G (2016) Pseudoscorpion diversity and distribution in the West Indies: sequence data confirms single island endemism for some clades, but not others. Journal of Arachnology, in press.

Crews SC, Gillespie RG (2010) Molecular systematics of *Selenops* spiders (Araneae: Selenopidae) from North and Central America: implications for Caribbean biogeography. Biological Journal of the Linnean Society 101: 288–322. doi: 10.1111/j.1095-8312.2010.01494.x

Crews SC, Puente-Rolón AR, Rutstein E, Gillespie RG (2010) A comparison of populations of island and adjacent mainland species of Caribbean *Selenops* (Araneae: Selenopidae) spiders. Molecular Phylogenetics and Evolution 54: 970–983. doi: 10.1016/j.ympev.2009.10.012

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9: 772–772. doi: 10.1038/nmeth.2109

Diamond JM, Gilpin ME, Mayr E (1976) Species-Distance Relation for Birds of Solomon Archipelago, and Paradox of Great Speciators. Proceedings of the National Academy of Sciences of the United States of America 73: 2160–2164. doi: 10.1073/pnas.73.6.2160

Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology 7. doi: 10.1186/1471-2148-7-214

Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian Phylogenetics with BEAUti and the BEAST 1.7. Molecular Biology and Evolution 29: 1969–1973. doi: 10.1093/molbev/mss075

Dziki A, Binford GJ, Coddington JA, Agnarsson I (2015) Spintharus flavidus in the Caribbean-a 30 million year biogeographical history and radiation of a 'widespread species'. Peerj 3. doi: 10.7717/peerj.1422

Eckert CG, Samis KE, Lougheed SC (2008) Genetic variation across species' geographical ranges: the central–marginal hypothesis and beyond. Molecular Ecology 17: 1170–1188. doi: 10.1111/j.1365-294X.2007.03659.x

Ersts PJ (2016) Geographic Distance Matrix Generator(version 1.2.3). American Museum of Natural History, Center for Biodiversity and Conservation. http://biodiversityinformatics.amnh.org/open_source/gdmg [accessed on 2016-8-31]

Esposito LA, Bloom T, Caicedo-Quiroga L, Alicea-Serrano AM, Sanchez-Ruiz JA, May-Collado LJ, Binford GJ, Agnarsson I (2015) Islands within islands: Diversification of tailless whip spiders (Amblypygi, *Phrynus*) in Caribbean caves. Molecular Phylogenetics and Evolution 93: 107–117. doi: 10.1016/j.ympev.2015.07.005

Fabricius JC (1775) Systema Entomologiae, sistens Insectorum classes,ordines,genera,species,adiectis,synonymis,locis descriptionibus observationibus. [Publ. not given], Flensbergi et Lipsiae.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidaes subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294–299.

Gillespie RG (2005) The ecology and evolution of Hawaiian spider communities. American Scientist 93: 122–131. doi: 10.1511/2005.52.961

Gillespie RG, Claridge EM, Goodacre SL (2008) Biogeography of the fauna of French Polynesia: diversification within and between a series of hot spot archipelagos. Philosophical Transactions of the Royal Society B-Biological Sciences 363: 3335–3346. doi: 10.1098/rstb.2008.0124

Gillespie RG, Roderick GK (2002) Arthropods on islands: Colonization, speciation, and conservation. Annual Review of Entomology 47: 595–632. doi: 10.1146/annurev.ento.47.091201.145244

Green P (2009) Phrap. 1.090518 ed.

Green P, Ewing B (2002) PHRED. 0.020425c ed. http://phrap.org/

Hamilton JA, Eckert CG (2007) Population genetic consequences of geographic disjunction: a prairie plant isolated on Great Lakes alvars. Molecular Ecology 16: 1649–1660. doi: 10.1111/j.1365-294X.2007.03241.x

Hebert PDN, Cywinska A, Ball SL, DeWaard JR (2003) Biological identifications through DNA barcodes. Proceedings of the Royal Society of London Series B-Biological Sciences 270: 313–321. doi: 10.1098/rspb.2002.2218

Heinicke MP, Duellman WE, Hedges SB (2007) Major Caribbean and Central American frog faunas originated by ancient oceanic dispersal. Proceedings of the National Academy of Sciences of the United States of America 104: 10092–10097. doi: 10.1073/pnas.0611051104

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754–755. doi: 10.1093/bioinformatics/17.8.754

Iturralde-Vinent M, MacPhee R (1999) Paleogeography of the Caribbean region: implications for cenozoic biogeography. Bulletin of the American Museum of Natural History 238: 1–95.

Iturralde-Vinent MA (2006) Meso-Cenozoic Caribbean Paleogeography: Implications for the Historical Biogeography of the Region. International Geology Review 48.

Jaeger P (2012) A review on the spider genus Argiope Audouin 1826 with special emphasis on broken emboli in female epigynes (Araneae: Araneidae: Argiopinae). Beitraege zur Araneologie 7: 272–331.

Jardon-Barbolla L, Delgado-Valerio P, Geada-Lopez G, Vazquez-Lobo A, Pinero D (2011) Phylogeography of *Pinus* subsection Australes in the Caribbean Basin. Annals of Botany 107: 229–241. doi: 10.1093/aob/mcq232

Katoh S (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30: 772–780. doi: 10.1093/molbev/mst010

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16: 111–120. doi: 10.1007/BF01731581

Kuntner M, Agnarsson I (2011a) Biogeography and diversification of hermit spiders on Indian Ocean islands (Nephilidae: *Nephilengys*). Molecular Phylogenetics and Evolution 59: 477–488. doi: 10.1016/j.ympev.2011.02.002

Kuntner M, Agnarsson I (2011b) Phylogeography of a successful aerial disperser: the golden orb spider *Nephila* on Indian Ocean islands. BMC Evolutionary Biology 11: 119. doi: 10.1186/1471-2148-11-119

Levi HW (1968) The spider genera Gea and Argiope in America (Araneae: Araneidae). Harvard University, Cambridge.

Levi HW (1983) The orb-weaver genera *Argiope*, *Gea* and *Neogea* from the western Pacific region (Araneae: Argiopinae,Araneidae). Bulletin of the Museum of Comparative Zoology 150: 247–338.

Levi HW (2004) Comments and new records for the American genera Gea and Argiope with the description of a new species (Araneae: Araneidae). Bulletin of the Museum of Comparative Zoology 158: 47–65. doi: 10.3099/0027-4100(2004)158[47:canrft]2.0.co;2

Librado P, Rozas J (2009) DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25: 1451–1452. doi: 10.1093/bioinformatics/btp187

Maddison DR, Maddison WP (2011a) Chromaseq: a Mesquite module for analyzing sequence chromatograms. version 1.0. http://mesquiteproject.org/packages/chromaseq

Maddison W, Maddison D (2011b) Mesquite: a modular system for evolutionary analysis. 2.75 (build 566) ed. http://mesquiteproject.org

McHugh A, Yablonsky C, Binford G, Agnarsson I (2014) Molecular phylogenetics of Caribbean Micrathena (Araneae : Araneidae) suggests multiple colonisation events and single island endemism. Invertebrate Systematics 28: 337–349. doi: 10.1071/is13051

Pons J, Barraclough TG, Gomez-Zurita J, Cardoso A, Duran DP, Hazell S, Kamoun S, Sumlin WD, Vogler AP (2006) Sequence-based species delimitation for the DNA taxonomy of undescribed insects. Systematic Biology 55: 595–609. doi: 10.1080/10635150600852011

Posada D, Buckley TR (2004) Model selection and model averaging in phylogenetics: advantages of the AIC and Bayesian approaches over likelihood ratio tests. Systematic Biology 53: 793–808. doi: 10.1080/10635150490522304

R Core Team (2014) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

Ricklefs R, Bermingham E (2008) The West Indies as a laboratory of biogeography and evolution. Philosophical Transactions of the Royal Society B-Biological Sciences 363: 2393–2413. doi: 10.1098/rstb.2007.2068

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution 30: 2725–2729. doi: 10.1093/molbev/mst197

Warren BH, Simberloff D, Ricklefs RE, Aguilee R, Condamine FL, Gravel D, Morlon H, Mouquet N, Rosindell J, Casquet J, Conti E, Cornuault J, Maria Fernandez-Palacios J, Hengl T, Norder SJ, Rijsdijk KF, Sanmartin I, Strasberg D, Triantis KA, Valente LM, Whittaker RJ, Gillespie RG, Emerson BC, Thebaud C (2015) Islands as model systems in ecology and evolution: prospects fifty years after MacArthur-Wilson. Ecology Letters 18: 200–217. doi: 10.1111/ele.12398

Weeks BC, Claramunt S (2014) Dispersal has inhibited avian diversification in Australasian archipelagoes. Proceedings of the Royal Society B-Biological Sciences 281. doi: 10.1098/rspb.2014.1257

Zhang J-X, Maddison WP (2012) New euophryine jumping spiders from the Dominican Republic and Puerto Rico (Araneae: Salticidae: Euophryinae). Zootaxa 3476: 1–54.

Supplementary material 1

Figure S1

Authors: Ingi Agnarsson, Stephanie M. LeQuier, Matjaž Kuntner, Ren-Chung Cheng, Jonathan A. Coddington, Greta Binford

Data type: phylogeny data

Explanation note: Bayesian phylogeny based on analysis in MrBayes using CO1 data

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Supplementary material 2

Figure S2

Authors: Ingi Agnarsson, Stephanie M. LeQuier, Matjaž Kuntner, Ren-Chung Cheng, Jonathan A. Coddington, Greta Binford

Data type: phylogeny data

Explanation note: Bayesian phylogeny based on analysis in MrBayes using the two nuclear genes.

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Supplementary material 3

Figure S3

Authors: Ingi Agnarsson, Stephanie M. LeQuier, Matjaž Kuntner, Ren-Chung Cheng, Jonathan A. Coddington, Greta Binford

Data type: phylogeny data

Explanation note: Detailed results from the BEAST analysis summarized in Fig 1.

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Supplementary material 4

Table S1

Authors: Ingi Agnarsson, Stephanie M. LeQuier, Matjaž Kuntner, Ren-Chung Cheng, Jonathan A. Coddington, Greta Binford

Data type: specimen data

Explanation note: Specimen data including the Genbank accession number.

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Supplementary material 5

Table S2

Authors: Ingi Agnarsson, Stephanie M. LeQuier, Matjaž Kuntner, Ren-Chung Cheng, Jonathan A. Coddington, Greta Binford

Data type: specimen data

Explanation note: Results of Fst and Kxy (average number of nucleotide differences) analyses among specimens from all islands and mainland. The Cuban specimens, here described as a new species, stand out in both analyses indicating genetic isolation and divergence.

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Supplementary material 6

Table S3

Authors: Ingi Agnarsson, Stephanie M. LeQuier, Matjaž Kuntner, Ren-Chung Cheng, Jonathan A. Coddington, Greta Binford

Data type: specimen data

Explanation note: Genetic and Geographic distances among all ingroup specimens used in generating Figure 2.

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RESEARCH ARTICLE

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The imagos of some enigmatic members of the *Hermanella* complex (Ephemeroptera, Leptophlebiidae)

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Abstract

The imago stages of three species of the *Hermanella* complex are described mostly based on material from Roraima, northern Brazil: *Hydrosmilodon gilliesae*, *Hydromastodon sallesi* and *Leentvaaria palpalis*. Male imagos of *Hydrosmilodon gilliesae* and *Leentvaaria palpalis* both have a pair of large, broad projections at the posterior margin of the styliger plate, nearly covering the penis lobes; in *L. palpalis*, however, these projections are fused. The male imago of *Hydromastodon sallesi* resembles *Hydrosmilodon plagatus* in that both species have a styliger plate with a robust projection that is curved towards the penis lobes. DNA barcoding is likely to be a powerful investigative tool for identifying and understanding species limits among these Ephemeroptera taxa, especially if it is used within an integrative taxonomic context. An updated identification key to the genera of the *Hermanella* complex is proposed.

Keywords

Taxonomy, diversity, Atalophlebiinae, mayflies, Neotropical Region, key, barcoding

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Introduction

Since the delimitation of the *Hermanella* generic complex (Ephemeroptera: Leptophlebiidae: Atalophlebiinae) by Domínguez and Flowers (1989), significant new data have come to light, including the descriptions of several new taxa. The genus *Hydrosmilodon* Flowers & Domínguez (1992) was established for the species *Thraulus primanus* Eaton and the new species *H. saltensis* Domínguez & Flowers. Domínguez et al. (2001) redescribed the nymphs of the monotypic genus *Leentvaaria* Demoulin, which is known only from nymphs, and studied the phylogenetic relationships of the genera of the *Hermanella* complex as it was understood at the time. Thomas et al. (2004) described two new species of *Hydrosmilodon*, *H. gilliesae* Thomas & Péru and *H. mikei* Thomas & Boutonnet, based on nymphs from French Guiana. Later, Polegatto and Batista (2007) erected the new genus *Hydrosmastodon* for *H. mikei* and described a new species *Hydrosmastodon sallesi* Polegatto & Batista, also based solely on nymphs. More recently, Kluge (2007) considered *Hydrosmilodon* and *Paramaka* Savage & Domínguez, 1992 as junior synonyms of *Needhamella* Domínguez & Flowers, 1989 and placed all remaining genera as subgenera of *Hermanella* Needham & Murphy, 1924, a vision not followed by Nascimento and Salles (2013) nor in the present paper. Currently, therefore, the *Hermanella* complex is composed by the following taxa: *Hermanella*; *Hydromastodon*; *Hydrosmilodon*; *Hylister* Domínguez & Flowers, 1989; *Leentvaaria*; *Needhamella*; *Paramaka*; and *Traverella* Edmunds, 1948.

While expedient on one hand, the description of new leptophlebiid taxa based on nymphs alone has, on the other hand, generally added more uncertainty to our understanding of the delimitations and relationships of taxa within this incredibly diverse mayfly family. As part of ongoing taxonomic and phylogenetic studies of the *Hermanella* complex, an important group of Neotropical Leptophlebiidae is examined here. The male imagos of *Hydrosmilodon gilliesae* and *Hydromastodon sallesi*, as well as the male and female imagos of *Leentvaaria palpalis* are described for the first time. Additionally, the first DNA barcode sequences is reported for these species, and their use for stage associations is assessed as part of a combined morphological and molecular approach. Based on the discovery of these metamorphic stages, an updated identification key is provided to the genera of the *Hermanella* complex.

Methods

Habitus images of preserved specimens were taken using a Leica M165C stereomicroscope with a DFC420 digital camera or a Zeiss STEMI 2000-C stereomicroscope with a ERC5 digital camera. In order to produce final images with enhanced depth of field, a series of stacked images were processed with the program Leica Application Suite version 3.4.1 or Helicon Focus®. Living specimens were photographed in the field, in a small acrylic aquarium, with a Nikon D800, a 105 mm objective and a Nikon macro flash. Line drawings based on photographs were made with Adobe Illustrator CC® and were prepared according to Coleman (2003, 2006).

Sequence data

DNA was extracted using a Wizard SV Genomic DNA Purification System Kit (Promega®) based on the protocol for animal tissue. For imago specimens, the abdomen and wing were removed, and all remaining portions were placed in extraction buffer; for nymphs, three legs were used, and the rest of each specimen was retained as voucher material. A 658 base pair portion of COI was amplified for for each specimen, and PCR was performed in a 25-µL mixture containing: approximately 20 ng/µL DNA template, 1X PCR buffer, a 2.0 mM concentration of $MgCl_2$, and a 30µM concentration of each primer (LCO 1490 and HCO 2198) (Folmer et al. 1994), a 100µM concentration (each) of dATP, dCTP, dGTP, and dTTP), 1U Taq Platinum DNA Polimerase Invitrogen® and ultrapure water to complete 25µL. Initial PCR consisted of a preheating at 94°C for 5 min; 40 cycles of 94°C for 45 s, 47°C of annealing temperature for 45 s and 72°C for 45 s, and incubation at 72°C for 5 min. Negative controls were used that contained all elements of the reaction mixture except DNA. Successful bands were detected on 1.5% agarose gel in 1X TAE buffer. Products were purified using ExoSAP-IT® for PCR Product Cleanup (GE Heathcare). All samples were sequenced by Macrogen®. The alignment of sequences was relatively unambiguous as all specimens were length invariable. Sequences were aligned and trimmed to length using Geneious R8, resulting in 658 characters. The basic sequence statistics including nucleotide frequencies and transition/transversion (Ts/Tv) ratio; variabilities in different regions of sequences were analyzed using Jmodeltest V0.1 (Posada 2008), DAMBE (Xia and Xie 2001) and DnaSP v5.0 (Librado and Rozas 2009). Pairwise numbers of nucleotide differences were calculated with MEGA, version 6.06 (Tamura et al. 2013), using the 'Calculate distances' option and the Kimura 2-parameter model of evolution (Kimura 1980).

Voucher material is deposited in the following institutions:

MZUESC Universidade Estadual de Santa Cruz, Ilhéus, Brazil
INPA Instituto Nacional de Pesquisa da Amazônia Manaus, Brazil
FAMU Florida A&M University, Tallahassee, Florida, USA
IBN Instituto de Biodiversidad Neotropical, Tucumán, Argentina
CZNC Coleção Zoológica Norte Capixaba, São Mateus, Brazil.

Results

Hydrosmilodon gilliesae Thomas & Péru, 2004, in Thomas et al. 2004

Figures 1, 2

Diagnosis. The male imago of *Hydrosmilodon gilliesae* can be distinguished from the other species of the genus by the following combination of characters: 1) Eyes separated on meson of head by a short distance — less than 0.5 times width of median ocellus (Fig. 1a); 2) Fore wings hyaline, slightly tinged with brown at base (Fig. 2a);

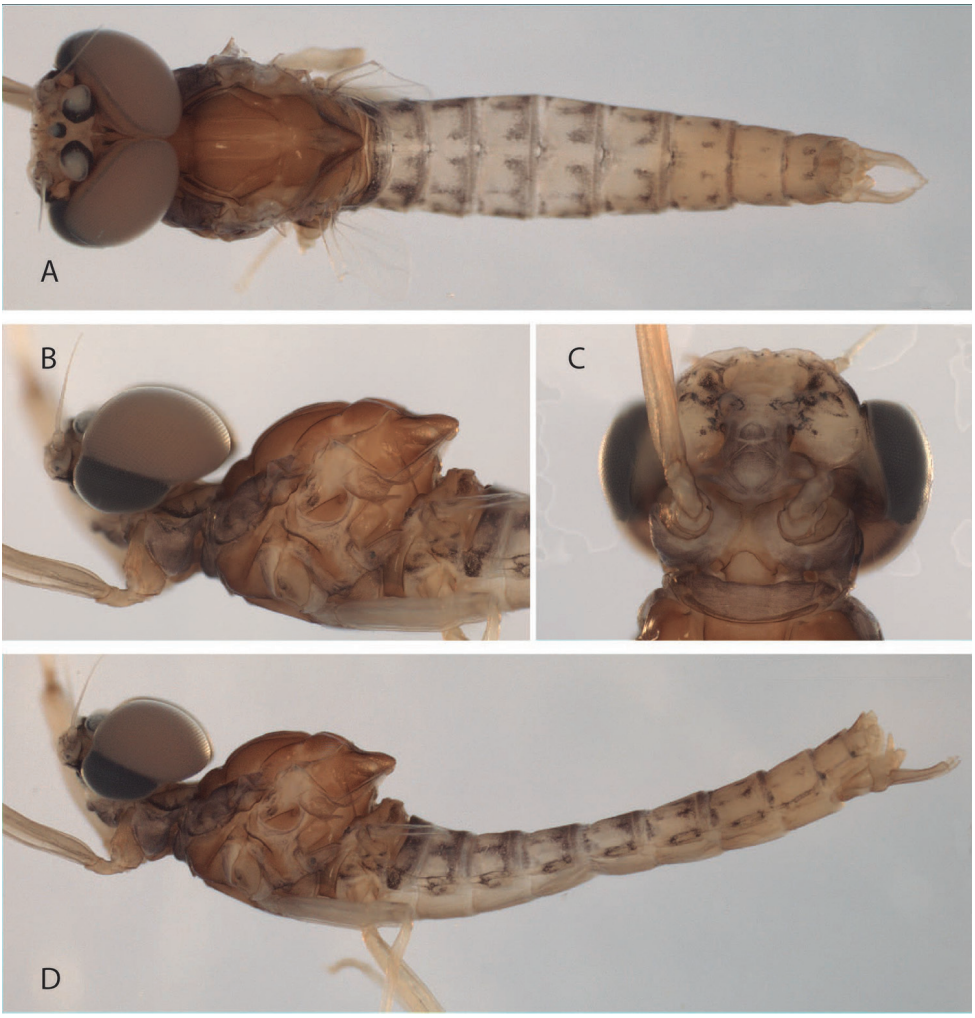

Figure 1. *Hydrosmilodon gilliesae*, male imago: **a** dorsal view **b** head and thorax, lateral view **c** head and prosternum, ventral view **d** lateral view.

3) Coloration of abdominal segments II – IX with blackish anterior and posterior stripes, and variable submedial marks as in Fig. 1a, b; 4) Styliger plate with two wide projections that nearly cover the penis (Fig. 2d); 5) Penis lobes totally divided with distomedial spines converging medially (Fig. 2d).

Description of male imago (in alcohol). *Lengths*: body, 4.1–5.5 mm; fore wings: 5.4-5.8 mm; hind wings: 0.7–0.9 mm.

Head (Fig. 1a, b): brown, upper portions of eyes light orange-brown, lower portions blackish. Eyes separated on meson of head by short distance — less than 0.5 times width of median ocellus. Ocelli white surrounded with black. Antennae: light brown.

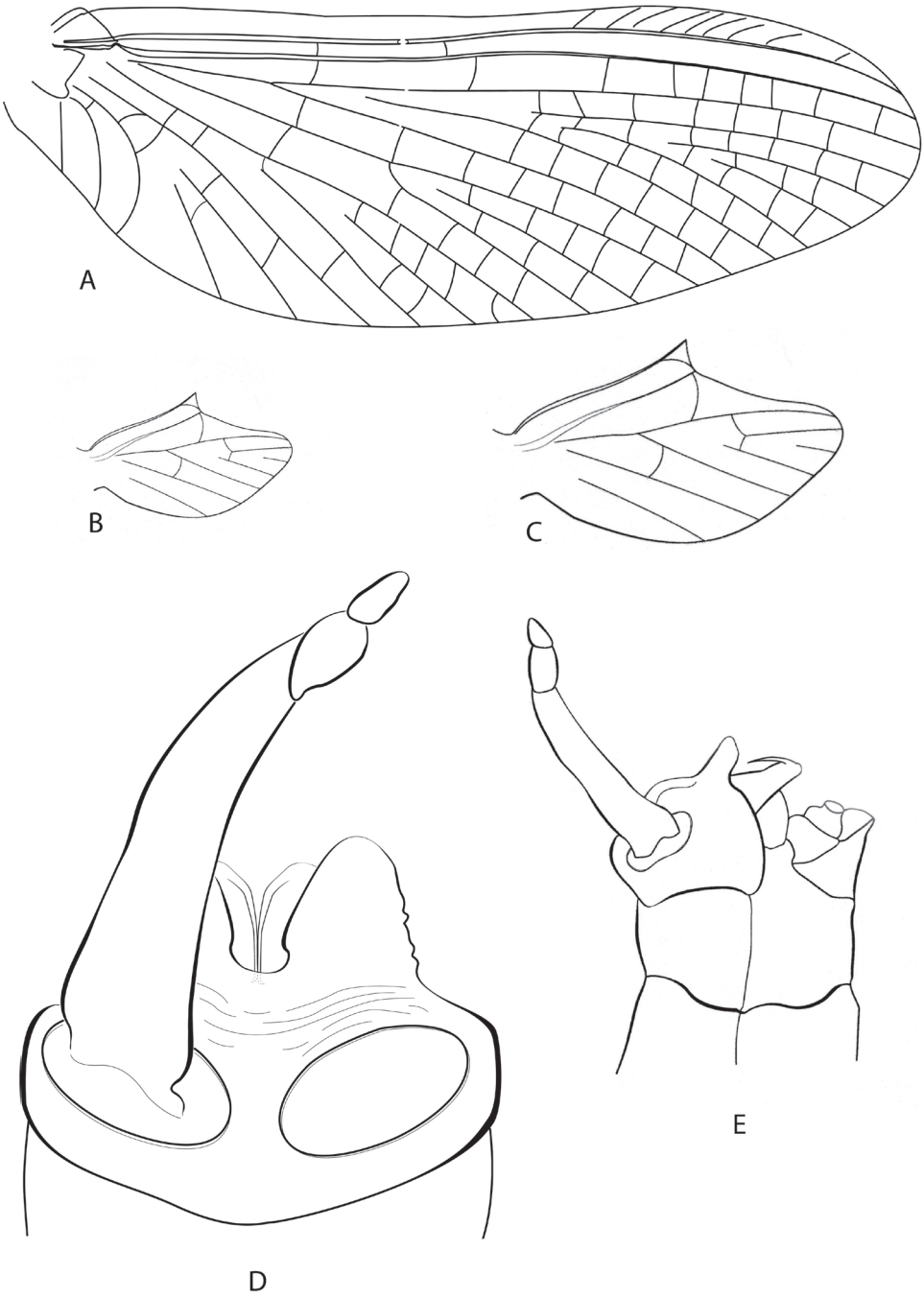

Figure 2. *Hydrosmilodon gilliesae*, male imago: **a** fore wing **b** hind wing **c** hind wing, enlarged **d** genitalia, ventral view **e** genitalia, lateral view.

Thorax (Fig. 1a, b): brownish with lighter sutures, mesoscutellum darker, and white spot on each anterolateral corner of posterior scutellar protuberance. Prosternum (Fig. 1c) similar to *Hydrosmilodon primanus* and *Hs. saltensis*, but with carina longer and slightly wider. Pleurae yellowish and heavily washed with black. Wings (Fig. 2a, b, c): membrane of fore and hind wings hyaline, slightly tinged with brown at bases, longitudinal veins yellowish-brown, cross veins yellowish. Fork of MA asymmetrical and fork of MP slightly asymmetrical (MP2 connected to MP1 by crossvein); crossvein above MA not slanted; vein ICu_2 attached at base to ICu_1 by crossvein. Legs: fore leg yellowish-brown, with apex of femur and base of tibia darker; mid and hind legs generally lighter.

Abdomen (Fig. 1a, d): terga light yellowish-brown, translucent on segments I–VII, segment I completely washed with black, segments II–IX with blackish anterior and posterior stripes, and variable submedial mark as in Fig. 1a; sterna translucent. Genitalia (Fig. 2d, e): styliger plate yellowish-brown, posterior margin blackish; two wide projections nearly covering penis. Forceps yellowish-brown, lightly washed with grey. *Penis*: yellowish; totally divided with distomedial spines converging medially. Caudal filaments: yellowish.

Material examined. Four ♂ imagos: Brazil, Mato Grosso State, Ribeirão Cascalheira, Gleba Maria Tereza, córrego "corgão", S12°43.040, W52°03.345, 09.x.2007, light trap, Pinho L.C., Mateus S., Torali L. & Silva F.R. (MZUESC). Two ♂ imagos: Brazil, Mato Grosso State, Nova Xavantina, córrego Ponte de Pedra, 06-XII-2006, light trap, Mariano, R., Calor, A.R. & Mateus, S. (MZUESC). Three ♂ imagos: Brazil, São Paulo State, Luis Antonio, Estação Ecológica de Jataí (PEJ), córrego Beija-Flor, 03.II.2004, Melo A. S. & Ferro V. G. (MZUESC). One ♂ imago: Brazil, São Paulo State, Santa Rosa do Viterbo, Fazenda Águas Claras, 12.XI.2000, light trap, Mendes H. F. & Andersen T. (MZUESC). One ♂ imago: Brazil, São Paulo State, Ribeirão Preto, Rio Pardo, próximo Ponte velha Jardinópolis, rancho Cesar & Nê 06.IX.2008, Calor A. (MZUESC). Eight ♂ imagos: Brazil, Bahia State, Lençois, Parque Nacional da Chapada Diamantina, Rio Santo Antônio,12°29'579"S, 41°19'752"W, 340m, 26.X.2008, Mariano, R., Calor, A.R. & Mateus, S. (MZUESC). Two ♂ imagos: Brazil, Bahia State, Barreiras, Rio das Ondas, 15.X.2008, Mariano, R., Calor, A.R. & Mateus, S. (MZUESC). 25 ♂ imagos: Brazil, Pernambuco State, Petrolina, rio da Vitória, afluente do Rio São Francisco, 09°21'814"S, 40°35'409"W, 440m, 22.X.2008, Mariano, R., Calor, A.R. & Mateus, S. (MZUESC). Ten nymphs, Brazil, Roraima, Boa Vista, Rio Cauamé, 2°52'5.30"N / 60°44'25.40"W, 76 m asl, 20.iii.2014, F.F. Salles, E. Domínguez, R. Boldrini, J. Gama-Neto col. (five nymphs CZNC, five nymphs IBN). One nymph: Brazil, Espírito Santo, Serra, 20°3'33"S/ W40°22'42', 20 m asl, 05/xi/2011, F. Massariol col. (CZNC). One nymph: Brazil, Espírito Santo, Bom Jesus do Norte, 21°6'53"S/41°41'31"W, 31/vii/2012, F. Massariol col. (CZNC). One nymph: Brazil, Espírito Santo, Iúna, 20°21'06"S/41°31'58"W, 08/v/2013, F. Massariol col. (CZNC).

Comments. The wide projections of the styliger plate readily distinguish *H. gilliesae* from all other members of the complex except for *Leentvaaria palpalis*, but this latter species has the projections fused (see "Discussion" below).

Variation in body lengths and colouration were encountered among specimens, with some individuals clearly darker than others. The overall shape of genitalia, however, was the same, and thus we are concluding for now that all of this material belongs to a single species. Unfortunately, since it could help in the identification of potential cryptic species, we were unable to extract and/or amplify DNA from all localities (see COI divergence section below).

Hydrosmilodon gilliesae was found to occur in several localities in Brazil, ranging from relatively close to its type-locale in French Guiana (state of Roraima), to central (Mato Grosso and Mato Grosso do Sul), Northeast (Pernambuco and Bahia) and southeast parts of the country (Espírito Santo and São Paulo) (Fig. 9).

With the description of this species, the diagnoses of the adults of the genus must be expanded in the following way: 1) Forks of veins MA and MP of fore wings asymmetrical; 2) cross vein close to MA fork slanted or not; 3) vein Sc of hind wings ending in transverse vein near base of costal projection; 3) vein MP of hind wings unforked; 4) costal projection of hind wings acute or rounded at apex; 5) tarsal claws of a pair dissimilar, one apically hooked, other blunt; 6) penis divided in apical 1/2 to totally divided, each lobe with median spine-like projection; 7) styliger plate with spines close to base of forceps or with two wide projections; 8) prosternum with short to long median carina; and 9) female sternum IX apically cleft.

Hydromastodon **Polegatto & Batista, 2007**

Figures 3, 4, 7

Diagnosis. The male imago of *Hydromastodon* can be distinguished from the other genera of the *Hermanella* complex by the following combination of characters: 1) Eyes meeting on meson of head (Fig. 3a); 2) Cross vein above fork of MA slanted (Fig. 4a); 3) Fork of MA asymmetrical and fork of MP slightly asymmetrical (MP2 connected to MP1 by a crossvein); 4) Styliger plate with a strong dorsally curved median projection (Fig. 4d, e); 5) Penis divided, each lobe with a long spine ventromedially directed (Fig. 4d, e).

Description of male imago (in alcohol). *Head* (Fig. 3a, b): Eyes meeting on meson of head, lower portion of eyes slightly < ½ length of upper portion.

Thorax: Prosternum with rather wide, X-shaped median carina, with similar anterior and posterior arms; similar to *Needhamella*, as shown by Domínguez and Flowers (1989: fig. 18).

Wings (Fig. 4a, b, c): Maximum width of fore wings 1/3 their maximum length; maximum width of hind wings about ½ their maximum length; maximum length of hind wings 1/6 maximum length of fore wings. Fore wings (Fig. 4a): vein Rs forked slightly > 1/4 distance from base of vein to margin, fork of vein MA asymmetrical and forked at ½ distance from base of vein to margin, cross vein above fork of MA slanted; fork of vein MP slightly asymmetrical and forked 1/3 distance from base of vein to margin; vein ICu_1 attached at base to vein CuA by crossvein; vein ICu_2 free basally.

Hind wings (Fig. 4b, c): costal projection well-developed, acute and located ½ distance from base to apex; vein MP unforked; apex of wings rounded; vein Sc ½ distance from base to wing margin, ending in crossvein; 5 cross veins present.

Legs. Ratio of segments of male forelegs, 0.6:1.0 (0.62 mm): 0.03:0.31:0.28:0.15: 0.08. Claws on each leg dissimilar, with one apically hooked and one blunt, pad-like.

Abdomen: Genitalia (Fig. 4d, e) with segment II of forceps subequal to segment III; segment II of forceps 1/5 length of segment I; styliger plate with strong, dorsally curved, median projection. *Penis* divided, each lobe with long spine ventromedially directed. Caudal filaments broken off and lost.

Hydromastodon sallesi Polegatto & Batista, 2007

Figures 3, 4, 7a, b

Diagnosis. This is the only species of the genus known from a male imago. Therefore, it is impossible to ascertain at this time the characteristics that will distinguish it from its congeners.

Description of male imago (in alcohol). *Lengths*: body, 4.6–5.6 mm; fore wings: 4.8–5.6 mm; hind wings: 0.8–0.9 mm. General coloration: light brown.

Head (Fig. 3a, b): yellowish-white, tinged with orange between ocelli; upper portion of eyes orangeish, lower portion black; ocelli white, surrounded with black and orange. Antennae light yellow-brown.

Thorax (Fig. 3a, b): yellowish-brown, sutures lighter. Wings (Fig. 4a, b, c): membranes of fore wing hyaline, base washed with light brown, veins C, Sc and R_1 tinged with orange, remainder of veins yellowish. Hind wing hyaline. Fore leg yellowish, washed with brown; mid and hind legs yellowish-white.

Abdomen (Fig. 3a): Terga I–V almost completely washed with black, segments II–V with sublateral circular mark less pigmented; segments VI–X yellowish-brown. Terga II–IX washed with black as in Fig. 3a, II–VI hyaline, VII–X yellowish. Sterna yellowish-brown, with pleura washed with black. Genitalia: styliger plate yellowish, washed with brown; forceps yellowish, washed with brown, but whitish distomedially. *Penis* yellowish; spines orangeish. Caudal filaments broken off and lost.

Material examined. One reared ♂ imago: Brazil, Roraima, Boa Vista, Rio Cauamé, 2°52'5.30"N / 60°44'25.40"W, 76 m asl, 21.v.2014, R. Boldrini col. (CZNC); one ♂ imago (partially molted) and two ♂ subimagos, same data as previous, except 03.ii.2007, J. Falcão col. (CZNC); 16 nymphs, same data as previous, except for 20.iii.2014, F.F. Salles, E. Domínguez, R. Boldrini, J. Gama-Neto col. (11 nymphs CZNC and 5 nymphs IBN); ten ♂ imagos: Brazil, Rondônia, Nova Londrina, Rio Urupá, 11°02'05"N / 62°08'34"W, 182 m asl, 02.ix.2012, N. Hamada leg. (5 INPA, 3 CZNC, 2 IBN).

Comments. Imagos of *Hydromastodon sallesi* are readily distinguished from all members of the complex, except for *Hydrosmilodon plagatus*, by the shape of the forceps and by the presence of a strong and dorsally curved, medial projection at the

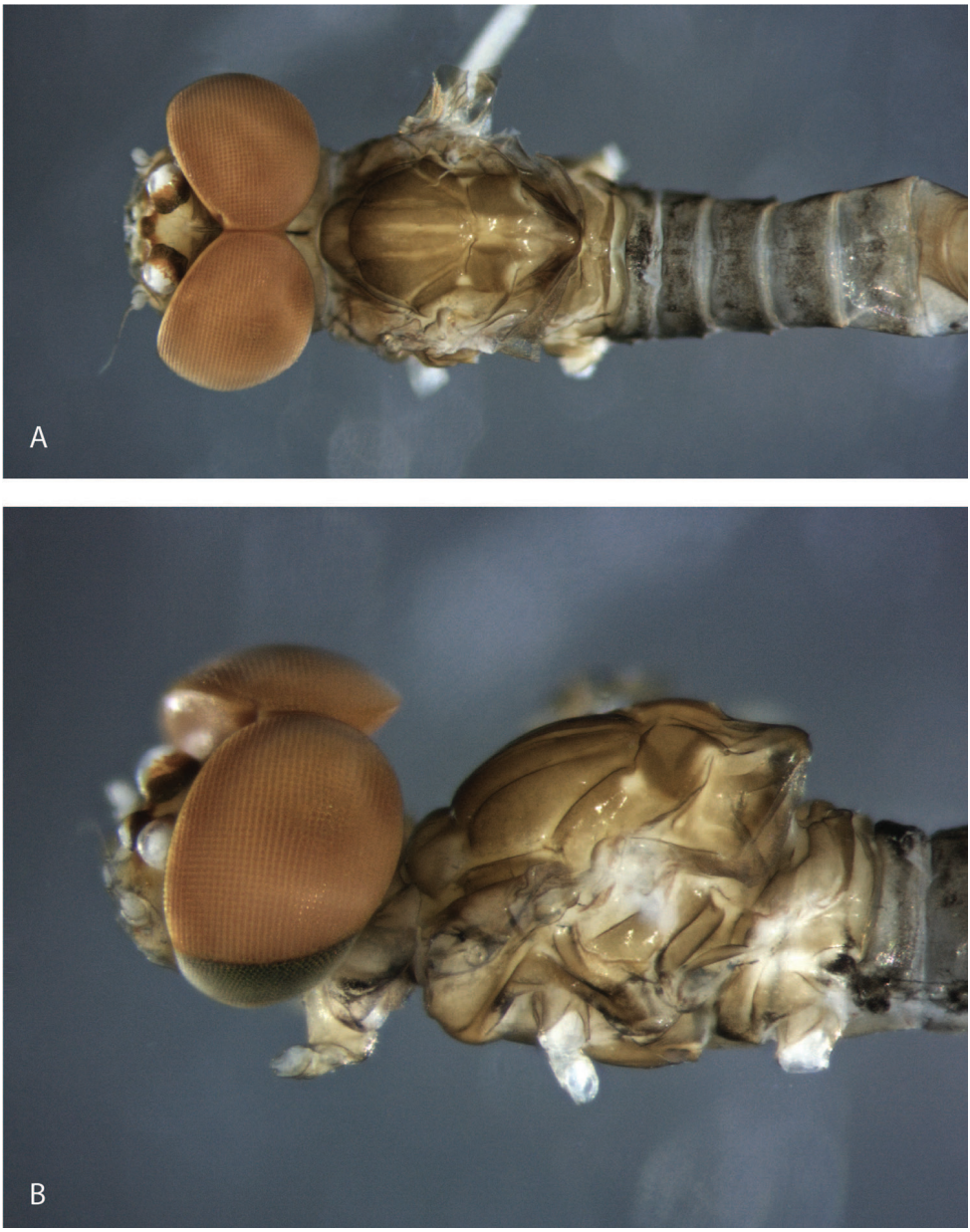

Figure 3. *Hydromastodon sallesi*, male imago: **a** dorsal view **b** head and thorax, lateral view.

styliger plate. Body color pattern (compare Fig. 3a herein to figs 2–4 of Lima et al. 2012), body length (around 5 mm in *Hm. sallesi*, but around 10 mm in *Hs. plagatus*) and details of penis morphology are enough to separate these two taxa. Geographic distribution may also prove helpful with identification, as *Hs. plagatus* is a typical Atlantic Forest species that seems to be restricted to the Brazilian coast, while *Hm. sal-*

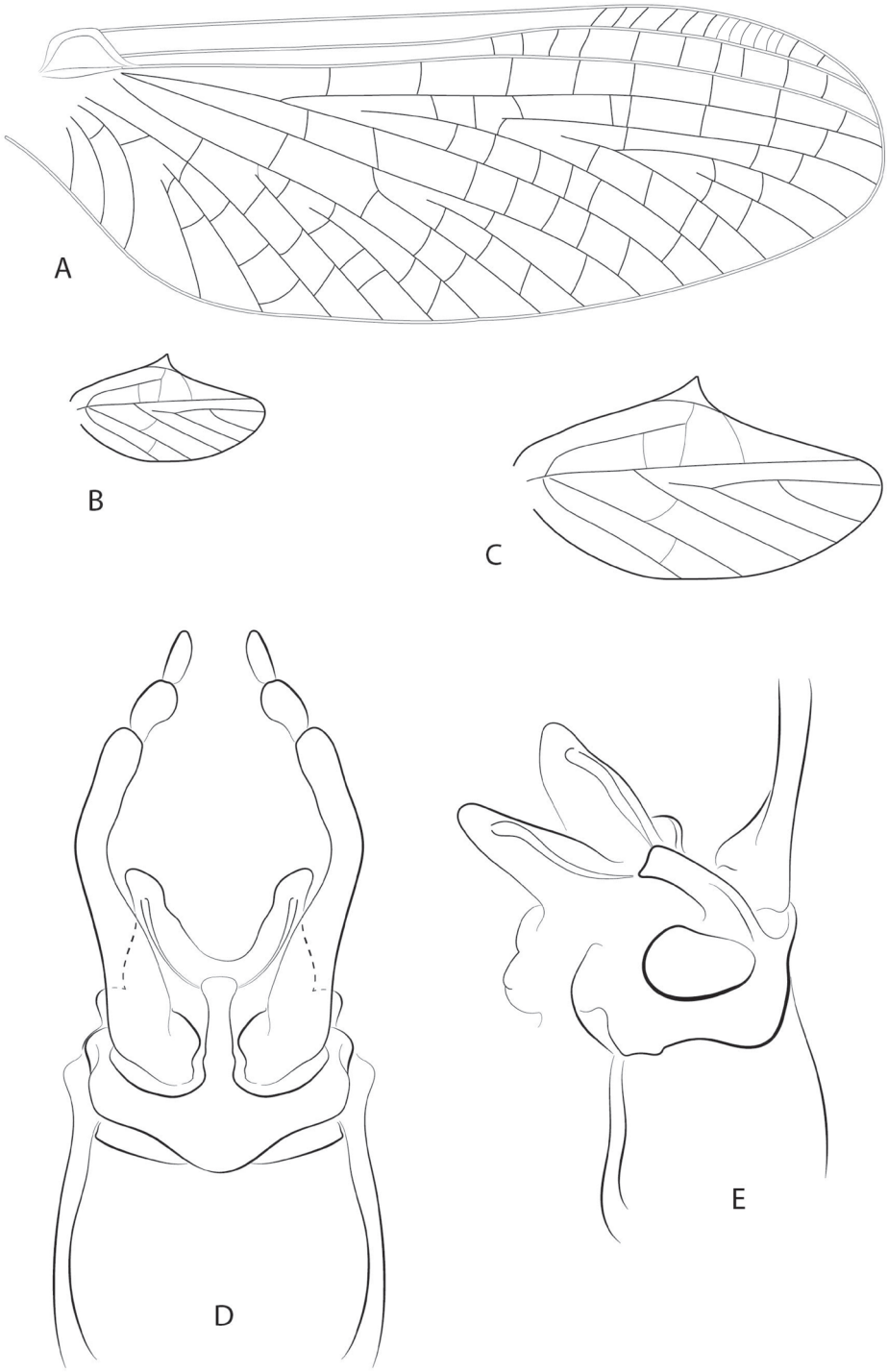

Figure 4. *Hydromastodon sallesi*, male imago: **a** fore wing **b** hind wing **c** hind wing, enlarged **d** genitalia, ventral view **e** genitalia, lateral view.

lesi is found in western and northern Brazil in transitional areas between the Amazon forest and Brazilian savannah.

Hydromastodon sallesi was described based on a few nymphs from Mato Grosso (Rio Pindaíba, Nova Xavantina) and Roraima (Bem Querer falls, Rio Branco, Caracaraí). The material examined in the present paper was collected from the states of Roraima and Rondônia, the latter of which extends the known distribution of the genus and species to the east.

In Roraima, nymphs were predominantly captured on a small stream leading to Rio Branco, at the Bem Querer falls, and in Boa Vista, at the Cauamé River (Fig. 8). In the Cauamé River, nymphs (Fig. 7a, b) of this species were found under rocks, close to the river margins, and they were much less abundant than the nymphs of *Leentvaaria palpalis* (see immediately below).

Leentvaaria **Demoulin, 1966**

Figures 5, 6, 7c, d

Diagnosis. The male imago of *Leentvaaria* can be distinguished from other genera of the *Hermanella* complex by the following combination of characters: 1) Eyes separated on meson of head by a short distance—less than 0.5 times the width of the median ocellus (Fig. 5a); 2) Fork of MA asymmetrical and fork of MP slightly asymmetrical (Fig. 6a); 3) Crossvein above fork of MA not slanted (Fig. 6a); 4) Styliger plate enlarged posteriorly, completely covering penis lobes in ventral view (Fig. 6d); 5) Penis divided, each lobe with a long spine apically curved (Fig. 6e).

Description of male imago (in alcohol). *Head* (Fig. 5a, b, c, e): Eyes separated on meson of head by short distance—less than 0.5 times width of median ocellus (Fig. 5a, b, c), lower portion of eyes slightly < ¼ length of upper portion.

Thorax: Prosternum with narrow, straight median carina, similar to *Hermanella* and *Hylister*, but with longer anterior arms, as in Fig. 5d.

Wings (Fig. 6a, b, c): Maximum width of fore wings 1/3 their maximum length; maximum width of hind wings about ½ their maximum length; maximum length of hind wings 1/5 maximum length of fore wings. Fore wings: vein Rs forked slightly > 1/6 distance from base of vein to margin, fork of vein MA asymmetrical and forked at ½ distance from base of vein to margin, cross vein above fork of MA not slanted; fork of vein MP slightly asymmetrical and forked 1/3 distance from base of vein to margin; vein ICu_1 attached at base to vein CuA by crossvein; vein ICu_2 attached at base to vein ICu_1 by crossvein. Hind wings: costal projection well-developed, acute; located ½ distance from base to apex; vein MP unforked; apex of wings rounded; vein Sc ½ distance from base to wing margin, ending in crossvein; 4–6 crossveins present.

Legs. Ratio of segments in male forelegs, 0.6:1.0 (1 mm): 0.03:0.35:0.30:0.15:0.06. Claws of each pair dissimilar, one apically hooked and one blunt, pad-like.

Abdomen. Genitalia (Fig. 6d, e): Styliger plate: length of segment II of forceps subequal to length of segment III; segment II of forceps 1/9 length of segment I; styl-

iger plate enlarged posteriorly, completely covering penis lobes in ventral view. *Penis* divided, each lobe with long spine apically curved. Caudal filaments: terminal filament longer than cerci.

Description of female imago (in alcohol). *Lengths*: body, 4.7–4.9 mm; fore wings, 4.9–5.2 mm; hind wings, 0.8–0.9 mm.

Head: Eyes (Fig. 5e) separated on meson of head by 6 times width of lateral ocellus.

Abdomen: Ninth sternum deeply cleft apically.

Leentvaaria palpalis Demoulin, 1966

Figures 5, 6, 7c

Diagnosis. This is the only species of the genus. Therefore, it is impossible to ascertain at this time the characteristics that will distinguish it from its congeners.

Description of male imago (in alcohol). *Lengths*: body, 4.7–4.9 mm; fore wings, 4.6–4.8 mm; hind wings, 0.8–0.9 mm.

General coloration: grayish-brown.

Head (Fig. 5a, b, c): yellowish-brown, upper portion of eyes reddish-brown, lower portion black; ocelli white, surrounded with black. Antennae light yellow-brown.

Thorax (Fig. 5a, b, c): brown, washed with black (faded in figures) with lighter sutures. Wings (Fig. 6a, b, c): membranes of fore and hind wings hyaline with base tinged with yellow. Base of C of both wings tinged with black basally. Longitudinal veins yellowish-brown, cross veins yellowish. Legs: fore leg yellowish, with base of coxa washed with black. Femur washed with grey. Remainder of fore leg and mid & hind legs yellowish.

Abdomen (Fig. 5a, b): Tergum I blackish; terga II–VI hyaline and washed with black; terga VII–X yellowish and washed with black. Sterna hyaline. Genitalia: styliger plate yellowish washed with grey; forceps greyish-black. *Penis* yellowish with orangeish spines. Caudal filaments yellowish, washed with gray.

Description of female imago (Fig. 5e) (in alcohol). *Lengths*: body, 4.4–4.7 mm; fore wings, 4.9–5.2 mm; hind wings, 0.8–0.9 mm.

Similar to male imago, except as follows: head yellowish-orange, except central longitudinal line on posterior part of dorsum of head; anterior margin of head, line connecting ocelli and area behind lateral ocelli washed with black. Eyes black. Ninth sternite yellowish-white.

Material examined. Three ♂ imagos: Brazil, Mato Grosso State, Nova Xavantina, córrego Benedito Ferreira, 06.xii.2006, light trap, Mariano R., Calor A.R. & Mateus S. (MZUESC). Eleven ♂ imagos: Brazil, Mato Grosso State, Ribeirão Cascalheira, Fazenda Campina Verde, Rio Suiamissu, 28-30.xii.2006, light trap, Mariano R., Calor A.R. & Mateus S. (MZUESC). Eleven ♂ imagos: Brazil, Mato Grosso State, Ribeirão Cascalheira, Fazenda Campina Verde, Rio Suiamissu, S12°48.591 W52°06.925, 10.x.2007, light trap, Pinho L.C., Mateus S., Torati L. & Silva F.R. (MZUESC). One reared ♂ imago, three ♂ imagos, two ♀ imagos: Brazil, Roraima, Boa Vista, Rio Cauamé, 2°52'5.30 N / 60°44'25.40"W, 76 m asl, 17.iii.2014, F.F. Salles, E. Domínguez,

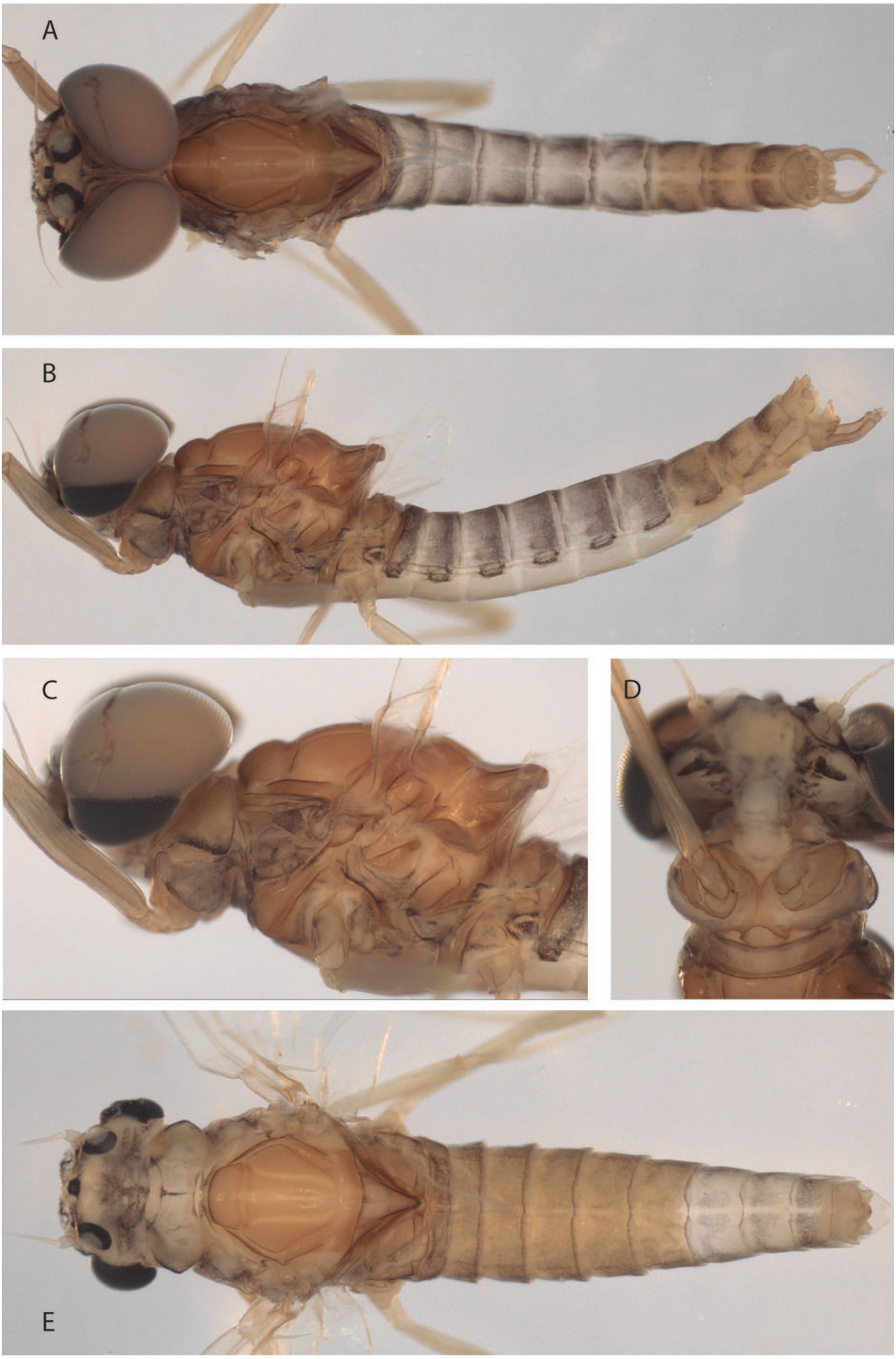

Figure 5. *Leentvaaria palpalis*, imagos: **a** dorsal view of male **b** lateral view of male **c** head and pronotum of male, lateral view **d** head and prosternum of male, ventral view **e** dorsal view of female.

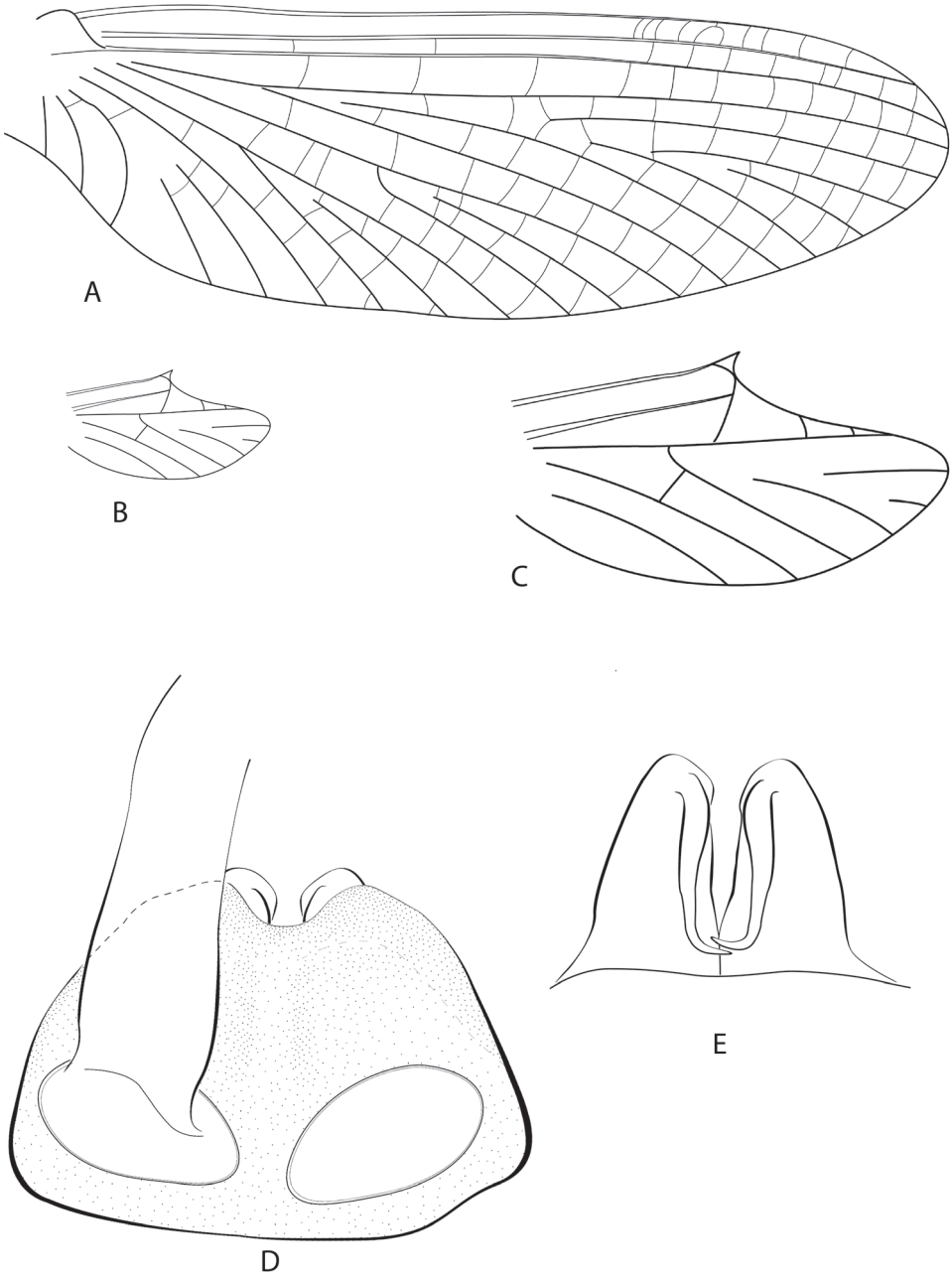

Figure 6. *Leentvaaria palpalis*, male imago: **a** fore wing **b** hind wing **c** hind wing, enlarged **d** genitalia, ventral view **e** penis, ventral view.

Figure 7. Living specimens: **a** *Hydromastodon sallesi*, female nymph **b** *Hydromastodon sallesi*, male nymph **c** *Leentvaaria palpalis*, male nymph.

R. Boldrini, J. Gama-Neto col. (reared imago, one ♂ imago, one ♀ imago CZNC; remainder at IBN); one reared ♀ imago, six ♂ imagos: Brazil, Roraima, Boa Vista, Rio Cauamé, 2°52'5.30"N / 60°44'25.40"W, 76 m asl, 03.ii.2007, J.N. Falcão col. (CZNC); 20 nymphs, sama data as previous, except for 20.iii.2014, F.F. Salles, E. Domínguez, R. Boldrini, J. Gama-Neto col. (ten nymphs CZNC and ten nymphs IBN); one ♂ imago, one nymph: Brazil, Roraima, Bonfim, Rio Arraia, 3°21'4" N / 59°54'13"W, 80 m asl, 21.iii.2013, J.Nascimento col. (CZNC).

Comments. This species appears to be unique, in particular reference to the development of the labial palpi in the nymph (Domínguez et al. 2001) and the subgenital plate in the male imago. The wide projections of the styliger plate are fused into a single structure (Fig. 6d), as mentioned in the discussion of *Hs. gilliesae* (see above), which readily distinguishes *L. palpalis* from all the other members of the complex.

Leentvaaria palpalis was originally described from Surinam, but it seems to be a widespread species. Recently Lima et al. (2012) reported its presence from the states of Espírito Santo and Pernambuco, representing the Brazilian coast and Atlantic Forest. In the present paper we report material from Mato Grosso and Roraima, western and northern Brazil, which represents the Amazon and Cerrado transition zones.

Nymphs (Fig. 7c) were found under rocks. In the case of the Cauamé River (where all the species treated herein were found, Fig. 8), *L. palpalis* is one of the most abundant species of mayflies.

Figure 8. General aspect of the Cauamé River, Roraima, Brazil.

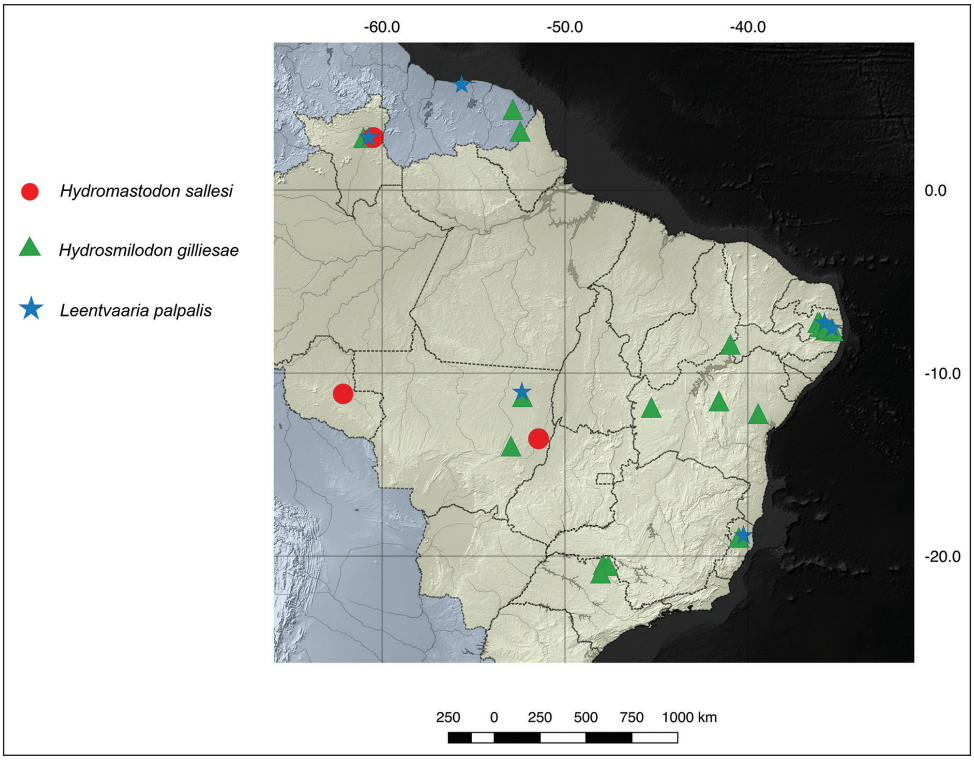

Figure 9. Partial view of South America, with emphasis on Brazil (yellow), showing the distribution of the species treated herein. Dashed lines, Brazilian states limits.

COI intra- and interspecific divergence

Genbank Accession numbers are given in Table 1. Identifications of the three morphologically defined species treated herein, their metamorphic stages, and the average sequence distance (K2P) among haplotypes are given in Table 2. Intraspecific distances ranged from 1.10–1.86% (values in bold in Table 2) with an average of 1.32%. Distances between species ranged from 16.50–21.50% with an average of 18.60%.

Genetic species delimitations were highly congruent with our morphological species identifications and showed a high level of confidence. Sequence differences smaller than 3% are frequently observed in intraspecific distances of DNA barcodes (Ferguson 2002;

Table 1. Collection information for specimens analysed in this study. Specimen information includes: species name, voucher number, locality (ES, State of Espírito Santo; RR, State of Roraima; BR, Brazil) and GenBank Accession Number.

Species	Voucher	Locality	GenBank
Hydrosmilodon gilliesae	4014 a	Serra, 20°3'33"S/ W40°22'42', ES - BR	KX831900
Hydrosmilodon gilliesae	4014 b	Serra, 20°3'33"S/ W40°22'42', ES - BR	KX831901
Hydrosmilodon gilliesae	4015 a	Bom Jesus do Norte, 21°6'53"S/41°41'31"W, ES - BR	KX831902
Hydrosmilodon gilliesae	6100 a	Iúna, 20°21'06"S/41°31'58"W, ES, BR	KX831903
Hydromastodon sallesi	5607 e	Boa Vista, 2°52'5"N/60°44'25"W, RR - BR	KX831904
Hydromastodon sallesi	5607 h	Boa Vista, 2°52'5"N/60°44'25"W, RR - BR	KX831905
Hydromastodon sallesi	5607 i	Boa Vista, 2°52'5"N/60°44'25"W, RR - BR	KX831906
Hydromastodon sallesi	5607 k	Boa Vista, 2°52'5"N/60°44'25"W, RR - BR	KX831907
Hydromastodon sallesi	5607 l	Boa Vista, 2°52'5"N/60°44'25"W, RR - BR	KX831908
Hydromastodon sallesi	5607 n	Boa Vista, 2°52'5"N/60°44'25"W, RR - BR	KX831909
Leentvaaria palpalis	5761 a	Bonfim, 3°21'4"N/59°545'13"W, RR - BR	KX831910
Leentvaaria palpalis	6086 a	Bonfim, 3°21'4"N/59°545'13"W, RR - BR	KX831911

Table 2. Kimura-2-Parameter (K2P) genetic distances for COI barcodes between Ephemeroptera specimens; specimens represented by voucher numbers (see Table 2). Intraspecific distances represented in bold. Lp, *Leentvaaria palpalis*; Hg, *Hydrosmilodon gilliesae*; and Hs, *Hydromastodon sallesi*.

	Lp 5761a	**Lp 6086a**	**Hg 4014a**	**Hg 4014b**	**Hg 4015a**	**Hg 6100a**	**Hs 5607e**	**Hs 5607h**	**Hs 5607i**	**Hs 5607k**	**Hs 5607n**
Lp 6086a	**0.011**										
Hg 4014a	0.176	0.184									
Hg 4014b	0.176	0.184	**0.000**								
Hg 4015a	0.173	0.180	**0.003**	**0.003**							
Hg 6100a	0.172	0.179	**0.019**	**0.019**	**0.017**						
Hs 5607e	0.164	0.171	0.218	0.218	0.215	0.211					
Hs 5607h	0.158	0.164	0.215	0.215	0.211	0.207	**0.031**				
Hs 5607i	0.158	0.164	0.218	0.218	0.215	0.211	**0.028**	**0.003**			
Hs 5607k	0.168	0.175	0.215	0.215	0.211	0.207	**0.008**	**0.028**	**0.025**		
Hs 5607l	0.158	0.164	0.218	0.218	0.215	0.211	**0.028**	**0.003**	**0.000**	**0.025**	
Hs 5607n	0.164	0.171	0.226	0.226	0.222	0.218	**0.017**	**0.025**	**0.022**	**0.014**	**0.022**

Hebert et al. 2003; Hebert et al. 2004; Ball et al. 2005; Cardoni et al. 2015; Gattolliat et al. 2015; Angeli et al. 2016). Whereas the examined specimens of *Hm. sallesi* for the barcode analysis were from the type-locality and those of *L. palpalis* were from an area relatively close to its type-locality, the specimens of *Hs. gilliesae* were from southeastern Brazil. Genetic distance of these specimens, when compared to those found in French Guyana (type-locality of the species), could be high due to geographic distance (e.g. Webb et al. 2012).

Discussion

Since the description of *Hs. gilliesae* and *Hs. mikei* the diagnosis and consequently the monophyly of the genus *Hydrosmilodon* have been questioned (Sartori 2005). This idea was later confirmed when Polegatto and Batista (2007) transferred *Hs. mikei* to the new genus *Hydromastodon*. Lima et al. (2012) described *Hydrosmilodon plagatus,* which presented some conflicting characters with the diagnosis of the genus (see below), as also happened with the adult of *Hs. gilliesae* described for the first time in this paper.

The imago of *Hs. gilliesae* described here does not conform with the diagnosis of the male imago of *Hydrosmilodon* given by Flowers and Domínguez (1992) in the following features: 1) Eyes of male separated on meson of head by a short distance—less than 0.5 times width of median ocellus; 2) Crossvein above MA not slanted; 3) Two wide projections almost covering the penis; and 4) Distomedial spines of penis converging medially. Most of these characteristics, in fact, are also present in *Leentvaaria palpalis*, indicating that these two species are probably closely related. The only difference is that the styliger projections are divided in *Hs. gilliesae*, while they are fused in *L. palpalis*.

The male imago of *Hydromastodon sallesi*, in turn, shares some important characteristics with the male imago of another recently described species of *Hydrosmilodon*, *Hs. plagatus*. Besides the shape and morphology of forceps segment I, which is more elongate than in other members of the complex (Fig. 4d), there is a medial projection at the posterior margin of the styliger plate, which is curved and directed towards the penis lobes (Fig. 4d, e).

Despite the similarities between *Hs. gilliesae* and *L. palpalis*, and between *Hs. plagatus* and *Hm. sallesi*, we will follow the classification scheme of Nascimento and Salles (2013). When describing species and commenting on the status of the generic arrangement in the *Hermanella* complex, Nascimento and Salles (2013) argued that no further classification changes should be made without a formally constructed phylogeny for the group. As there is a cladistic analysis in progress, we will wait to make any necessary changes until after formal hypotheses of relationships are presented. Also for this reason, no emendations to the generic diagnosis of *Hydrosmilodon* are presented here.

The species in the *Hermanella* complex group present a tendency to bear some kind of projections on the styliger plate. These projections can be paired, submedial and of different width, from narrow and pointed (as in *Needhamella* and some species of *Hermanella*) to broad (*Hydrosmilodon gilliesae*), or single and medial as in *Paramaka convexa*

(Spieth), *Hydromastodon sallesi* and *Hydrosmilodon plagatus*. With the imagos described here, interesting questions could be raised: is the plate that completely covers the penis found in *Leentvaaria palpalis* (and also in *Traverella insolita* Nascimento & Salles) a single projection resulting from the medial fusion of the mentioned paired projections, of which *Hs. gilliesae* is an intermediate development (from narrow, to wide projections to totally fused)? Is the origin of the expansion of a medial projection similar to that of *Paramaka convexa,* or is there a different explanation for this character? We hope that these questions will be answered with the new evidence we are gathering from several new taxa recently collected and with the ongoing phylogenetic analysis of the group.

Updated Key to the male imagos of the *Hermanella* complex

1 Styliger plate without projections (Fig. 151d of Domínguez et al. 2006) ....***Hylister*** (in part, *plaumanni*)

- Styliger plate with sublateral (Figs 144j, 144l, 150e of Domínguez et al. 2006) or medial projections (Figs 4d, 4e and 174e of Domínguez et al. 2006).....**2**

2 Styliger plate with single medial projection (Fig. 4d, e and fig. 174e of Domínguez et al. 2006) **3**

- Styliger plate with paired sublateral projections (Figs 2d, 6d and figs144j, l, 150e of Domínguez et al. 2006) **5**

3 Medial projection of styliger plate of various shapes, but never curved toward penis lobes (Fig. 174e of Domínguez et al. 2006) .... ***Paramaka* (*convexa*, *pearljam*, *incognita*)**

- Medial projection of styliger plate robust, curved towards penis lobes (Fig. 4d, e) **4**

4 Length of body ca. 5 mm; costal area of fore wing hyaline .... ***Hydromastodon* (*sallesi*)**

- Length of body ca. 10 mm; costal area of fore wing brown ....***Hydrosmilodon* (*plagatus*)**

5 Paired projections wide, partially or almost completely covering the penis lobes (Figs 2d, 6d) **6**

- Paired projections subtriangular, not covering the penis lobes (figs144j, l, 150e of Domínguez et al. 2006) **9**

6 Paired projections fused (Fig. 6d) **7**

– Paired projections separated (Fig. 2d) **8**

7 Abdominal coloration contrasting, with segments II–VI translucent and segments VII–X reddish-brown (fig. 13a, b of Nascimento and Salles 2013); paired projections forming three small plates (fig. 14d of Nascimento and Salles 2013) ..... ***Traverella*** (in part, *insolita*)

– Abdominal coloration not contrasting, segments II–X all similarly washed with black (Fig. 5a); paired projections forming two small plates (Fig. 6d) ... .***Leentvaaria* (*palpalis*)**

8 Paired projections with small distal spines; penis lobes each with a strong spine-like projection, which is medially bowed and ventrally directed (fig. 35 of Kluge 2007).....***Hylister*** (in part, *chimaera*)

- Paired projections without small distal spines; penis lobes each with a strong spine-like projection posteriorly directed (Fig. 2d)..... .....***Hydrosmilodon*** (in part, *gilliesae*)

9 Eyes meeting on meson of head..... ... ***Traverella*** (in part, *bradley*, *calingastensis*, *longifrons*, *montium*, *valdemari*)

– Eyes not meeting on meson of head (separated by a distance equal to 1.5 times width of lateral ocellus).....**10**

10 Projections of penis lobes broad and parallel (figs 144k, 144l of Domínguez et al. 2006) ..... ***Hermanella*** (in part, *amere*, *guttata*, *thelma*)

- Projections of penis lobes spine-like and convergent (figs144j, m, 150e, 169e of Domínguez et al. 2006).....**11**

11 Spine-like projection of penis lobes straight (sometimes slightly curved at apex) (fig. 150e of Domínguez et al. 2006, fig. 24 of Lima et al. 2012) **12**

- Spine-like projection of penis lobes strongly curved (Figs144j, m, 169e of Domínguez et al. 2006).....**14**

12 Projections of styliger plate short and blunt (fig. 150e of Domínguez et al. 2006)..... ***Hydrosmilodon*** (in part, *primanus*, *saltensis*)

– Projections of styliger plate long and pointed (fig. 24 of Lima et al. 2012)..... **13**

13 Apex of penis lobes pointed; projections of styliger plate relatively short (fig. 24 of Lima et al. 2012); distribution Neotropical...***Hermanella*** (in part, *mazama*)

– Apex of penis lobes somwehat truncate; projections of styliger plate relatively long (fig. 6 of Edmunds 1948); distribution Nearctic .... ***Traverella*** (in part, *albertana*)

14 Projections of styliger plate long; distolateral corner of penis lobe less developed than inner corner (fig. 169e of Domínguez et al. 2006); prosternum wide, median carina X-shaped (fig. 18 of Domínguez and Flowers 1989)..... .....***Needhamella* (*ehrhardti*)**

– Projections of styliger plate short; distolateral corner of penis lobe more developed than inner corner (as in figs 144j, m of Domínguez et al. 2006); prosternum with narrow, straight median carina (fig. 144r of Domínguez et al. 2006) .....***Hermanella*** (in part, *froehlichi*, *maculipennis*, *nigra*) / ***Hylister*** (in part, *obliquus*)

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References

Angeli KB, Salles FF, Paresque R, Molineri C, Lima LRC (2016) Stage description, new combination and new records of Neotropical Brachycercinae (Ephemeroptera: Caenidae). Zootaxa 4088: 268–278. doi: 10.11646/zootaxa.4088.2.8

Ball SL, Hebert PDN, Burian SK, Webb JM (2005) Biological identifications of mayflies (Ephemeroptera) using DNA barcodes. Journal of the North American Benthological Society 24: 508–524. doi: 10.1899/04-142.1

Cardoni S, Tenchini R, Ficulle I, Piredda R, Simeone MC, Belfiore C (2015) DNA barcode assessment of Mediterranean mayflies (Ephemeroptera), benchmark data for a regional reference library for rapid biomonitoring of freshwaters. Biochemical Systematics and Ecology 62: 36–50. doi: 10.1016/j.bse.2015.07.035

Coleman CO (2003) Digital inking: how to make perfect line drawings on computers. Organisms, diversity & evolution 3: 303–304. doi: 10.1078/1439-6092-00081

Coleman CO (2006) Substituting time consuming pencil drawings in arthropod taxonomy using stacks of digital photographs. Zootaxa 1360: 61–68.

Demoulin G (1966) Contribution à l'étude des Ephéméroptères du Surinam. Bulletin de l'Institut Royal des Sciences Naturelles de Belgique 42: 1–22.

Domínguez E, Molineri C, Pescador ML, Hubbard MD, Nieto C (2006) Ephemeroptera of South America. Aquatic Biodiversity of Latin America. Vol. 2. Sofia-Moscow, 646.

Domínguez E, Ferreira MJ, Nieto C (2001) Redescription and Phylogenetic Relationships of *Leentvaaria* Demoulin (Ephemeroptera: Leptophlebiidae). In: Domínguez E (Ed.) Trends in Research in Ephemeroptera and Plecoptera, 313–320. doi: 10.1007/978-1-4615-1257-8_35

Domínguez E, Flowers RW (1989) A Revision of *Hermanella* and Related Genera (Ephemeroptera: Leptophlebiidae; Atalophlebiinae) from Subtropical South America. Annals of the Entomological Society of America 82: 555–573. doi: 10.1093/aesa/82.5.555

Edmunds GF Jr. (1948) A new genus of mayflies from western North America (Leptophlebiinae). Proceedings of the Biological Society of Washington 61: 141–148.

Ferguson JWH (2002) On the use of genetic divergence for identifying species. Biological Journal of the Linnean Society 75: 509–516. doi: 10.1046/j.1095-8312.2002.00042.x

Flowers RW, Dominguez E (1992) New Genus of Leptophlebiidae (Ephemeroptera) from Central and South America. Annals of the Entomological Society of America 85: 655–661. doi: 10.1093/aesa/85.6.655

Gattolliat J-L, Cavallo E, Vuataz L, Sartori M (2015) DNA barcoding of Corsican mayflies (Ephemeroptera) with implications on biogeography, systematics and biodiversity. Arthropod Systematics Phylogeny 73: 3–18.

Hebert PDN, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. Proceedings of the Royal Society of London. Series B. Biological Sciences 270: 313–321. doi: 10.1098/rspb.2002.2218

Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. Proceedings of the National Academy of Sciences of the United States of America 101: 14812–14817. doi: 10.1073/pnas.0406166101

Kluge NJ (2007) A new taxon Hermanellonota, or subtribe Hermanellini subtr.n. (Ephemeroptera: Leptophlebiidae: Hagenulini), with description of three new species from Peruvian Amazonia. Russian Entomological Journal 16: 385–400.

Lima LRC, Nascimento JMC, Mariano R, Pinheiro US, Salles FF (2012) New species and new records of *Hermanella* complex (Ephemeroptera: Leptophlebiidae) from Eastern Brazilian Coast. Annales de Limnologie 48: 201–213. doi: 10.1051/limn/2012011

Nascimento JMC, Salles FF (2013) New species of *Hermanella* complex (Ephemeroptera: Leptophlebiidae) from Brazilian Atlantic Forest. Zootaxa 3718: 1–27. doi: 10.11646/zootaxa.3718.1.1

Polegatto CM, Batista JD (2007) *Hydromastodon sallesi*, new genus and new species of Atalophlebiinae (Insecta: Ephemeroptera: Leptophlebiidae) from West and North of Brazil, and notes on systematics of Hermanella group. Zootaxa 1619: 53–60.

Sartori M (2005) A new species of the genus *Paramaka* Savage & Domínguez, 1992 with some comments on related genera (Ephemeroptera, Leptophlebiidae, Atalophlebiinae). Studies on Neotropical Fauna & Environment 40: 237–245. doi: 10.1080/01650520500208481

Thomas A, Boutonnet J, Péru N, Horeau V (2004) Les Ephemere`s de la Guyane Française. 9. Descriptions d'*Hydrosmilodon gilliesae* n. sp. et d'*H. mikei* n. sp. (Ephemeroptera, Leptophebiidae). Ephemera (2002) 4: 65–80.

Webb JM, Jacobus LM, Funk DH, Zhou X, Kondratieff B, Geraci CJ (2012) A DNA Barcode Library for North American Ephemeroptera: Progress and Prospects. PLoS ONE 7(5): e38063. doi: 10.1371/journal.pone.0038063

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RESEARCH ARTICLE

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Effects of an increase in population of sika deer on beetle communities in deciduous forests

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Abstract

The overabundance of large herbivores is now recognized as a serious ecological problem. However, the resulting ecological consequences remain poorly understood. The ecological effects of an increase in sika deer, *Cervus nippon* Temminck (Cervidae), on three insect groups of beetles was investigated: ground beetles (Carabidae), carrion beetles (Silphidae), and dung beetles (Scarabaeidae and Geotrupidae) on Nakanoshima Island, Hokkaido, northern Japan. We collected beetles on Nakanoshima Island (experimental site) and lakeshore areas (control site) and compared the species richness, abundance, diversity index, and community composition of beetles between the sites. Results showed that although both species diversity and abundance of carabid beetles were significantly higher at the lakeshore site, those of dung and carrion beetles were higher at the island site. It was additionally observed that abundance of larger carabid beetles was higher at the lakeshore site, whereas that of small-sized carabid beetles did not differ between the lakeshore and island sites. For dung beetles, abundance of smaller species was higher at the island site, whereas that of large species did not differ between the lakeshore and island sites. Abundance of two body sizes (small and large) of carrion beetles were both higher at the island site. Overall, the findings of this study demonstrated that an increase in deer population altered the insect assemblages at an island scale, suggesting further changes in ecosystem functions and services in this region.

Keywords

Ecosystem functions, ecosystem management, forest ecosystems, herbivores overgrazing, species traits

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Introduction

The overabundance of large herbivores is now recognized as one of the serious ecological issues worldwide, especially in the northern hemisphere (Gill 1992, Côté et al. 2004, Takatsuki 2009). Indeed, there is mounting evidence demonstrating the serious consequences of large herbivore overabundance on forest ecosystems (Takatsuki 2009, Foster et al. 2014). As large herbivores selectively browse palatable species, browsing has negative effects on understory structure and species composition (Rooney 2009, Martin et al. 2011, Tanentzap et al. 2011). Large herbivores additionally alter forest structure (e.g. Kanda et al. 2005, White 2012) by limiting tree regeneration (Akashi and Nakashizuka 1999, Horsley et al. 2003), thereby inducing a cascading effect on animal species (Kanda et al. 2005, Bressette et al. 2012). Indeed, it has been reported that smaller herbivorous invertebrates are negatively influenced by large herbivore overabundance because of interspecies competition for food resources (Gómez and González-Megías 2002, Wheatall et al. 2013). In addition, browsing results in the degradation of habitat for invertebrates and birds (Stewart 2001, Allombert et al. 2005, Chollet and Martin 2013).

To evaluate the ecological impacts of large herbivore overabundance on ecosystems, previous studies have commonly used manipulations with inclusion/exclusion treatments of large herbivores (Goméz and González Megías 2002, Mysterud et al. 2010, Holt et al. 2011, Bush et al. 2012). However, these previous studies have two major limitations. First, as past studies investigated the effects of large herbivore overabundance up to 10 years (e.g. Suominen et al. 2003, Beguin et al. 2011), the effects on ecosystems were unlikely to be completely detected. Indeed, it is established that the effects of browsing by large herbivores on vegetation structure can persist for an extended period (Tanentzap et al. 2011, Nuttle et al. 2014). Second, to understand the responses of ecosystems to perturbation, it is necessary to conduct a large-scale experiment (Carpenter 1998), whereas previous studies have employed relatively small-scale experiments (e.g. Dennis et al. 1998, Gómez and González-Megías 2002; Iida et al. 2016). Nevertheless, field studies that employed a relatively large-scale experiment remain scarce, with most having been conducted in Canadian coniferous forest (e.g. Allombert et al. 2005, Martin et al. 2010).

Lake Toya located in western Hokkaido, northern Japan, provides an ideal study site to investigate the long-term impacts of deer overabundance on ecosystems (Fig. 1). On Nakanoshima Island (476.7 ha, hereafter, island), which is one of the islands situated within Lake Toya, three sika deer individuals [*Cervus nippon* Temminck (Cervidae)] were introduced approximately 50 years ago and deer density at the island has now reached over 50 deer/km2 (Ikeda et al. 2013). Takahashi and Kaji (2001) reported a significant impoverishment of understory vegetation at the island because of the rapid growth in the deer population (Fig. 1B), suggesting a further consequence for other animal species. In the areas around Lake Toya (hereafter, lakeshore), and at the time of the current study, deer density was approximately 1.1 × 10–2 deer/km2 (Akaba et al. 2014), which is considerably lower than the density at the island site (Fig. 1C). As the island is

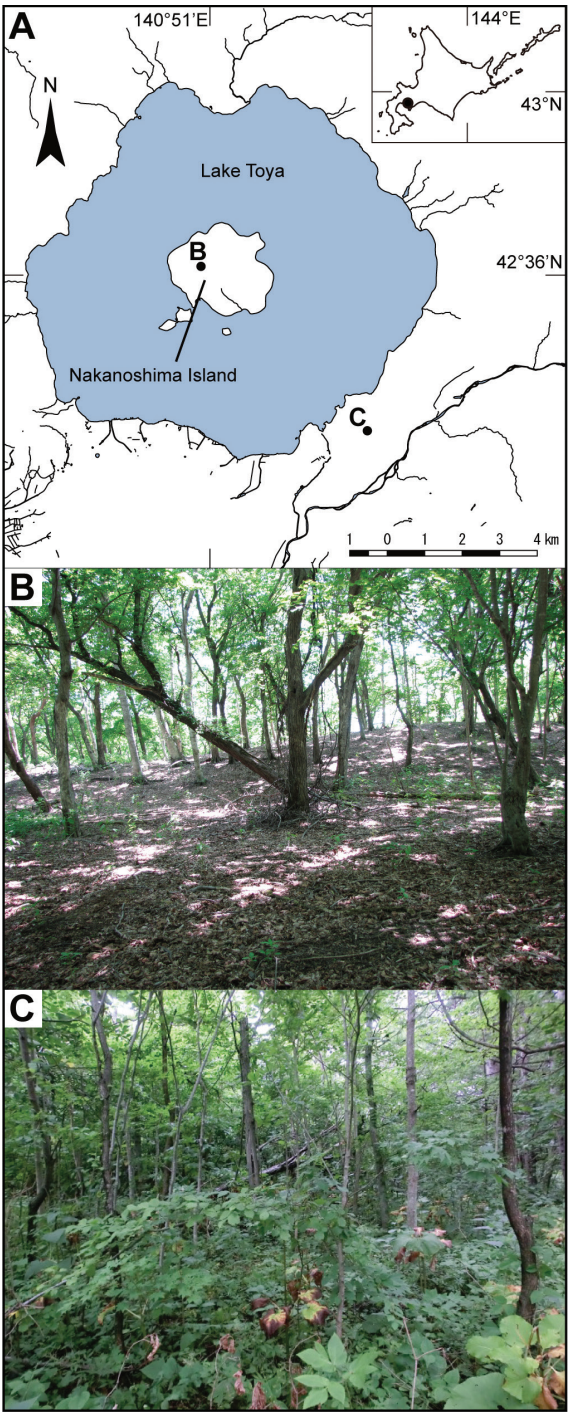

Figure 1. The location and forest floor of the study area. **A** The location of Lake Toya **B** Forest floor of Nakanoshima Island **C** Forest floor of the lakeshore of Lake Toya. The map of Lake Toya was modified from the Geospatial Information Authority of Japan 2015.

geographically isolated from lakeshore areas, the island ecosystem could be considered as an experimental site for examining the effects of an increase in deer population on natural ecosystems.

Here, we evaluated the effects of an increase in sika deer population on four taxonomic groups of beetles: carabid (Carabidae), carrion (Silphidae) and dung beetles (Scarabaeidae and Geotrupidae). These beetle groups were selected for several reasons. First, as these beetles inhabit the forest floor and are known to be sensitive to microclimatic changes (Rainio and Niemelä 2003, Arellano et al. 2005), they are likely to sharply respond to the increase in the large herbivores (Rooney 2009, Yamada and Takatsuki 2015). Second, many beetles display species-specific ecological traits, which facilitates the investigation of the association between species life-history traits and their responses to large herbivore overabundance (Sumways 2007, Bachand et al. 2014). Third, the clarification of the responses of beetles to environmental changes is crucial for understanding the overall changes in forest ecosystems, as beetles constitute a large proportion of biomass in forest ecosystems and play important roles that maintain ecosystem functions, such as nutrient cycling and pollination (Kevan and Baker 1983, Speight et al. 2008, Barton et al. 2013).

Materials and methods

Study area

Our study area is located in Western Hokkaido, northern Japan. The deer density at the island site (42°36'N, 140°51'E (DDM)) is dramatically higher than that at the lakeshore site because of artificial introduction. The mean annual temperature of this area is 7.3 °C and the mean monthly temperature ranges from –5.1 °C to 20.2 °C. The mean annual precipitation is 984.8 mm and the mean annual snow depth is 30 cm. The study area is situated in a deciduous forest dominated by *Quercus crispula* Blume (Fagaceae); *Kalopanax septemlobus* (Thunb.) Koidz. (Araliaceae); *Magnolia obovata* Thunb. (Magnoliaceae); *Acer pictum* Thunb. (Sapindaceae); *Maackia amurensis* Rupr. et Maxim. (Fabaceae); *Tilia japonica* (Miq.) Simonk. (Tiliaceae) and *Ostrya japonica* Sarg. (Betulaceae) (Kaji et al. 1991). Because of overgrazing at the island site, the structure of understory vegetation and the forest floor differ between the island and lakeshore sites (Fig. 1), with the island site dominated by *Senecio cannabifolius* Less. (Asteraceae); *Sagina japonica* (Sw.) Ohwi (Caryophyllaceae); *Pachysandra terminalis* Siebold et Zucc. (Buxaceae) and *Chloranthus serratus* (Thunb.) Roem. et Schult. (Chloranthaceae), which sika deer find unpalatable (Takahashi and Kaji 2001, Miyaki and Kaji 2009). The forest floor of the lakeshore site is dominated by *Dryopteris crassirhizoma* Nakai (Dryopteridaceae); *Cardamine leucantha* (Tausch) O.E. Schulz (Brassicaceae); *Cephalotaxus harringtonia* (Knight ex Forbes) K.Koch *var. nana* (Nakai) Rehder (Cephalotaxaceae) and *Sasa senanensis* (Franch. et Sav.) Rehder (Poaceae) (observed by authors). Although other large animals including *Vulpes vulpes* Linnaeus (Canidae);

Nyctereutes procyonoides Gray (Canidae) and *Procyon lotor* (Linnaeus) (Canidae) occur at the lakeshore site, their abundance is negligible compared to sika deer (Akaba et al. 2014). Besides the sika deer, the island site contains no medium- or large-sized mammals (Akaba et al. 2014). We established 30 sampling plots which are 100 m apart from each other (along a 3 km sampling transect) at each of the island and lakeshore sites (42°34'N, 141°54'E (DDM)).

Beetles sampling

At both sites, we sampled carabid, carrion and dung beetles using pitfall traps baited with cattle dung and fermented milk. Fermented milk is one of the major bates in collecting ground-dwelling beetles in Japan (Suttiprapan and Nakamura 2007). Pitfall traps were constructed using plastic containers (22.5 cm diameter and 26.6 cm deep) and plastic cups (8.3 cm diameter and 11.5 cm deep). Each container of fermented milk was buried to the rim in the ground. Plastic cups containing cattle dung were then hung inside the container using wires. We set plastic roofs on the traps to prevent interference from rain and fallen leaves. These traps were set at a density of one trap per plot. Surveys were conducted during early September 2012 and 2013.

Species classification

For each beetles group, the insect species collected in the field were divided into different size groups according to body length (see Fujita et al. 2008, Koike et al. 2014). Information on body size was collected from Ueno et al. (1985). Carabid and carrion beetles were divided into groups for small, medium or large species (small, <10 mm; medium, ≥10 and <20 mm and large, ≥20 mm), and dung beetles were divided into groups for small or large species (small, <10 mm; large, ≥10 mm). However, because carrion beetle species which are divided into small size group were not sampled, carrion beetles were divided into the medium or large size groups.

Data analyses

All analyses were performed using R ver. 3.2.1 (R core team 2015). In order to test the differences in abundance and species richness of each taxonomic group or body length between the island and lakeshore sites, we used generalized linear models (GLMs), using the 'glm' function. For GLMs, the abundance and species richness of each taxonomic or body size group were used by a response variable with a Poisson distribution and a log link function. Sampling sites were used as categorical explanatory variables (we used the lakeshore site as a reference). To test the difference in species diversity between two sites, Shannon-Wiener diversity index of each taxonomic group which were calculated

by using package 'vegan' (Oksanen et al. 2015) were also used as a response variable with a Gaussian (normal) distribution and an identity function. Species diversity indices of each functional group were not calculated and were excluded from the analysis because of the small sample size. Because we could not identify *Synchus* spp. to the species level, these species were excluded from the analysis of species richness and diversity. We did not find large carabid species in the island sites, which hinders the fitting of our data using GLMs. Hence, we excluded large carabid species in this analysis. Because of small sampling sizes, we used 'zeroinfl' function in the package 'pscal' for abundance and species richness of small carabid species, and medium and large carrion beetles. We calculated estimated abundance, species richness and Shannon-Wiener indices using the estimates of parameters in GLMs.

To investigate the difference in species composition between the island and lakeshore sites, we performed a non-metric multidimensional scaling ordination (NMDS) using the metaMDS function with the Bray–Curtis measure within the package 'labdsv' (Roberts 2015) as well as cluster analysis. The validity of clustering was evaluated using the Calinski–Harabasz criterion. The indicator species value (IndVal) was calculated to identify species, which are indicators of assemblages of each site as illustrated by NMDS and cluster analysis.

Results

3,876 individuals in total were collected, comprising 824 carabid beetles (18 species), 148 carrion beetles (four species) and 2,902 dung beetles (five species) (see Suppl. material 1).

Comparisons between species richness, abundance, and diversity index of insect species between the island and lakeshore sites were summarized in Figs 2–4 and Tables 1–3 (see also Supplementary Tables 2–4 for the differences between estimated and observed values). For carabid beetles, the abundance of individuals at the lakeshore site was higher than that at the island site ($p < 0.001$; Fig. 2A; Table 1). Although the abundance of medium species were higher at the lakeshore site than at the island site ($p < 0.001$), that of small species did not significantly differ between the two sites ($p = 0.24$; Fig. 2A; Table 1). The species richness of all carabid species ($p = 0.49$), small ($p = 0.42$) and medium species ($p = 0.38$) did not significantly differ between the island and lakeshore sites (Fig. 3A; Table 2). The species diversity index was higher at the lakeshore site than at the island site ($p = 0.05$; Fig. 4A; Table 3).

The abundance and species richness of carrion beetles at the island site were higher than those at the lakeshore site ($p < 0.001$; Figs 2B and 3B; Tables 1 and 2). The abundance of the two body size groups (medium and large species) were higher at the island site than at the lakeshore site ($p < 0.001$; Fig. 2B; Table 1). The species richness of the two body size groups were also higher at the island site than at the lakeshore site (medium: $p = 0.04$; large: $p = 0.003$). The mean species diversity index was higher at the island site than at the lakeshore site ($p = 0.005$; Fig. 4B; Table 3).

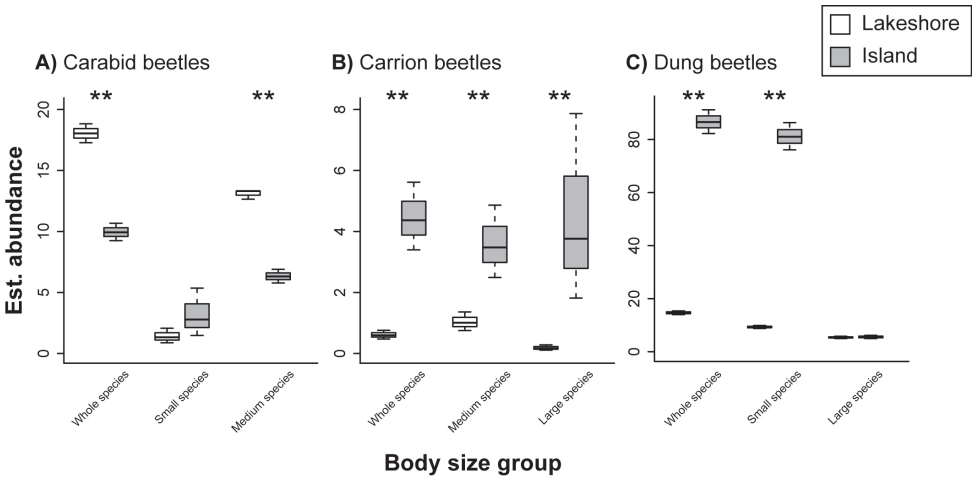

Figure 2. Abundance of each insect taxonomic and functional group per plot at the island and lakeshore sites estimated by using GLMs. **A** Carabid **B** Carrion **C** Dung beetles. Carabid and carrion beetles were classified into small, medium and large species and dung beetles were classified into small and large species (see the main text). Asterisks indicate a significant difference ($p < 0.05$).

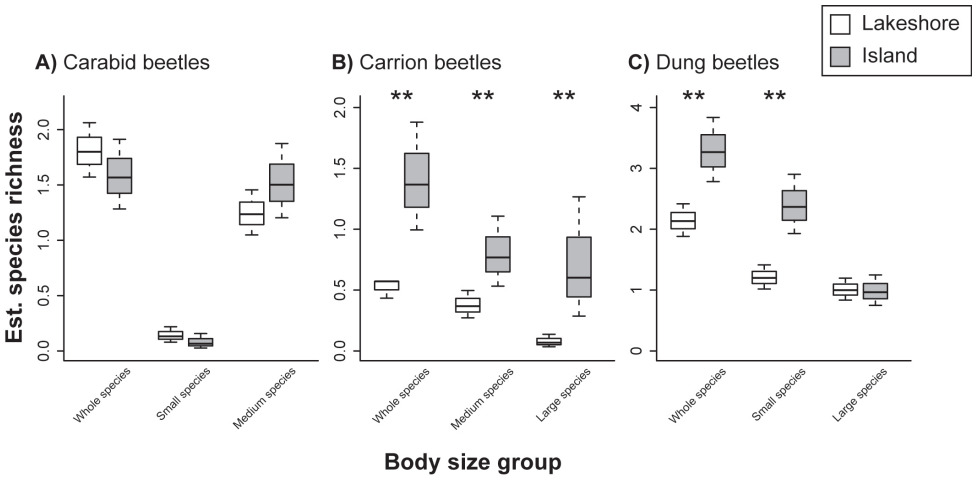

Figure 3. Species richness of each insect taxonomic and functional group per plot at the island and lakeshore sites estimated by using GLMs. **A** Carabid **B** Carrion and **C** Dung beetles. Carabid and carrion beetles were classified into small, medium and large species and dung beetles were classified into small and large species (see the main text). Asterisks indicate a significant difference ($p < 0.05$).

For dung beetles, the abundance ($p < 0.001$) and species richness ($p = 0.008$) at the island site were higher than those at the lakeshore site (Figs 2C, 3C; Tables 1 and 2). Although the abundance and species richness of small species were similarly higher at the island site than at the lakeshore site ($p < 0.001$; Figs 2C, 3C; Tables 1and 2), those were not the case for large species (abundance: $p = 0.78$, species: $p = 0.90$; Fig. 2C, 3C;

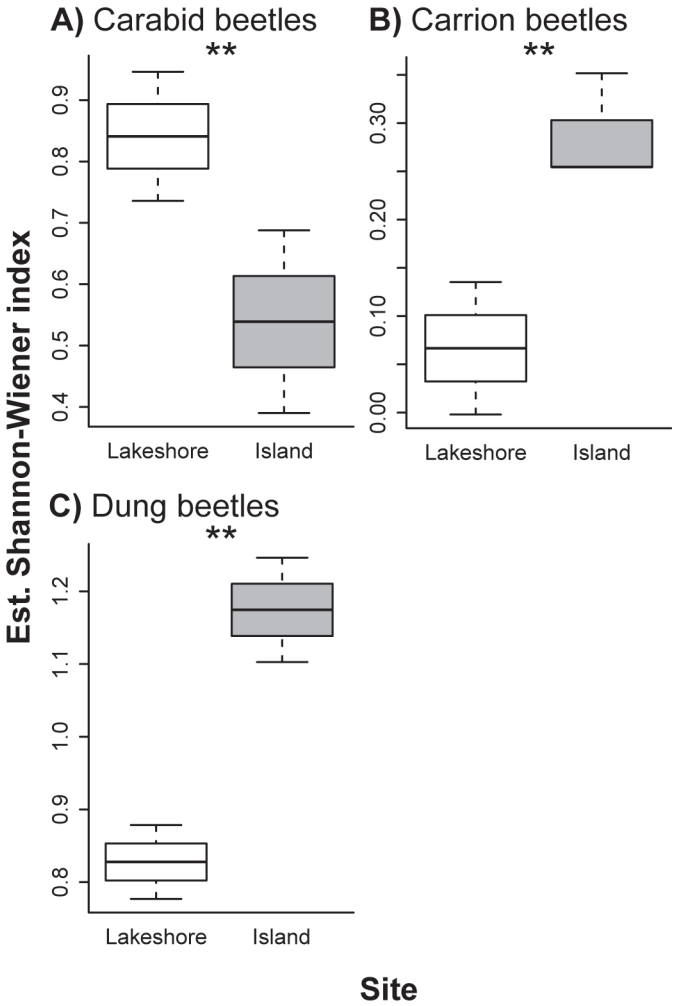

Figure 4. Shannon–Wiener index of each insect taxonomic group per plot at the island and lakeshore sites estimated by using GLMs. **A** Carabid **B** Carrion and **C** Dung beetles. Asterisks indicate a significant difference ($p < 0.05$).

Tables 1 and 2). The mean species diversity index was higher at the island site than at the lakeshore site ($p < 0.001$; Fig. 4C; Table 3).

NMDS and cluster analysis identified a significant difference in species composition between the island and lakeshore sites (Fig. 5). In addition, according to indicator species analysis, indicator species were different between the island and lakeshore sites. Indicator species analysis showed that *Caccobius jessoensis* Harold; *Onthophagus ater* Waterhouse; *Liatongus phanaeoides* (Westwood); *Copris ochus* Motschulsky; *Eusilpha japonica* (Motschulsky); *Silpha perforata* Gebler and *Pterostichus leptis* Bates had significant IndVals for the island site (Table 4). Conversely, Synuchus spp. Gyllenhal; *Pterostichus thunbergi* Morawitz and *Leptocarabus arboreus* (Lewis) had significant IndVals for the lakeshore site.

Table 1. Estimates of parameter differences between sites (reference = lakeshore site, i.e., coefficients of lakeshore site are zero), standard errors (SEs), z values, and p values in generalized linear models of each of taxonomic group and body size group abundance. Bold letters represent p < 0.05.

	Parameters	Estimates	SEs	z values	p values
Carabid beetles					
Whole species	(Intercept)	2.892	0.043	67.271	< 0.001
	Island	-0.596	0.072	-8.266	< 0.001
Small species	(Intercept)	0.170	0.484	0.351	0.73
	Island	0.799	0.682	1.172	0.24
Medium species	(Intercept)	2.526	0.052	48.911	< 0.001
	Island	-0.707	0.090	-7.864	< 0.001
Carrion beetles					
Whole species	(Intercept)	-0.511	0.236	-2.168	0.03
	Island	1.985	0.251	7.897	< 0.001
Medium species	(Intercept)	-0.051	0.316	-0.160	0.88
	Island	1.280	0.340	3.767	< 0.001
Large species	(Intercept)	-2.226	0.736	-3.023	0.003
	Island	3.536	0.745	4.758	< 0.001
Dung beetles					
Whole species	(Intercept)	2.686	0.048	56.330	< 0.001
	Island	1.776	0.052	34.450	< 0.001
Small species	(Intercept)	2.230	0.060	37.250	< 0.001
	Island	2.165	0.063	34.260	< 0.001
Large species	(Intercept)	1.680	0.079	21.319	< 0.001
	Island	0.031	0.111	0.276	0.78

Discussion

The current study demonstrated that an increase in sika deer at Lake Toya significantly changed both the abundance and species richness of beetle species belonging to three different taxonomic groups. The abundance and diversity index of carabid beetles at the island site were significantly lower than that at the lakeshore site (Figs 2, 4). In addition, we observed lower species richness and diversity indices at the island site; however they were not statistically significant. As carabid beetles utilize the forest floor as a habitat, the decline of understory vegetation because of overbrowsing is likely to result in the alteration of micro-climate conditions of their habitat, such as a decrease in humidity and an increase in light availability, and a higher mortality by predation (Rooney and Waller 2003, Melis et al. 2007, Cerda et al. 2015). For example, Melis et al. (2007) reported that shade tolerant and hygrophilous carabid species were negatively affected by moose browsing as browsing resulted in a decrease of bilberry coverage and changed the humidity and light intensity. Although we did not measure vegetation and other environmental variables, a significant decline in understory cover has been reported at the island site (Takahashi and Kaji 2001; Fig. 1B).

Table 2. Estimates of parameter differences between sites (reference = lakeshore site, i.e., coefficients of lakeshore site are zero), standard errors (SEs), z values, and p values in generalized linear models of each of taxonomic group and body size group species richness. Bold letters represent p < 0.05.

	Parameters	Estimates	SEs	z values	p values
Carabid beetles					
Whole species	(Intercept)	0.588	0.136	4.319	< 0.001
	Island	-0.139	0.200	-0.696	0.49
Small species	(Intercept)	-2.015	0.500	-4.028	< 0.001
	Island	-0.693	0.866	-0.800	0.42
Medium species	(Intercept)	0.210	0.164	1.276	0.20
	Island	0.196	0.222	0.882	0.38
Carrion beetles					
Whole species	(Intercept)	-0.836	0.277	-3.015	0.003
	Island	1.149	0.318	3.609	< 0.001
Medium species	(Intercept)	-1.003	0.302	-3.327	< 0.001
	Island	0.738	0.367	2.012	0.04
Large species	(Intercept)	-2.708	0.707	-3.830	< 0.001
	Island	2.197	0.745	2.948	0.003
Dung beetles					
Whole species	(Intercept)	0.758	0.125	6.061	< 0.001
	Island	0.426	0.161	2.651	0.008
Small species	(Intercept)	0.154	0.169	0.912	0.36
	Island	0.693	0.207	3.348	< 0.001
Large species	(Intercept)	-0.034	0.186	-0.183	0.86
	Island	-0.035	0.265	-0.132	0.90

Table 3. Estimates of parameter differences between sites (reference = lakeshore site, i.e., coefficients of lakeshore site are zero), standard errors (SEs), t values, and p values in generalized linear models of each of taxonomic group and body size group Shannon-Wiener diversity index. Bold letters represent p < 0.05.

	Parameters	Estimates	SEs	t values	p values
Carabid beetles	(Intercept)	0.841	0.105	7.995	< 0.001
	Island	-0.302	0.149	-2.030	0.05
Carrion beetles	(Intercept)	0.067	0.069	0.971	0.34
	Island	0.285	0.097	2.935	0.005
Dung beetles	(Intercept)	0.828	0.051	16.300	< 0.001
	Island	0.347	0.072	4.828	< 0.001

Conversely, carrion and dung beetles responded positively to deer overabundance, with abundance, species richness and the diversity index higher at the island site (Figs 2–4). Adult and larvae dung beetles utilize the faces of mammals as their main food resource (Andresen and Laurance 2007, Nichols et al. 2009). Hence, deer overabundance at the island site is likely to produce a greater quantity of deer faces, thereby facilitating a more abundant food source for dung beetles as compared to the lakeshore

Table 4. The indicator species values of each species. Bold letters represent $p < 0.05$.

	Taxa	Community	IndVals	*p* values
***Caccobius jessoensis* Harold**	**Scarabaeidae**	**island**	**0.988**	**0.001**
***Onthophagus ater* Waterhouse**	**Scarabaeidae**	**island**	**0.822**	**0.001**
***Eusilpha japonica* (Motschulsky)**	**Silphidae**	**island**	**0.604**	**0.001**
***Silpha perforata* Gebler**	**Silphidae**	**island**	**0.405**	**0.01**
***Pterostichus leptis* Bates**	**Carabidae**	**island**	**0.375**	**0.001**
***Liatongus phanaeoides* (Westwood)**		**island**	**0.345**	**0.001**
Nicrophorus quadripunctatus Kraatz	Silphidae	island	0.177	0.23
***Copris ochus* Motschulsky**	**Scarabaeidae**	**island**	**0.172**	**0.02**
Chlaenius pallipes Gebler		island	0.115	0.39
Pterostichus planicollis (Motschulsky)	Carabidae	island	0.103	0.11
Pterostichus yoritomus Bates	Carabidae	island	0.069	0.21
Pterostichus haptoderoides (Tschitscherin)	Carabidae	island	0.034	0.49
Chlaenius variicornis Morawitz		island	0.034	0.49
Lithochlaenius noguchii (Bates)		island	0.034	0.49
Pterostichus samurai (Lutshnik)	Carabidae	island	0.034	0.48
Hemicarabus tuberculosus (Dejean et Boisduval)		island	0.034	0.48
Oiceoptoma thoracicum (Linnaeus)		island	0.034	0.47
Pterostichus prolongatus Morawitz	Carabidae	island	0.031	0.74
***Synuchus* spp. Gyllenhal**	**Carabidae**	**lakeshore**	**0.827**	**0.001**
***Pterostichus thunbergi* Morawitz**	**Carabidae**	**lakeshore**	**0.806**	**0.001**
Geotrupes laevistriatus Motschulsky	Geotrupidae	lakeshore	0.477	0.56
***Leptocarabus arboreus* (Lewis)**	**Carabidae**	**lakeshore**	**0.355**	**0.001**
Trichotichnus longitarsis Morawitz	Carabidae	lakeshore	0.075	0.58
Pterostichus orientalis (Motschulsky)	Carabidae	lakeshore	0.065	0.50
Damaster blaptoides Kollar	Carabidae	lakeshore	0.065	0.50
Cychrus morawitzi Gehin		lakeshore	0.065	0.49
Leptocarabus opaculus (Putzeys)	Carabidae	lakeshore	0.032	1.00

site. Indeed, Kanda et al. (2005) reported a positive relationship between dung beetle abundance and deer density. In addition, as adult and larvae carrion beetles utilize animal carcasses as a food resource (Scott 1998, Dekeirsschieter et al. 2011), deer overabundance may provide greater abundance of food resources for carrion beetles. In support of these assertions, it has similarly been reported that a deer carcass increases the activity of necrophagous beetles (Melis et al. 2004).

Although small carabid species were not affected by deer overabundance, medium and large carabid species were negatively affected by deer overabundance (note: large carabid species were not sampled at the island site). This result suggests that larger carabid species are more sensitive to changing habitat condition than smaller species (Magura et al. 2006, Jelaska and Durbes˘ić 2009). Because the understory vegetation protects these species against extreme microclimate conditions (Ikeda et al. 2005), understory decline because of large herbivore overbrowsing results in a harsh ground floor environment for larger carabid species (Melis et al. 2007). Indeed, a higher susceptibil-

ity of larger species to other environmental changes, including habitat fragmentation, deforestation and urbanization, has been reported (Magura et al. 2006, Fujita et al. 2008).

Although the abundance of small dung beetle species was significantly higher at the island site than at the lakeshore site, that of large species did not differ between the two sites. This result suggests that small species are likely to favor a deer abundant environment. In contrast to our results, in Japan, Koike et al. (2014) observed that small species did not prefer environments with higher deer density. One possible reason for the difference would be the difference of dung usage among study species. Koike et al. (2014) found that small species tended to be dwellers which simply lay their eggs into the dung on the ground (Camberfort and Hanski 1991). Therefore, drying because of sunlight exposure, which results from deer overbrowsing, negatively affects these species. Conversely, in our study, the majority of small species were tunnelers, which are species burying dung under the ground before oviposition (Camberfort and Hanski 1991). Because of dung burying, tunnelers are not influenced by the drying of the ground surface.

In our study, NMDS showed a difference in species composition between the island and lakeshore sites (Fig. 5). In addition, indicator species analysis suggested that whereas beetle assemblage at the lakeshore site was characterized by carabid beetle species, that at the island site was characterized by carrion and dung beetle species (Table 4). These results, along with other reported findings indicate a biological homogenization of insect communities at the island site (McKinney and Lockwood 1999). In our study, large herbivore overabundance is likely to cause the homogenization of the beetle assemblage through the increased number of carrion and dung beetles and the decreased number of carabid beetles (Figs 2–4). Moreover, our findings suggest a shift in body size in insect communities; as the number of deer increase, insect communities are more likely to be dominated by smaller individuals.

Although our study brings a valuable contribution, the survey data is limited in its ability for generalizing the current results. Indeed, since the data used in this study was obtained from short term surveys with a limited number of samples (see also the species accumulation curves in Suppl. material 5: Figure 1), our results would provide only partial support for our hypotheses. Thus, further longitudinal studies must be needed to understand the ecological impacts of deer overabundance on insect communities more comprehensively.

Conclusions

In the present study, evidence is provided that an increase in deer population altered species richness, abundance and diversity of beetles within three different taxonomical groups. Whether such changes affect the ecosystem functions provided by these beetles is unknown. Nevertheless, the observed change in compositions of the three taxonomic beetles groups raises the potential that ecosystem functions may be altered through cascading effects (Larsen et al. 2005, Rouabah et al. 2014). To conserve and

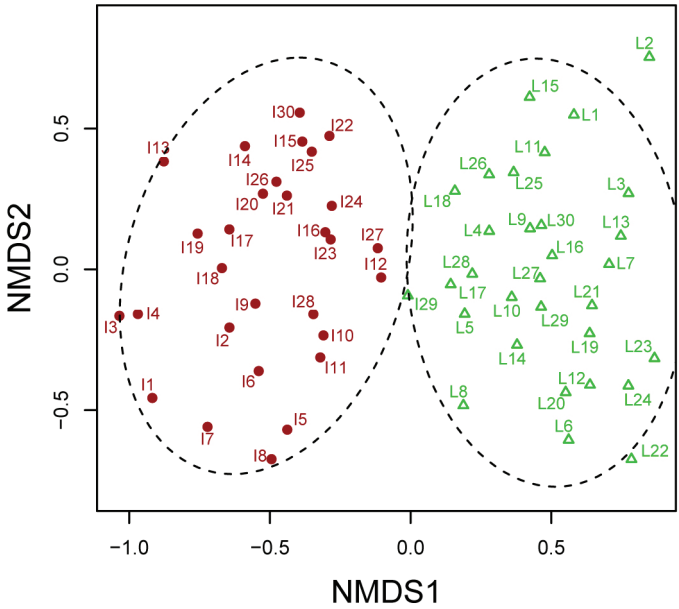

Figure 5. Non-metric multidimensional scaling ordination of each plot of the island and lakeshore sites. From I1 to I30: plots at the island site; from L1 to L30: plots at the lakeshore site. The differences of color and symbol represent the result of clustering.

maintain overall forest ecosystems, an investigation into the responses of other taxonomic species, including birds or amphibians, to the rapid increase in deer population and its impacts on ecosystem functions is required.

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References

Akaba S, Hino T, Yoshida T (2014) Impacts of high density of sika deer on dung beetle community. Japanese Journal of Applied Entomology and Zoology 58: 269–274. doi: 10.1303/jjaez.2014.269

Akashi N, Nakashizuka T (1999) Effects of bark-stripping by sika deer (Cervus nippon) on population dynamics of a mixed forest in Japan. Forest Ecology and Management 113: 75–82. doi: 10.1016/S0378-1127(98)00415-0

Allombert S, Stockton S, Martin J-L (2005) A natural experiment on the impact of overabundant deer on forest invertebrates. Conservation Biology 19: 1917–1929. doi: 10.1111/j.1523-1739.2005.00280.x

Andresen E, Laurance SGW (2007) Possible indirect effects of mammal hunting on dung beetle assemblages in Panama. Biotropica 39: 141–146. doi: 10.1111/j.1744-7429.2006.00239.x

Arellano L, Favila ME, Huerta C (2005) Diversity of dung and carrion beetles in a disturbed Mexican tropical montane cloud forest and on shade coffee plantations. Biodiversity and Conservation 14: 601–615. doi: 10.1007/s10531-004-3918-3

Bachand M, Pellerin S, Moretti M, Aubin I, Tremblay J-P, Côté SD, Poulin M (2014) Functional responses and resilience of boreal forest ecosystem after reduction of deer density. PLoS ONE 9: e90437. doi: 10.1371/journal.pone.0090437

Barton PS, Cunningham S a., Lindenmayer DB, Manning AD (2013) The role of carrion in maintaining biodiversity and ecological processes in terrestrial ecosystems. Oecologia 171: 761–772. doi: 10.1007/s00442-012-2460-3

Beguin J, Pothier D, Côté SD (2011) Deer browsing and soil disturbance induce cascading effects on plant communities: A multilevel path analysis. Ecological Applications 21: 439–451. doi: 10.1890/09-2100.1

Bressette JW, Beck H, Beauchamp VB (2012) Beyond the browse line: Complex cascade effects mediated by white-tailed deer. Oikos 121: 1749–1760. doi: 10.1111/j.1600-0706.2011.20305.x

Bush ER, Buesching CD, Slade EM, Macdonald DW (2012) Woodland recovery after suppression of deer: Cascade effects for small mammals, wood mice (Apodemus sylvaticus) and bank voles (Myodes glareolus). PLoS ONE 7: e31404. doi: 10.1371/journal.pone.0031404

Camberfort Y, Hanski I (1991) Dung beetle population biology. In: Hanski I, Camberfort Y (Eds) Dung Beetle Ecology. Princeton University Press, Princeton, 36–50.

Carpenter SR (1998) The need for large-scale experiments to assess and predict the response of ecosystems to perturbation. In: Successes, Limitations, and Frontiers in Ecosystem Science. Springer New York, New York, 287–312. doi: 10.1007/978-1-4612-1724-4_12

Cerda Y, Grez AA, Simonetti JA (2015) The role of the understory on the abundance, movement and survival of Ceroglossus chilensis in pine plantations: An experimental test. Journal of Insect Conservation 19: 119–127. doi: 10.1007/s10841-015-9752-y

Chollet S, Martin J-L (2013) Declining woodland birds in North America: Should we blame Bambi? Diversity and Distributions 19: 481–483. doi: 10.1111/ddi.12003

Côté SD, Rooney TP, Tremblay J-P, Dussault C, Waller DM (2004) Ecological impacts of deer overabundance. Annual Review of Ecology, Evolution, and Systematics 35: 113–147. doi: 10.1146/annurev.ecolsys.35.021103.105725

Dekeirsschieter J, Verheggen F, Lognay G, Haubruge E (2011) Large carrion beetles (Coleoptera, Silphidae) in Western Europe: A review. Les grands coléoptères nécrophages (Coleoptera, Silphidae) en Europe occidentale: Synthèse bibliographique 15: 435–447. http://www.scopus.com/inward/record.url?eid=2-s2.0-80052580984&partnerID=40&md5=c91f47becba8a56ffd60beacd96c1384

Dennis P, Young MR, Gordon IJ (1998) Distribution and abundance of small insects and arachnids in relation to structural heterogeneity of grazed, indigenous grasslands. Ecological Entomology 23: 253–264. doi: 10.1046/j.1365-2311.1998.00135.x

Foster CN, Barton PS, Lindenmayer DB (2014) Effects of large native herbivores on other animals. Journal of Applied Ecology 51: 929–938. doi: 10.1111/1365-2664.12268

Fujita A, Maeto K, Kagawa Y, Ito N (2008) Effects of forest fragmentation on species richness and composition of ground beetles (Coleoptera: Carabidae and Brachinidae) in urban landscapes. Entomological Science 11: 39–48. doi: 10.1111/j.1479-8298.2007.00243.x

Geospatial Information Authority of Japan (2015) Website of Fundamental Geospatial Data. http://www.gsi.go.jp/kiban/ [accessed 9 December 2015]

Gill RMA (1992) A review of damage by mammals in north temperate forests: 3. Impact on trees and forests. Forestry 65: 363–388. doi: 10.1093/forestry/65.4.363-a

Gómez JM, González-Megías A (2002) Asymmetrical interactions between ungulates and phytophagous insects: Being different matters. Ecology 83: 203–211. doi: 10.1890/0012-9658(2002)083[0203:AIBUAP]2.0.CO;2

Holt CA, Fuller RJ, Dolman PM (2011) Breeding and post-breeding responses of woodland birds to modification of habitat structure by deer. Biological Conservation 144: 2151–2162. doi: 10.1016/j.biocon.2011.05.004

Horsley S, Stout S, DeCalesta D (2003) White-tailed deer impact on the vegetation dynamics of a northern hardwood forest. Ecological Applications 13: 98–118. doi: 10.1890/1051-0761

Iida T, Soga M, Hiura T, Koike S (2016) Life history traits predict insect species responses to large herbivore overabundance: a multitaxonomic approach. Journal of Insect Conservation 20: 295–304. doi: 10.1007/s10841-016-9866-x

Ikeda H, Homma K, Kubota K (2005) Biotic and abiotic factors affecting the structures of ground invertebrate communities in Japanese cedar dominant forests. Eurasian Journal of Forest Research 8: 1–13. http://133.87.26.249/dspace/handle/2115/22186

Ikeda T, Takahashi H, Yoshida T, Igota H, Kaji K (2013) Evaluation of camera trap surveys for estimation of sika deer herd composition. Mammal Study 38: 29–33. doi: 10.3106/041.038.0103

Jelaska LS, Durbes˘ić P (2009) Comparison of the body size and wing form of carabid species (Coleoptera: Carabidae) between isolated and continuous forest habitats. Annales de la Société Entomologique de France 45: 327–338. doi: 10.1080/00379271.2009.10697618

Kaji K, Yajima T, Igarashi T (1991) Forage selection by deer introduced on Nakanoshima Island and its effect on the forest vegetation. In: Maruyama N, Bobek B, Ono Y, Regelin W, Bartos L, Ratcliffe PR (Eds) Proceedings of International Symposium on Wildlife Conservation, the 5th International Congress of Ecology (INTECOL 1990). Japan Wildlife Research Center, Tokyo, 52–55.

Kanda N, Yokota T, Shibata E, Sato H (2005) Diversity of dung-beetle community in declining Japanese subalpine forest caused by an increasing sika deer population. Ecological Research 20: 135–141. doi: 10.1007/s11284-004-0033-6

Kevan PG, Baker HG (1983) Insects as flower visitors and pollinators. Annual Review of Entomology 28: 407–453. doi: 10.1146/annurev.en.28.010183.002203

Koike S, Soga M, Nemoto Y, Kozakai C (2014) How are dung beetle species affected by deer population increases in a cool temperate forest ecosystem? Journal of Zoology 293: 227–233. doi: 10.1111/jzo.12138

Larsen TH, Williams NM, Kremen C (2005) Extinction order and altered community structure rapidly disrupt ecosystem functioning. Ecology Letters 8: 538–547. doi: 10.1111/j.1461-0248.2005.00749.x

Magura T, Tóthmérész B, Lövei GL (2006) Body size inequality of carabids along an urbanisation gradient. Basic and Applied Ecology 7: 472–482. doi: 10.1016/j.baae.2005.08.005

Martin J-L, Stockton SA, Allombert S, Gaston AJ (2010) Top-down and bottom-up consequences of unchecked ungulate browsing on plant and animal diversity in temperate forests: Lessons from a deer introduction. Biological Invasions 12: 353–371. doi: 10.1007/s10530-009-9628-8

Martin TG, Arcese P, Scheerder N (2011) Browsing down our natural heritage: Deer impacts on vegetation structure and songbird populations across an island archipelago. Biological Conservation 144: 459–469. doi: 10.1016/j.biocon.2010.09.033

McKinney ML, Lockwood JL (1999) Biotic homogenization: A few winners replacing many losers in the next mass extinction. Trends in Ecology & Evolution 14: 450–453. doi: 10.1016/S0169-5347(99)01679-1

Melis C, Sundby M, Andersen R, Moksnes A, Pedersen B, Røskaft E (2007) The role of moose *Alces alces* L. in boreal forest – the effect on ground beetles (Coleoptera, Carabidae) abundance and diversity. Biodiversity and Conservation 16: 1321–1335. doi: 10.1007/s10531-005-6230-y

Melis C, Teurlings I, Linnell JDC, Andersen R, Bordoni A (2004) Influence of a deer carcass on Coleopteran diversity in a Scandinavian boreal forest: A preliminary study. European Journal of Wildlife Research 50: 146–149. doi: 10.1007/s10344-004-0051-2

Miyaki M, Kaji K (2009) Shift to litterfall as year-round forage for sika deer after a population crash. In: McCullough DR, Takatsuki S, Kaji K (Eds) Sika Deer. Springer Japan, Tokyo, 171–180. doi: 10.1007/978-4-431-09429-6_12

Mysterud A, Aaserud R, Hansen LO, Åkra K, Olberg S, Austrheim G (2010) Large herbivore grazing and invertebrates in an alpine ecosystem. Basic and Applied Ecology 11: 320–328. doi: 10.1016/j.baae.2010.02.009

Nichols E, Gardner Ta, Peres Ca, Spector S (2009) Co-declining mammals and dung beetles: An impending ecological cascade. Oikos 118: 481–487. doi: 10.1111/j.1600-0706.2009.17268.x

Nuttle T, Ristau TE, Royo AA (2014) Long-term biological legacies of herbivore density in a landscape-scale experiment: Forest understoreys reflect past deer density treatments for at least 20 years. Journal of Ecology 102: 221–228. doi: 10.1111/1365-2745.12175

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H (2015) vegan: Community Ecology Package. R package version 2.3-1. http://CRAN.R-project.org/package=vegan [accessed 5 November 2015]

Rainio J, Niemelä J (2003) Ground beetles (Coleoptera: Carabidae) as bioindicators. Biodiversity and Conservation 12: 487–506. doi: 10.1023/A:1022412617568

R Core Team (2015) R ver. 3. 2. 1: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

Roberts DW (2015) labdsv: Ordination and Multivariate Analysis for Ecology. R package version 1.7-0. http://CRAN.R-project.org/package=labdsv [accessed 19 November 2015]

Rooney TP (2009) High white-tailed deer densities benefit graminoids and contribute to biotic homogenization of forest ground-layer vegetation. Plant Ecology 202: 103–111. doi: 10.1007/s11258-008-9489-8

Rooney TP, Waller DM (2003) Direct and indirect effects of white-tailed deer in forest ecosystems. Forest Ecology and Management 181: 165–176. doi: 10.1016/S0378-1127(03)00130-0

Rouabah A, Lasserre-Joulin F, Amiaud B, Plantureux S (2014) Emergent effects of ground beetles size diversity on the strength of prey suppression. Ecological Entomology 39: 47–57. doi: 10.1111/een.12064

Scott MP (1998) The ecology and behavior of burying beetles. Annual Review of Entomology 43: 595–618. doi: 10.1146/annurev.ento.43.1.595

Speight MR, Hunter MD, Watt AD (2008) Ecology of Insects. Concepts and Applications. 2nd ed. Wiley-Blackwell, Oxford, 640 pp.

Stewart AJA (2001) The impact of deer on lowland woodland invertebrates: A review of the evidence and priorities for future research. Forestry 74: 259–270. doi: 10.1093/forestry/74.3.259

Suttiprapan P, Nakamura H (2007) Species composition and seasonal abundance of carabid beetles by three sampling methods on the campus of the faculty of agriculture, Shinshu University. Japanese Journal of Environmental Entomology and Zoology 18: 83–90.

Sumways MJ (2007) Insect Conservation Biology (1st edn). Springer Netherlands, Heidelberg, 358 pp.

Suominen O, Niemelä J, Martikainen P, Niemelä P, Kojola I (2003) Impact of reindeer grazing on ground-dwelling Carabidae and Curculionidae assemblages in Lapland. Ecography 26: 503–513. doi: 10.1034/j.1600-0587.2003.03445.x

Takahashi H, Kaji K (2001) Fallen leaves and unpalatable plants as alternative foods for sika deer under food limitation. Ecological Research 16: 257–262. doi: 10.1046/j.1440-1703.2001.00391.x

Takatsuki S (2009) Effects of sika deer on vegetation in Japan: A review. Biological Conservation 142: 1922–1929. doi: 10.1016/j.biocon.2009.02.011

Tanentzap AJ, Bazely DR, Koh S, Timciska M, Haggith EG, Carleton TJ, Coomes DA (2011) Seeing the forest for the deer: Do reductions in deer-disturbance lead to forest recovery? Biological Conservation 144: 376–382. doi: 10.1016/j.biocon.2010.09.015

Ueno S, Kurosawa Y, Sato M (1985) The coleoptera of Japan in color, vol. 2. Hoikusha publishing, Osaka, 514 pp.

Wheatall L, Nuttle T, Yerger E (2013) Indirect effects of pandemic deer overabundance inferred from caterpillar-host relations. Conservation Biology 27: 1107–1116. doi: 10.1111/cobi.12077

White MA (2012) Long-term effects of deer browsing: Composition, structure and productivity in a northeastern Minnesota old-growth forest. Forest Ecology and Management 269: 222–228. doi: 10.1016/j.foreco.2011.12.043

Yamada H, Takatsuki S (2015) Effects of deer grazing on vegetation and ground-dwelling insects in a larch forest in Okutama, Western Tokyo. International Journal of Forestry Research 2015: 1–9. doi: 10.1155/2015/687506

Supplementary material 1

Table 1
Authors: Taichi Iida, Masashi Soga, Shinsuke Koike
Data type: Data table
Explanation note: A list of beetle species observed in our study.
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Supplementary material 2

Table 2
Authors: Taichi Iida, Masashi Soga, Shinsuke Koike
Data type: Data table
Explanation note: Estimated and observed values of beetle abundance at the island and lakeshore sites.
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Supplementary material 3

Table 3
Authors: Taichi Iida, Masashi Soga, Shinsuke Koike
Data type: Data table
Explanation note: Estimated and observed values of beetle species richness at the island and lakeshore sites.
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Supplementary material 4

Table 4

Authors: Taichi Iida, Masashi Soga, Shinsuke Koike

Data type: Data table

Explanation note: Estimated and observed values of beetle diversity (Shannon-Wiener diversity index) at the island and lakeshore sites.

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Supplementary material 5

Figure 1

Authors: Taichi Iida, Masashi Soga, Shinsuke Koike

Data type: Figure

Explanation note: Species accumulation curves for carabid, carrion and dung beetles at the island (blue lines) and lakeshore sites (red lines).

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RESEARCH ARTICLE

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New species of *Teratolytta* Semenov, 1894 from Turkey and a key to the females (Coleoptera Meloidae)

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Abstract

Teratolytta krejciki **sp. n.** from Munzur range (E Turkey) and a female belonging to a possible new species from the eastern Pontus range (N Turkey) are described and figured. New records of *T. gentilis* from southern Turkey are also provided. Difficulties to recognize females of this genus are discussed and a tentative key to the females of all species is proposed with the addition of a photographic plate.

Keywords

Anatolia, key, new species, taxonomy, *Teratolytta krejciki* sp. n.

Introduction

The blister beetle genus *Teratolytta* Semenov, 1894 was revised by Bologna and Di Giulio (2006) who described four new species and the first instar larva (never studied in this genus), synthesized the information on ecology and ethology, included a key to males, proposed some taxonomic changes and a classification of the genus divided in two sections with five groups of species. Since this study only a faunistic and ecological contribution on *T. kulzeri* from Turkey has been published (Kemal and Koçak 2011). Females of this genus are extremely difficult to identify if they are not associated with males, which is why the key of the mentioned revision was based on the males only.

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The genus is biogeographically centered in the Near and Middle East and shows the richest diversity in the Anatolian peninsula, where 10 out of 17 species occur. Just ten years after the revision of the genus a new species from eastern Turkey is described and a possible new species from northern Turkey is briefly discussed and figured in this work. These novelties increase the diversity of this genus in Anatolia.

Aims of this paper are: a) to describe and figure *Teratolytta krejciki* sp. n.; b) to briefly describe and figure a possible new species from eastern Pontus; c) to publish new records for *T. gentilis*; d) to provide a tentative key to females of the genus, enriched by a photographic synoptic table.

Results

***Teratolytta krejciki* sp. n.**
http://zoobank.org/FD38A467-B10F-4582-BE20-A53200E6568C
Figs 1–3

Type material. Holotype ♂ (S. Krejcik collection), labelled "26–27.6.2009; TR; Turkey, Pülümür, 2–7 km NW Pülümür, T. Tichny; 1550-2100 m; Tunceli". A second label specifies "*Teratolytta* sp. det. Stanislav Krejcik 2011-2". The holotype lacks the last right protarsomere.

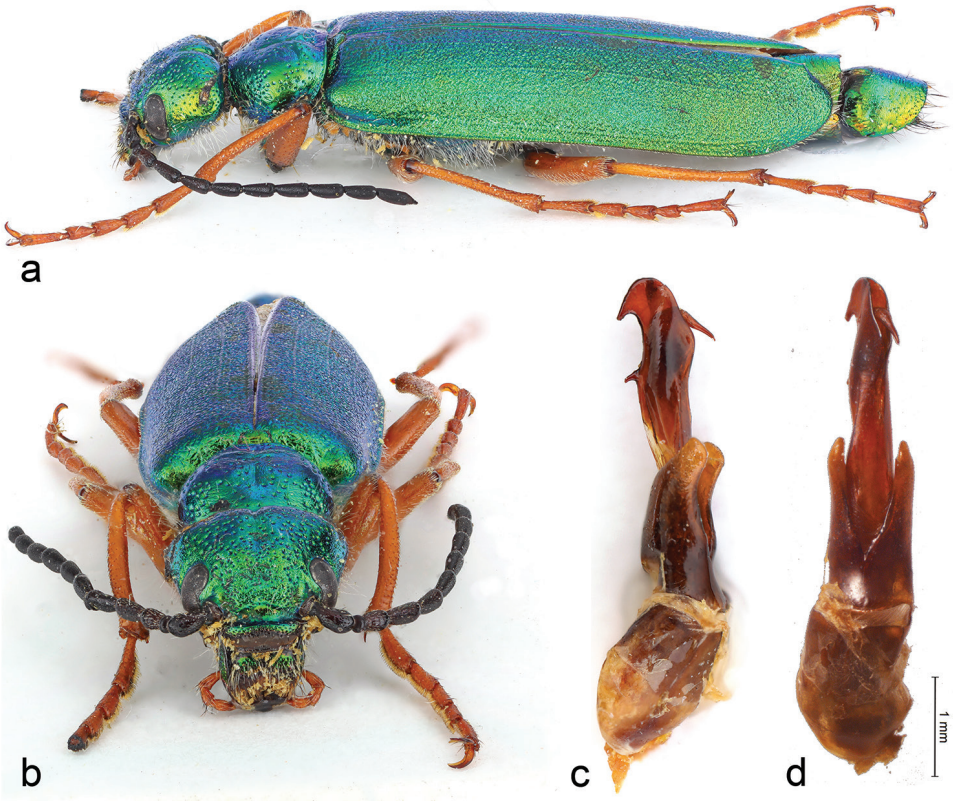
Type locality. "2–7 km NW Pülümür, T. Tichny; 1550–2100 m". Pülümür is a small village of the Tunceli vilayet (province), in the eastern Turkey, placed at base of the southern slope of the Pülümür geçidi (pass), along the Munzur dağları (range). This locality is characterized by subtermophilic pastures and sparse woodlands.

The single specimen was collected early in the morning in a sparsely vegetated flood zone just north of Pülümür village. Although this locality has a very rich fauna of blister beetles and we collected about 30 species of meloids, other attempts to collect further specimens of *Teratolytta krejciki* in the same area during subsequent years (May, June) were not successful.

Diagnosis. Large sized and slender species (22 mm) (Figs 1, 2a) belonging to the Section I as defined by Bologna and Di Giulio (2006). Body monochromatic metallic green, but legs red with metallic green coxa and black trochanter. Setation short, black on sides of pronotum and head, ventrally long and white. Head puncturation scattered, surface shiny. Sexual dimorphism not evaluable, female unknown. Male mesotibia not modified at apex but deeply curved on the apical half of inner side, and with microtubercles on external side. Male mesotarsomere I not modified and without modified black setae. Male metatrochanters triangularly elongate without modified apical setae; pro- and mesotibiae with two apical spurs, outer metatibial spur very large; protibiae only slightly curved. Parameres with robust apical lobes; aedeagus with two apical hooks greatly distanced to each other; endophallic hook slightly curved at apex.

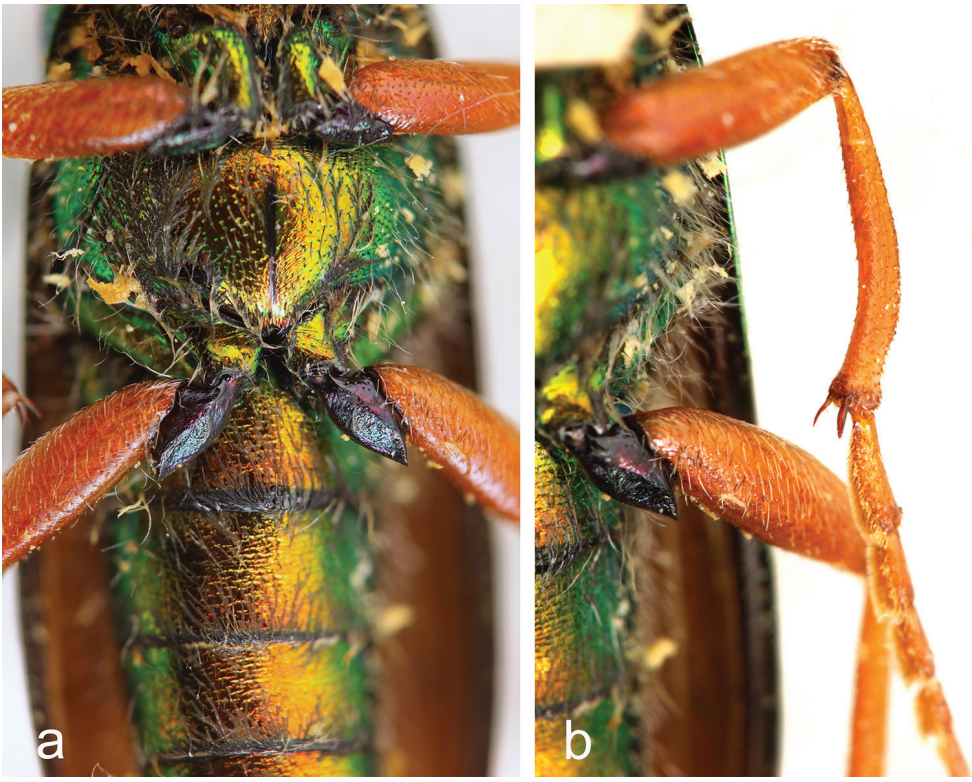
Description. *Body* (coxae included) shiny metallic green (Figs 1, 2a–b), abdomen metallic cupreous with posterior margin of sterna strictly black (Fig. 3a) and last two

Figure 1. *Teratolytta krejciki* sp. n., habitus, male, dorsal view.



Figures 2. *Teratolytta krejciki* sp. n., male: **a** habitus lateral view **b** head, frontal view **c** tegmen and aedeagus, lateral view **d** tegmen and aedeagus, ventral view.

urites metallic green; maxillary palpi orange-red as well as legs, but coxae metallic green and trochanters black; mouthparts black, antennae subopaque black. Setation dorsally very short, particularly on elytra, black and slightly longer on the pronotum sides, genae and mouthparts. Modified setae of male last abdominal urite black. Body length (apex of mandibles to apex of elytra) 21.70 mm; head maximum width 3.74 mm; pronotum length 2.77 mm, width 3.25 mm; elytral greatest width at posterior third 5.46 mm. *Head* short subtrapezoidal (Fig. 2b), distinctly wider than long, maximum width at temples; sides of head obliquely narrowed from base to eyes; frons transversely depressed before the suture, convex in the middle and slightly depressed at level of the posterior margin of eyes; mandibles short, robust and curved; temples convex without postocular depression; clypeus convex; labrum slightly depressed and slightly emarginated at fore margin, with moderately deep scattered punctures, surface between punctures shiny; frontal suture almost straight; maxillary and labial palpomeres slender; last maxillary palpomeres longer than penultimate; antennae extending to basal third of elytra (Fig. 1, 2a); antennomere I about twice as long as II, subequal to III; III–X elongate, cylindrical; III slightly longer than the following; XI 1.5 times as long



Figures 3. *Teratolytta krejciki* sp. n., male: **a** metasternum and metatrochanters **b** mesotibiae and mesotarsomeres.

as X, cylindrical, narrowing in the apical third; antennomeres I-III with longer black setae. *Pronotum* (Fig. 1) shortly transverse, almost hexagonal, maximal width at middle, wider than long slightly depressed longitudinally in middle, slightly depressed transversally along the base; pronotal punctures as on head or slightly sparser laterally. Scutellum wide, subquadrate, with round and slightly depressed apex. Elytra elongate, feebly convex, narrowly rounded at apex, with vague traces of venation, uniformly rugose, setation extremely short. *Metasternum* without tubercles (Fig. 3a). Tibiae of all legs with two spurs, both slender and pointed on pro- and mesotibiae (Fig. 3b); spurs of metatibiae robust, the inner pointed, the outer very large, subtruncate apically; male tibiae of all legs cylindrical, not modified at apex, with simple setation and with spiniform and obtuse mixed tubercles on external side, mesotibiae without supplementary spine-like brush of setae, greatly curved on inner side in the posterior half (Fig. 3b), metatibiae slightly curved on inner side; male mesotarsomere not modified (Fig. 3b), tarsomere II not modified, with regular setae; male metatrochanters simple, triangularly elongate without modified apical setae (Fig. 3a). Last visible sternite of male abdomen emarginated, with modified apical setae on both sides, shorter than the entire sternite. *Parameres* (Fig. 2c–d) robust and with robust apical lobes; aedeagus with two

apical hooks, distinctly distanced, different in shape and size, distal one smaller than proximal one (Fig. 2c); endophallus hook straight, acutely and shortly curved apically.

Etymology. The new species is named after Stanislav (Standa) Krejčík, an active Czeck entomologist and excellent photographer, very interested in blister beetles, who, after recognizing the novelty of this *Teratolytta,* kindly sent us the single specimen of this new species with some nice photos (Figs 1, 2, 3a).

Taxonomic remarks. According to Bologna and Di Giulio (2006) two sections are recognized in the genus, based on the absence (section I), or presence (section II) of two tubercles on metasternum, just posterior to the base of the middle legs. *Teratolytta krejciki* clearly belongs to section I due to certain male symplesiomorphic features such as lack of tubercles on metasternum and presence of two spurs on pro- and mesotibiae. This species is immediately distinct from *T. gentilis* group because mesotibiae and mesotarsomeres are not modified, and from *T. pilosella* group by lack of depression on males genae. It differs from *T. klapperichi* group because of the presence of two pro- and mesotibial slender spurs and two big aedeagal hooks, and from *T. kaszabi* group because of the distanced aedeagal hooks.

The new species does not shows any peculiar modification on male mesotibiae, mesotarsomeres, metathorax, which are present in several *Teratolytta* species (Bologna and Di Giulio 2006), a condition similar to *T. carlae*, but this last species greatly differs from *T. krejciki* at least because of the following features: smaller size and stout body shape; body colour, metallic blue or metallic green with a cupreous stripe; aedeagal hooks both at apex and smaller.

Teratolytta sp. A (possible new species related to *T. carlae* Bologna, 2006)

Fig. 4a

Material examined. 1 ♀ (M.A. Bologna collection), labelled "Turkey 12. Gümüṣhane, 14 km N Kelkit, 6 km N Ülüpinar, 40.1533N -39.2847E, 1850–2150 m, 28.6.2013, M.A. Bologna, P. Rapuzzi & P. Audisio" (by pitfall traps positioned 30 days before).

This locality is placed on the southern slope of the eastern Pontus range, in a sub-mesophilic mountain habitat, characterized by a mosaic of *Quercus* woodlands and pastures, with *Crataegus* and other Rosaceae in the ecotonal borders.

Diagnostic characters and comparative analysis. Middle sized species (Fig. 4a), length 17 mm; body integument metallic green but head, pronotum (except base, which is green), one very wide longitudinal stripe along each elytron, meso- and metathoracic sternites and abdominal sternites metallic cupreous; clypeus, labrum, mandibles and antennae black; maxillary and labial palpomeres respectively light and dark red-orange, last maxillary palpomere black at apex; legs red-orange, but coxae, trochanters, apex of femurs and base of tibiae black.

Head transversely trapezoidal, with dense and deep punctures, slightly depressed in front; black elongate setae on temples. Antennae short (Fig. 4a), extending to or a little beyond the base of pronotum; antennomeres short, particularly V-VII. *Pronotum* transversely subexagonal (Fig. 4a), maximal width just anterior to the middle, sides

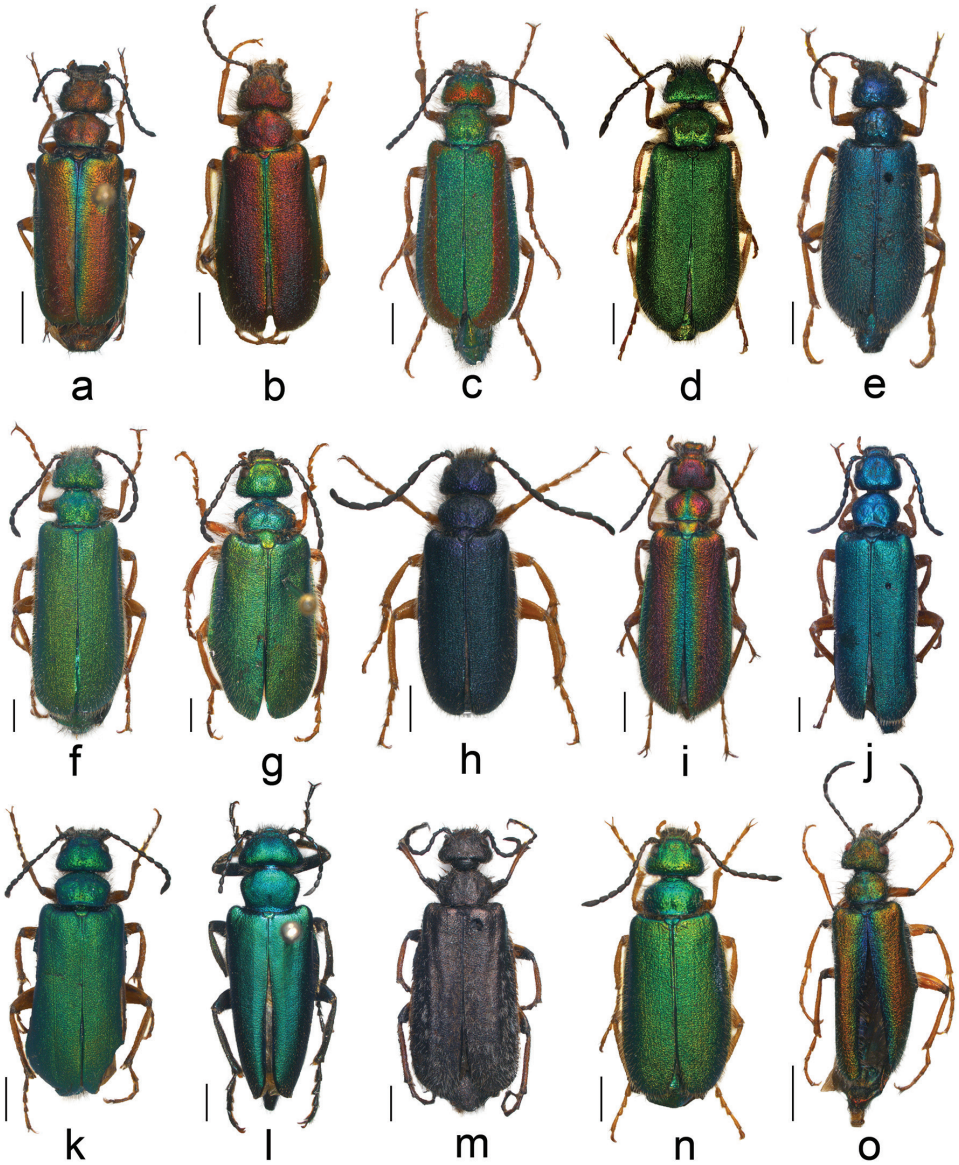


Figure 4. Females of the genus *Teratolytta*: **a** *Teratolytta* sp. A (sp. n. ?) **b** *T. carlae* **c** *T. dives*, striped phenotype **d** *T. dives*, unicoloured phenotype **e** *T. eylandti* **f** *T. flavipes* **g** *T. gentilis*, typical phenotype **h** *T. gentilis*, blue phenotype **i** *T. kaszabi* **j** *T. klapperichi* **k** *T. monticola* **l** *T. optabilis* **m** *T. pilosella* **n** *T. taurica* **o** *T. vanensis*. Scale bars 2 mm.

subrounded in the basal half; in the middle with a slight mid-longitudinal depression; punctures slightly sparser than on head. Scutellum subrectangular, rounded at apex, with long black setae particularly on sides. Elytra weakly convex only on the basal third, subrugose, with long whitish setae, denser posteriorly and on lateral margins.

Ventral side with dense and long whitish setation. *Legs* not modified; tibiae straight; all legs with two tibial spurs, fore and middle slender, posterior spurs spatuliform, external spur wider; claws distinctly curved.

The single female does not correspond to any described species and possibly represents a new undescribed species. We prefer not to describe it and wait for the discovery of the male.

Six other species in both Section I and II have a cupreous longitudinal stripe on the elytra: (a) *Teratolytta carlae* Bologna, 2006 (Fig. 4b); *T. kaszabi* Kryzhanovskij, 1959 (Fig. 4i); *T. regina* Kaszab, 1958; (b) *T. dives* (Brullé, 1832) (Fig. 4c), *T. tricolor* (Haag-Rutenberg, 1880), *T. vanensis* Kaszab, 1968 (Fig. 4o). Species A differs from the species in Section II because it has a narrower cupreous elytral stripe, metallic green head and pronotum, distinctly longer antennae, extending to the fore third of elytra, more slender and elongate antennomeres V-VII. Moreover in *T. dives* and *T. tricolor* the pronotum is wider in front, slightly concave, not rounded with whitish setae and head and pronotum punctures are bigger. Due to the more expanded black coloration of knees and black setation on pronotum and head, the probable new species is more similar to *T. vanensis*, which differs by its notably smaller size, narrower pronotum and distinctly longer antennae.

Comparing this new *Teratolytta* species with other striped species of Section I, *T. kaszabi* and *T. regina*, have slightly longer antennae, similar body size and both are distributed in Central Asia. Moreover, *T. kaszabi* has red unicolour knees and more hexagonal and narrower pronotum. The probable new species is similar to *T. carlae* in colour of setae on head and pronotum, colour of knees and body and the length of antennae, but differs by smaller size, less slender body, and slightly widened posterior portion of elytra . The striped form in *T. carlae* is a variant and other specimens are uniformly blue.

Teratolytta gentilis (Frivaldszky, 1877)

Fig. 4g–h

New records. (Eğirdir) Yukangökdere, 37.42964N, -30.49899E, Kasnak forest, window-trap 17, Hollow *Quercus*, 17.5.2007, N. Jonsson & M. Avci (Konya), Güneyyurt Km 2 SE, 1.6.2011, F, Angelini (Antalya) Akseki, 1500 m, 22.5.1997, P. Rapuzzi. All specimens are housed at the M. A. Bologna collection (University Roma Tre).

These new records improve the distribution of this Anatolian species in southern Turkey and confirm a doubtful record from Antalya province cited by Bologna and Di Giulio (2006).

Key to the females of the genus *Teratolytta* (see Fig. 4)

In the revision of the genus (Bologna and Di Giulio 2006), the identification key was provided only for males due to the difficulties in detecting distinctive characters of females. In the following key we tentatively distinguish the females of all species except *T. dvoraki* and *T. krejciki*, which are still unknown. The key is mostly based on the

colour of body parts, because we did not find diagnostic characters such as in males. In order to help with the identification of females, a colour plate (Fig. 4) of most of species is provided except *T. dvoraki*, *T. krejciki*, and *T. regina* and *T. tricolor*, which were unavailable.

1 Elytra metallic green with a longitudinal, more or less widened cupreous stripe **2**

– Elytra unicolourous **8**

2 Antennae distinctly extending to the basal third of elytra; cupreous stripe narrow (Fig. 4c, o); pronotum metallic green; middle antennomeres (V–VIII) slender cylindrical **3**

– Antennae extending to the base of pronotum or only slightly beyond; cupreous stripe wide (Fig. 4b, i); pronotum cupreous; middle antennomeres (V–VIII) obtusely cylindrical **5**

3 Pronotum maximal width in the apical third, distinctly subhexagonal, sides slightly concave at basal half **4**

– Pronotum maximal width at middle, not subhexagonal, sides slightly convex externally at basal half ..... ***T. vanensis* Kaszab, 1968**

4 Species distributed in Balkans an Anatolia ..... ***T. dives* (Brullé, 1832)** (pars)

– Species distributed in NE Iran and SW Turkmenistan ..... ***T. tricolor* (Haag-Rutenberg, 1880)**

5 Apex of femur and base of tibia uniformly red-orange ..... ***T. kaszabi* Kryzhanovskij, 1959**

– Apex of femur and base of tibia more or less extensively black **6**

6 Antennae distinctly extending beyond the base of pronotum, antennae elongate. Species distributed in Afghanistan ..... ***T. regina* Kaszab, 1958**

– Antennae short (Fig. 4a), extending to or a little beyond the base of pronotum, antennomeres short. Species distributed in Turkey **7**

7 Body stout, posterior third of elytra slightly widened ..... ***T. carlae* Bologna, 2006** (pars)

– Body slender, the posterior third of elytra parallel ..... ***Teratolytta* sp. A**

8 Head, pronotum and elytra dark bronze ..... ***T. pilosella* (Solsky, 1881)**

– Body colour different **9**

9 Head, pronotum and elytra dark metallic blue **10**

– Head, pronotum and elytra metallic green or green-bluish, not uniformly dark blue **12**

10 Apex of femur and base of tibia uniformly red-orange ..... ***T. gentilis* (Frivaldszky, 1877)** (pars)

– Apex of femur and base of tibia black **11**

11 Head and pronotum subopaque; antennomeres III-VI short ..... ***T. carlae* Bologna, 2006** (pars)

– Head and pronotum shiny; antennomeres III-VI elongate ..... ***T. eylandti* Semenow, 1894**

12 Legs black; dorsal body surface sparsely micropunctate, subopaque .... ***T. optabilis*** **(Falderman, 1832)**

– Legs totally or partially red-orange; dorsal body surface with middle sized punctures, more or less scattered, shiny..... **13**

13 Apex of femur and base of tibia black; pronotum transverse, subhexagonal, slightly wider than head at temples ..... ***T. monticola*** **Bologna, 2006**

– Apex of femur and base of tibia red-orange or base of tibia vaguely dark; pronotum variously shaped **14**

14 Pronotum not transverse, slightly narrower than head width at temples, sides subrounded or parallel, or slightly angulate..... **15**

– Pronotum clearly transverse, slightly wider than head at temples, notably subtrapezoidal, sides distinctly angulate **18**

15 Head distinctly depressed in the middle; middle antennomeres with vague green-blue metallic reflections. Species distributed in Afghanistan .... ***T. klapperichi*** **Kaszab, 1958**

– Head not distinctly depressed in the middle; antennomeres black, subopaque, except for black metallic reflection of antennomere I. Species distributed in Turkey and Syria **16**

16 Pronotum with sparse punctation; pronotal sides arcuate subrounded; dorsal surface greenish-blue; head and pronotal setae mostly black, mixed with short whitish setae, dorsal elytral setae black ..... ***T. kulzeri*** **Kaszab, 1958**

– Pronotum with dense punctuation and in some areas subrugose; pronotal sides subparallel or slightly angulate; dorsal surface distinctly green; head and pronotal setae white and long, dorsal elytral setae white..... **17**

17 Hind trochanter red-orange..... ***T. senilis*** **(Abeille de Perrin, 1895)**

– Hind trochanter black .... ***T. flavipes*** **(Mulsant & Rey, 1858) and** ***T. dives*** **(Brullé, 1832)** (pars)

18 Pronotum with sparse punctation, basal margin straight; antennomere I black; dorsal elytral setae sparse; mesotibiae slightly curved..... ..... ***T. taurica*** **Bologna, 2006**

– Pronotum with dense punctuation, basal margin slightly sinuate in the middle; antennomere I with metallic violet-cupreous reflections; dorsal elytral setae denser; mesotibiae distinctly curved..... ***T. gentilis*** **(Frivaldszky, 1877)** (pars)

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References

Bologna MA, Di Giulio A (2006) Revision of the genus *Teratolytta* (Coleoptera: Meloidae). European Journal of Entomology 103: 137–161. doi: 10.14411/eje.2006.019

Kemal M, Koçak AO (2011) *Teratolytta kulzeri*, a little known Blister Beetle from East Turkey (Coleoptera, Meloidae). Cesa News 64: 7–11.

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RESEARCH ARTICLE

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Occurrence of cavernicolous ground beetles in Anhui Province, eastern China (Coleoptera, Carabidae, Trechinae)

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Abstract

Two new species of anophthalmic ground beetles belonging to the subfamily Trechinae are described: *Cimmeritodes* (*Zhecimmerites*) *parvus* Tian & Li, **sp. n.** and *Wanoblemus wui* Tian & Fang, **gen. n., sp. n.** Both were discovered in the limestone caves of Anhui Province in eastern China. *C.* (*Z.*) *parvus* was found in caves Ziwei Dong, Xianren Dong and Qingtai Dong, whereas *W. wui* was discovered in cave Baiyun Dong. This is the first record of cavernicolous ground beetles in Anhui Province, eastern China.

Keywords

Carabid beetle, new genus, new species, troglobitic

Introduction

Trechine beetles are the most speciose group of insects in cave fauna (Moldovan 2012) and are distributed across every continent (Casale et al. 1998). Within the tribe Trechini, the phyletic series (Jeannel 1928; Casale and Laneyrie 1982; Casale et al. 1998) or complex (Uéno and Pawlowski 1981) *Trechoblemus* appears to be among the most diverse groups, comprising about 300 known species belonging to 16 genera. The first

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genus of this series described in China, *Cimmeritodes*, is represented by a single species endemic to the Hunan Province (Deuve 1996). The genera *Wulongoblemus* and *Microblemus* were newly described in the Zhejiang Province (Uéno 1997), whereas the endemic genera *Balazucellus* and *Sinocimmerites* were described from the Hubei and Fujian Provinces, respectively (Deuve 2001, 2007). More recently, Tian and Yin (2013) reported *Sidublemus* from the Hunan Province and Deuve and Tian (2015) described the new subgenus *Zhecimmerites* (*Cimmeritodes*) from eastern Zhejiang.

More than 600 caves are located in Anhui Province (eastern China), the majority of which spans both banks of the Yangtze River (Lu 2012). Unlike the karstic landscape characteristic of southern China provinces, the limestone landforms of Anhui Province are regularly distributed. Their geographic scale and relevant topographic features are primarily influenced by stratum's lithology and structure, as well as by hydrogeological conditions (Sun et al. 2000). Despite the presence of suitable cavernicolous environments, no trechine ground beetles have been recorded in Anhui so far (Tian et al. 2016).

In 2015, 11 anophthalmic trechine beetles were collected from caves in the Chaohu–Wuwei–Xuancheng karstic areas, as part of a series of biological surveys conducted in Anhui's caves. Further studies confirmed that these beetles belonged to two taxa not recorded in this Province: the subgenus *Zhecimmerites* (genus *Cimmeritodes*) and the new genus *Wanoblemus* gen. n., which is proposed in this paper. This is the first report of cavernicolous trechine beetles in Anhui Province.

Material and methods

Specimens were collected in cave by aspiration and kept in 55% ethanol until their dissection and observation under a Leica S8AP0 stereomicroscope. Dissected genital pieces, including the median lobe and the parameres of the aedeagus, were glued onto small transparent plastic plates and pinned beneath the specimen to which they belonged. Habitus were photographed using a Keyence VHX-5000 digital microscope and the genital pieces were photographed using a Canon EOS 40D camera connected to a Zeiss AX10 microscope. Photographs were then stacked and processed using Adobe Photoshop CS5 software. Distribution maps were obtained in MapInfo.

Body length was measured from the apex of the right mandible (in the open position) to the elytral apex; body width corresponded to the maximum width of the elytra.

Abbreviations of other measurements are as follows, following Tian et al. (2016):

HLm head length including mandibles, measured from the apex of the right mandible to the occipital suture;

HLl head length excluding mandibles, measured from the front of the labrum to the occipital suture;

HW maximum head width;

PnL pronotum length along the median line;

PnW maximum pronotum width;
PfW pronotum width at front;
PbW pronotum width at base;
EL elytra length, from base of scutellum to elytral apex;
EW maximum width of combined elytra.

Taxonomy

***Cimmeritodes* (*Zhecimmerites*) *parvus* Tian & Li, sp. n.**
http://zoobank.org/F0002E70-E282-4E32-8D43-90393C99344A
Figs 1–3

Material. Holotype, male, Ziwei Dong (also called Shuangjing Dong) Cave, Chaohu Shi, 31.6479N, 117.8632E, 83 m altitude, IV-21-2015, leg. Yunhe Wu and Wenbo Li, deposited in the insect collections of South China Agricultural University (SCAU), Guangzhou. Paratypes: one male and three females, ibid., deposited in SCAU and the animal collections of Anhui University (ANU), Hefei, respectively.

Additional material. One male, Cave Xianren Dong, suburbs of southern Chaohu Shi, 31.4633N, 117.8413E, 110 m altitude, IV-27-2015, leg. Yunhe Wu and Wenbo Li, deposited in SCAU; one female, Boshan Dong Cave, Xiaboshan, 38 km SW of the main town of Wuwei County, 31.1851N, 117.5582E, 28 m altitude, IV-28-2015, leg. Yunhe Wu and Wenbo Li, deposited in SCAU; two males, Qingtai Dong Cave, Qingtaishan, NW Shijian Zhen, Wuwei County, 31.5330N, 117.0244E, 79 m altitude, IV-22-2015, leg. Yunhe Wu and Wenbo Li, deposited in SCAU; one female, Boshan Dong Cave, Xiaboshan, Shushan Zhen, Wuwei County, 31.1851N, 117.5582E, 28 m altitude, IV-28-2015, leg. Yunhe Wu and Wenbo Li, in SCAU.

Diagnosis. Small-sized, anophthalmic trechine beetles, with yellowish brown and stout body, short antennae and legs, fore body distinctly shorter than elytra.

Description. Length: 3.0–3.7 mm, including mandibles (or 2.8–3.5 mm, excluding mandibles); width: 1.0 mm. Habitus as in Fig. 1.

Small-sized trechine species. Body yellowish brown and palps, antennae, and tarsi pale; moderately shiny; finely pubescent though frons; vertex, ventral head, and prosternum glabrous; micro sculptural meshes moderately isodiametric on head, moderately transverse on pronotum, and strongly transverse on elytra. Mandibles much shorter than elytra, EL/(HLm + PnL) = 2.31, in the front part of the body

HLm/HW = 1.22, HLl/HW= 0.94, right mandible tridentate, median mandible reduced; labial suture absent; submentum 6-setose; mentum bisetose, without basal pits; tooth short, bifid at apex; palps short and stout; labial palpomere 2 longer than 3, which is very thin, bisetose on inner margin, with two or three additional setae at the subapex; palpomere 3 much more slender than 2; maxillary palpomeres 3 and 4 subequal in length; ligula short and rounded at apex, 8-setose. Antennae short, extending to the middle of the elytra.

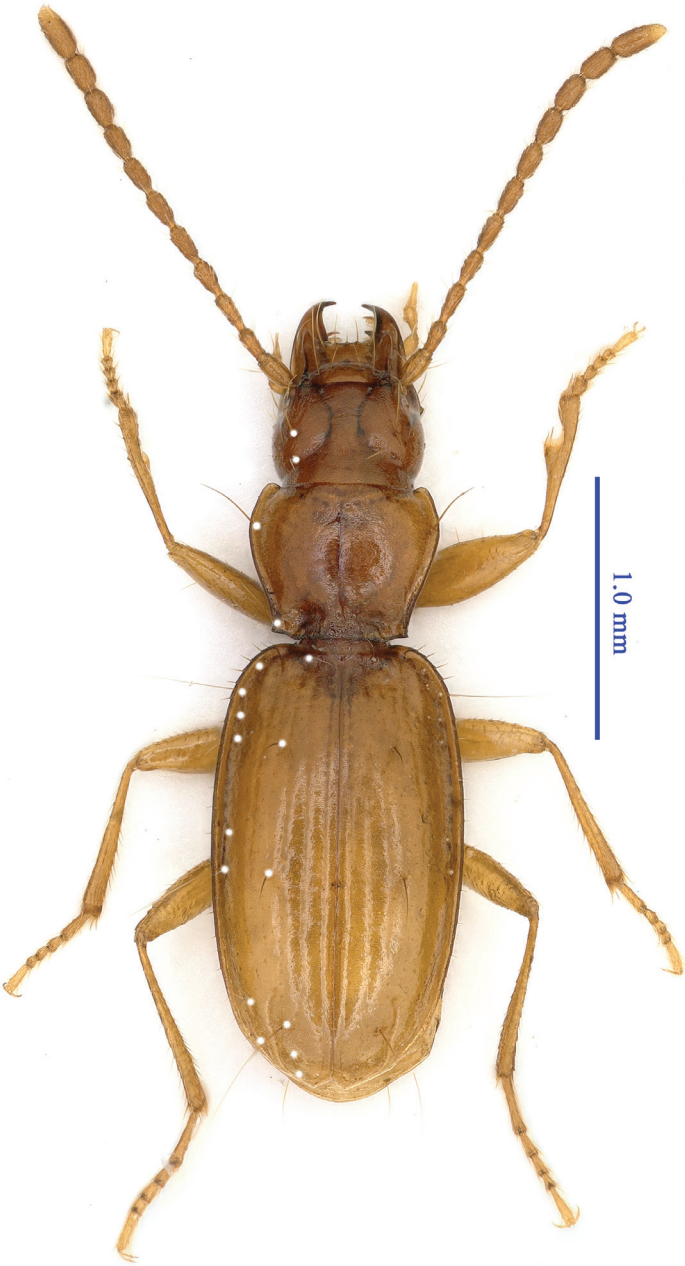

Figure 1. Habitus of *Cimmeritodes* (*Zhecimmerites*) *parvus* Tian & Li, sp. n., female, paratype.

Pronotum transverse, PnL/PnW = 0.83, wider than the head, PnW/HW = 1.24, shorter than the head (including mandibles), HLm/PnL = 1.19, or as long as the head (excluding mandibles); disc moderately convex; pronotum widest at about 1/3 from the apex, fore and hind lateromarginal setae located just before the widest point and

Figure 2. Male genitalia of *Cimmeritodes* (*Zhecimmerites*) *parvus* Tian & Li, sp. n., **a** median lobe and parameres, lateral view **b** apical lobe, dorsal view.

the hind angle, respectively; fore angles rounded, basal angles sharp; pronotum base narrower than front, PfW/PbW = 1.12; front almost straight, base nearly straight medially, obtusely sinuate near hind angles. Scutellum small and short.

Elytra elongate, thin, moderately convex, much longer than wide, EL/EW =1.74, sides ciliated, gently expanded laterally, widest near the middle, evenly narrowed towards the base and the subapex; elytra base wide, shoulders rounded; disc moderately convex, deeply striated, apical striae well-marked, intervals slightly convex; basal pore present; anterior dorsal pore located on 3rd stria at about 2/9 of the base, middle pore on the 4th interval, a little behind the middle of the elytra; pre-apical pore located exactly at the end of the 4th stria, at about 1/7 of elytra apex, subequal to the apex and to the suture; humeral group of marginal umbilicate series equidistant, both pores of the middle group closely located.

Legs moderately long, covered with dense and short hairs; protarsi short, 1st tarsomere slightly wider than the others, as long as the 2nd and 3rd combined, 4th protarsomere as long as wide; 1st tarsomere as long as, or longer than 2nd – 4th tarsomere combined in meso- and metatarsi, respectively. Ventrites IV–VI bearing a pair of paramedian setae; ventrite VII 4-setose in female, but bisetose in male.

Male genitalia (Fig. 2): median lobe of the aedeagus well-sclerotized, small and thin, strongly curved ventrally in the middle, gradually narrowed towards the apex, which is blunt; base moderately large, sagittal aileron small and thin, inner sac provided with a short and very thin copulatory piece, which is about one fifth of the aedeagus in length; in dorsal view, apical lobe short and broad; parameres elongate and thin, subequal, each armed with four (right paramere) and three (left paramere) long setae at apex.

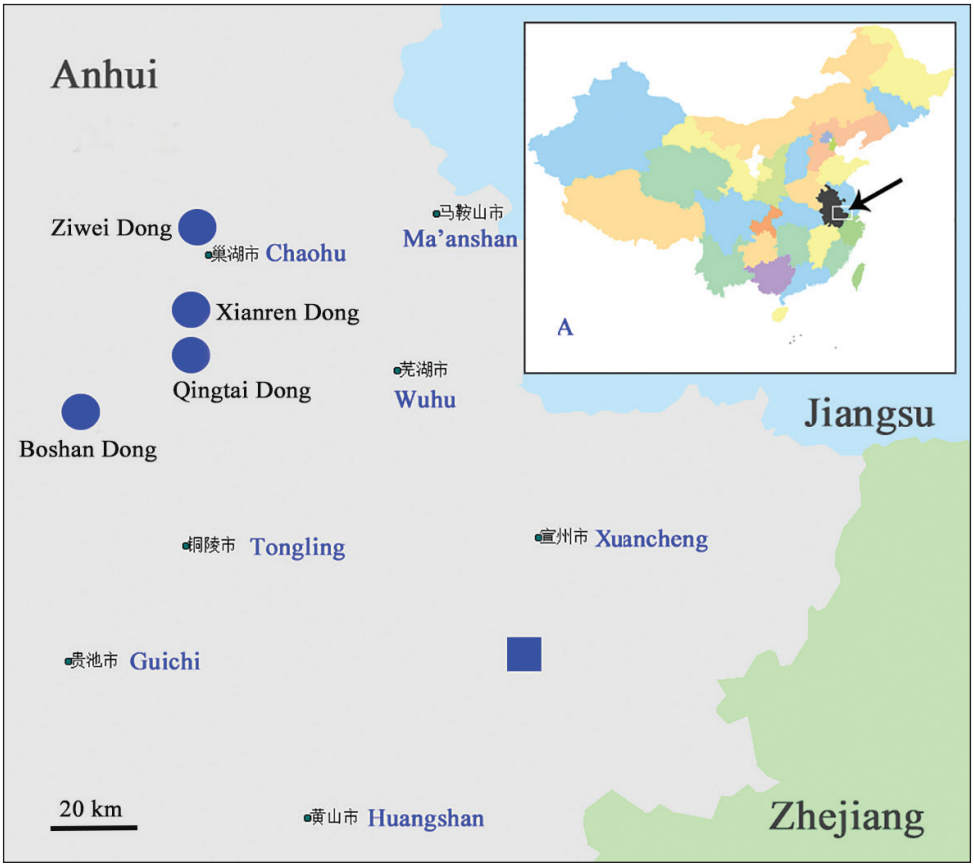

Figure 3. Distribution map of cave-dwelling trechines in Anhui Province **A** map of China, arrowhead showing the cave biological study area; circle localities of *Cimmeritodes* (*Zhecimmerites*) *parvus* Tian & Li, sp. n., square localities of *Wanoblemus wui* Tian & Fang, gen. n., sp. n.

Remarks. Similar to *C.* (*Z.*) *zhejiangensis* Deuve & Tian, 2015, which occurs in eastern Zhejiang Province, but with different male genital structures. Specifically, in *C.* (*Z.*) *parvus*, the median lobe is distinctly curved, and shorter and stouter than in *C.* (*Z.*) *zhejiangensis*.

Etymology. Indicates the small-sized body of this new species.

Distribution. China (Anhui) (Fig. 3). Xianren Dong Cave is a well-developed cave, approximately 450 m in length. Numerous stalactites and white peonies are found within the cave. Ziwei Dong Cave is a typical subterranean river cave, more than 3,000 m long. It is a show cave with uneven ground and numerous stalactites. Boshan Dong Cave is approximately 500 m long and has an uneven and complex tunnel. It is also a show cave, containing stone flowers and waterfalls. Qingtai Dong Cave is approximately 4,000 m in length, has a deep and long tunnel and a unique and outstanding interior. Beetle specimens were sampled from the ground surfaces of the caves, which were covered with litter and/or bat guano.

***Wanoblemus* Tian & Fang, gen. n.**
http://zoobank.org/15A4B7B5-33B0-4AF9-8951-7248849F46D7

Type species. *Wanoblemus wui* Tian & Fang, sp. n. (Baiyun Dong Cave, Xuancheng Shi).

Generic characteristics. Medium-sized within the phyletic series (Jeannel 1928; Casale and Laneyrie 1982; Casale et al. 1998) or complex (Uéno and Pawlowski 1981) *Trechoblemus*; anophthalmic beetles; body and appendages fairly thin and elongate; head subquadrate, longer (excluding mandibles) than wide, and longer than pronotum; frontal furrows complete, two pairs of supra-orbital and one pair of suborbital pores present; right mandible bidentate; labial suture completely absent, resulting in fused mentum and submentum; mentum bisetose, base strongly concave, tooth simple and short, blunt at apex; submentum 10-setose; antennae fairly long, nearly reaching the middle of the elytra; pronotum subcordate, slightly shorter than wide, widest near the front, about 1/5 of the apex, with two pairs of lateromarginal setae; lateral margin of the pronotum just before the hind angles nearly parallel, hind angles rectangular and sharp, the base nearly straight; elytra elongate, much longer (including mandibles) than the fore body, widest near the middle, surface moderately convex, shoulders distinct and angularly rounded where finely subserrate; lateral margins ciliated throughout; striae obliterated in stria 1, partly traceable in striae 2 and 3; two dorsal and the pre-apical pores present on each elytron; humeral group of marginal umbilicate pores irregular; protibia with external longitudinal groove; in males, protarsomeres 1 and 2 modified, distinctly denticulate inward, at the apex; ventrite VII with two pairs of setae in females and one pair of setae in males; male genitalia short and stout, strongly curved.

Discussion. *Wanoblemus* is not clearly related to any trechine genus associated with the *Trechoblemus* phyletic series described in China. It is probably closest to the Zhejiangese genus *Wulongoblemus* Uéno, 2006, as both have a similar pronotum and modified protarsomeres 1 and 2 in males. However, *Wanoblemus* differs from *Wulongoblemus* in the following characteristics: (1) right mandible bidentate in *Wanoblemus* but tridentate in *Wulongoblemus*; (2) elytra not serrate in the shoulders, less convex and narrower in *Wanoblemus* than in *Wulongoblemus*; (3) *Wanoblemus* is smaller than *Wulongoblemus*; (4) in *Wanoblemus* the pronotum is covered with fairly long pubescence, hind angles are right, postangular carinae are indistinct, and the lateral margin before hind angles is straight and nearly parallel, whereas in *Wulongoblemus* the pronotum is glabrous, hind angles are sharp, postangular carinae are distinct, and the lateral margin before hind angles is strongly sinuate, not parallel.

Wanoblemus might also be closely related to the subgenus *Zhecimmerites* (genus *Cimmeritodes*), although differing from the latter in the following key characteristics: (1) members of *Wanoblemus* are larger, and their body and appendages are more elongated than that of members of the genus *Cimmeritodes*; (2) the right mandible is bidentate in *Wanoblemus* and tridentate in *Cimmeritodes*; (3) in male *Wanoblemus* protarsomeres 1 and 2 are modified whereas in male *Cimmeritodes* only protarsomere 1 is modified; (4) *Wanoblemus* pronotum is as long as wide, with right hind angles whereas *Cimmeritodes* pronotum is transverse, with acute hind angles.

A number of characteristics also differ between *Wanoblemus* and the Zhejiangese genus *Microblemus* Uéno, 2007: (1) head quadrate in *Wanoblemus*, but not quadrate in *Microblemus*; (2) right mandible bisetose in *Wanoblemus* and 3-setose in *Microblemus*; (3) mentum and submentum completely fused in *Wanoblemus* and only partly fused, with labial suture traceable, in *Microblemus* ; (4) base of pronotum nearly straight in *Wanoblemus*, with right hind angles, and distinctly emarginated at the median section, with obtuse hind angles, in *Microblemus*; (5) elytra shoulders not dentate in *Wanoblemus* but remarkably dentate in *Microblemus*.

Wanoblemus is also easily separated from *Sidublemus* Tian & Yin, 2013, which is found in the southeast of the Hunan Province. In both genera, males protarsi are modified in joints 1 and 2, but: (1) *Wanoblemus* body is larger and more elongated, with slender appendages than *Sidublemus* body, which is small but stout, with short appendages; (2) right mandible bidentate in *Wanoblemus* and tridentate in *Sidublemus*; (3) simple hind angle in *Wanoblemus* and dentate in *Sidublemus*.

Wanoblemus is clearly distinct from the genus *Sinocimmerites* due to its elongate body, long and thin appendages, completely fused mentum and submentum, simple head tooth, longitudinally furrowed protibia, and stout and short aedeagus.

The endogean genus *Balazucellus*, which was recorded from Shennongjia, western Hubei Province, also differs from *Wanoblemus*. Among other features, *Balazucellus* body is smaller and stouter than *Wanoblemus*, the right mandible is tridentate (bidentate in *Wanoblemus*), and the antennae are moniliform (filiform in *Wanoblemus*).

Etymology. Indicates these beetles occur in Anhui Province; "Wan" is the short name for Anhui Province in Chinese.

Genus distribution range. China (Anhui) (Fig. 3).

Wanoblemus wui **Tian & Fang, sp. n.**

http://zoobank.org/5C9EF8D0-0D00-4C8B-880F-AC7685908D80

Material. Holotype: male, Baiyun Dong Cave, Huayang Xiang, Xuancheng, 30.3737N, 118.4457E, 300 m altitude, X-25-2015, leg. Yunhe Wu and Wenbo Li, deposited in SCAU. Paratypes: one male and one female, ibid., deposited in SCAU and ANU, respectively.

Diagnosis. Medium-sized *Trechoblemus* beetles, with brownish, sparsely pubescent and elongated body, and rather short and stout appendages.

Description. Length: 3.9–4.0 mm, including mandibles (3.5–3.6 mm, excluding mandibles); width: 1.0–1.1 mm. Habitus as in Fig. 4.

Body brownish, palps and tarsi pale; surface sparsely pubescent, setae on pronotum longer on the underside of head, prosternum and propleura glabrous; legs densely pubescent, covered with long setae; micro sculptural engraved meshes nearly isodiametric, although irregular on head and pronotum moderately transverse on elytra, meshes well marked on head, but absent on elytra.

Figure 4. Habitus of *Wanoblemus wui* Tian & Fang, gen. n., sp. n., male, holotype.

Head subquadrate, longer than wide, HLm/HW = 1.5, HLl/HW = 1.4; front and vertex moderately convex; frontal furrows fairly long and complete, nearly parallel medially; genae slightly expanded laterally; anterior supraorbital pores located in the middle of genae, sub-equidistant to lateral margin of genae and to posterior pore; clypeus 4-setose, labrum transverse, nearly straight in the front margin, 6-setose; mandibles distinctly curved at apex; ligula short, adnated with paraglossae, 6-setose at apex; palps short, penultimate joints much stouter than apical joints; labial palpomere 2 as

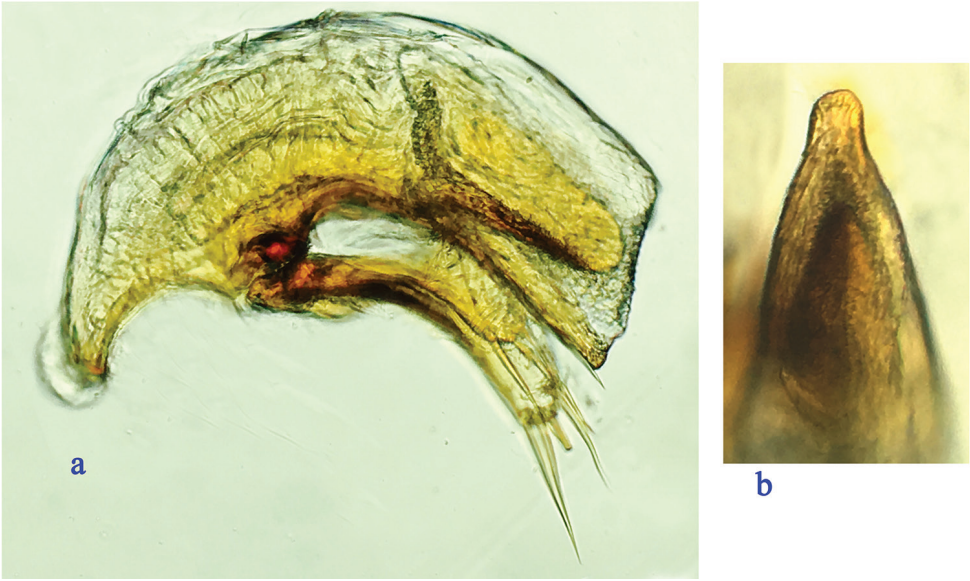

Figure 5. Male genitalia of *Wanoblemus wui* Tian & Fang, gen. n., sp. n. **a** median lobe and parameres, lateral view **b** apical lobe, dorsal view.

long as labial palpomere 3, bisetose on inner margin, with two or three additional setae at the outer margin of the subapex; maxillary palpomeres 3 and 4 subequal in length; suborbital pores located on ventral side of genae; antennomeres subequal in length, except for antennomere 11, which is longer than the others.

Pronotum slightly transverse, PnL/PnW = 0.97, shorter than head, PnL/HLm = 0.76, slightly wider than head, PnW/HW = 1.19, lateral margin finely beaded, anterior lateromarginal pores located at about 1/5 of the apex; posterior lateromarginal pores just before hind angles; base narrower than front, PbW/PfW = 0.87, both nearly straight and unbeaded; frontal impression faint, basal transverse sulcus well-marked; disc strongly convex; scutellum small and short.

Elytra elongate, distinctly longer than fore body, EL/(HLm + PnL) = 1.27, EL/(HLl + PnL) = 1.5, much longer than wide, EL/EW = 1.77; elytra much wider than pronotum, EW/PnW = 1.53; base not bordered; disc moderately convex but fairly depressed near the base; basal pores on either side of scutellum, apical stria absent; anterior and posterior dorsal pores located on stria 3, at about 2/7 and 4/7 from the base of the elytra, respectively; pre-apical pores at about 1/8 of the apex of the elytra, closer to the suture than to the elytra margin; pores 1 and 2 of the marginal umbilicate series close to marginal gutter, pore 2 closer to pore 1 than to pore 3; pores 5 and 6 of middle group closely located; pore 10 near apical margin.

Legs moderately long, densely pubescent; protarsi short, tarsomere 1 shorter than tarsomeres 2 and 3 combined, tarsomeres 3 and 4 as long as wide; tarsomere 1 subequal to, or longer than, tarsomeres 2 to 4 combined in meso- and metatarsi; each abdominal ventrite IV–VI bearing a pair of paramedian setae.

Male genitalia (Fig. 5): Median lobe of the aedeagus well-sclerotized, small but stout, strongly curved ventrally in the middle part, blunt at the apex; base fairly large, sagittal aileron very small and hyaline, inner sac with a large and thick copulatory piece, which is covered with scales, almost 1/3 the length of the aedeagus; in dorsal view, apical lobe short and broad; parameres elongate, right paramere longer than left paramere, each armed with three long setae at apex.

Etymology. In honor of Yunhe Wu (College of Life Science, Anhui University, Hefei), an active collector of cave insects.

Distribution. China (Anhui Province). Collected from a single limestone cave in Xuancheng Shi (Fig. 3). Baiyun Dong cave is approximately 1,000 m long and has a total area of about 20,000 m^2. This show cave contains many impressive stalactites. Beetle specimens were sampled from the ground surface, in a dark zone covered with abundant litter and bat guano.

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References

Casale A, Laneyrie R (1982) Trechodinae et Trechinae du Monde. Memoires de Biospeologie 9: 1–226.

Casale A, Vigna Taglianti A, Juberthie C (1998) Coleoptea: Carabidae. In: Juberthie C, Decu V (Eds) Encyclopedia Biospeologica III. Société Internationale Biospéologie, Moulis, Bucharest, 1047–1081.

Deuve Th (1996) Descriptions de trois Trechinae anophtalmes cavernicoles dans un karst du Hunan, Chine (Coleoptera, Trechidae). Revue française d'Entomologie (NS) 18: 41–48.

Deuve Th (2001) Nouveaux Trechinae des Philippines, du Sikkim, du Népal, de la Chine et de l'Equateur (Coleoptera, Trechidae). Bulletin de la Société entomologique de France 106: 43–50.

Deuve Th (2007) Nouveaux Paussidae, Carabidae et Trechidae de la Chine et du Sud-Est Asiatique (Coleoptera). Coléoptères 13: 51–62.

Deuve T, Tian MY (2015) Trois nouveaux Trechidae troglobies anophtalmes des karsts du Guizhou et du Zhejiang, en Chine (Coleoptera, Caraboidea). Bulletin de la Société entomologique de France 120(3): 397–402.

Jeannel R (1928) Monographie des Trechinae (Troisième livraison). L'Abeille 35: 1–808.

Lu YR (2012) Karst in China: A world of improbable peaks and wonderful caves. Higher Education Press, Beijing, 378 pp.

Moldovan OT (2012) Beetles. In: White WB, Culver DC (Eds) Encyclopedia of Caves (2nd edn). Elsevier, Amsterdam, 54–62. doi: 10.1016/b978-0-12-383832-2.00008-6

Sun FX, Xu XL, Jiang WJ, Gu F (2000) The cave resources in Anhui and their development and utilization. Geology of Anhui 2: 142–146.

Tian MY, Huang SB, Wang XH, Tang MR (2016) Contributions to the knowledge of subterranean trechine beetles in southern China's karsts: five new genera (Insecta: Coleoptera: Carabidae: Trechinae). ZooKeys 564: 121–156. doi: 10.3897/zookeys.564.6819

Tian MY, Yin HM (2013) Two new cavernicolous genera and species of Trechinae from Hunan Province, China (Coleoptera: Carabidae). Tropical Zoology 26: 154–165. doi: 10.1080/03946975.2013.869097

Uéno SI, Pawlowski J (1981) A new microphthalmic trechine beetle of the *Trechoblemus* Complex from Tian Shan. Annotationes Zoologicae Japonenses 54: 147–155.

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Eight new species of *Strongylophthalmyia* Heller from Vietnam with a key to species from Vietnam and neighbouring countries (Diptera, Strongylophthalmyiidae)

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Abstract

Seventeen species of *Strongylophthalmyia* are recognized in the fauna of Vietnam, including eight new species: *S. annulipes* **sp. n.**, *S. basisterna* **sp. n.**, *S. dichroa* **sp. n.**, *S. gavryushini* **sp. n.**, *S. obtecta* **sp. n.**, *S. orchidanthae* **sp. n.**, *S. stricta* **sp. n.**, *S. tomentosa* **sp. n.** *Strongylophthalmyia angusticollis* Frey, *S. fascipennis* Frey, *S. metatarsata* Meijere, *S. splendida* Yang & Wang, and *S. thaii* Papp are recorded for the first time from Vietnam. The male of *S. splendida* and female of *S. thaii* are described for the first time. A key to 34 species of *Strongylophthalmyia* of the fauna of Vietnam and continental southeast Asia, including the Oriental southern region of China is provided.

Keywords

Dipterous fauna, new species, *Strongylophthalmyia*, Strongylophthalmyiidae, systematics, Vietnam

Introduction

The Strongylophthalmyiidae is a small family of acalyptrate Diptera containing two genera, *Strongylophthalmyia* Heller, 1902 and *Nartshukia* Shatalkin, 1993. The genus *Nartshukia* is known only by the single female specimen of *Nartshukia musiva*

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Shatalkin, 1993 from Vietnam. The genus *Strongylophthalmyia* includes 54 species to date, occurring in the Nearctic (two species), Palaearctic (eight species), Oriental (40 species) and Australasian (9 species) regions (Iwasa and Evenhuis 2014). Two species described from Madagascar by Verbeke (1963, 1968) were transferred to Clusiidae by Barraclough (2000). As our studies show, the Vietnamese fauna of the genus includes 17 species. Eight of them are described in this paper as new, and five of them are reported for Vietnam for the first time.

Materials and methods

This study is part of an ongoing series of studies on the Vietnamese cyclorrhaphous fauna. A key is composed for species from Vietnam, Burma, Oriental southern Region of China and Thailand; the genus has not yet been recorded from Laos or Cambodia. The specimens of new species of *Strongylophthalmyia* described in this paper are rare in our collection, and the colouration of the abdomen is important for determination; hence, genitalic characters are not explored in the descriptions of new species.

Types of the new species are deposited in the collection of Zoological Museum of Moscow University (**ZMUM**).

In the key and descriptions of species, morphological terminology, abbreviations of wing veins, and wing cells, are after Cumming and Wood (2009). Measurements are given in millimetres. Labels of specimens are quoted verbatim. Frontal index = the ratio between height of the frons from its anterior margin to hind ocelli and from hind ocelli to vertex or vti.

Results

The genus *Strongylophthalmyia* includes strikingly elegant flies with elongated bodies and slender legs. These flies have body lengths from 2.3 mm to 7.5 mm (the smallest is *S. palpalis* Papp, 2006 the largest is *S. gigantica* Iwasa & Evenhuis, 2014).

Head (Figure 1a) is spherical or extended in profile (extended in *S. splendida* Yang & Wang, 1996); the gena is narrow; the facial sclerotization is interrupted by membrane medially; and the ocellar tubercle is moved forward and is often situated in the middle of the frons. Females often have a large bulbous clypeus, but it is smaller and band-like in males. First flagellomere is short, rounded.

Several species of this genus have sexual dimorphism in the antennal structure. The males of some *Strongylophthalmyia* differ in developing of the dorsal process of the first flagellomere (Figure 10d, e).

The arista is usually bare, but several Oriental species have small setulae on the arista. Males of some of these species groups have modified palpi, which can be yellow (compared to the black female palpus), bilobate and with black scales or thick setulae. Chaetotaxy of head: 2–3 orbital setae, usually with three hair-like small frontal

setae, one ocellar seta, one inner vertical seta and one outer vertical seta (absent in *S. splendida*), one postocellar seta, one paravertical seta (absent in some species), vibrissa absent.

Thorax is coloured from yellow (Figure 6) to black (Figure 2b). Several species are characterised by a black thorax, and a postpronotum and propleuron and often basisternum that are entirely or partly yellow. Several species are black with a pair of reddish yellow spots (in females) or one large spot (in males) on the basisternum between the fore coxae (Figure 2e). Finally, two species, described in this paper as new, *Strongylophthalmyia obtecta* sp. n. (Figure 5a,b) and *Strongylophthalmyia stricta* sp. n. (Figure 7a), are black with brownish or yellowish spots on the postpronotum (laterally), and with yellowish spots around the fore spiracles. These two species may be related to *Strongylophthalmyia papuana* Iwasa & Evenhuis, 2014 and *Strongylophthalmyia gigantica* Iwasa & Evenhuis, 2014, both from Papua New Guinea, because these four species are characterized by predominantly black legs. Since the black colouration of legs can fade in preserved specimens, these species appear in multiple parts of the key. The mesonotum is covered with yellowish setulae or it is bare with black setae located in rows.

Chaetotaxy of thorax: 1 anepisternal seta, 2 notopleural setae (one notopleural seta in Nearctic *S. pengellyi* Barber, 2006), 1 supraalar seta, 1 postalar seta, 1–2 dorsocentral setae, 1 apical scutellar seta. Postpronotum usually with some setulae, rarely with long setae. *Strongylophthalmyia splendida* has a strong black seta in the anterior part of the mesonotum, near the postpronotum (Figure 9b: marked by arrow), which we consider as sublateral (Hennig 1973: 184, Figure 109).

Legs are slender, usually bare. Hind legs of males of some species bear small papillae, having one papilla on trochanter and two papillae on femur (these papillate protuberances are variable; some species with only on femur, others on both trochanter and femur, some with just one, others up to three, some bare, others with long stiff setae), being bubble-like basally and conical (with yellow or black setulae) subbasally. Male fore femur sometimes with short stout spines dorsally (Figure 4a).

Wings have a distinct costal break just before merging of costa and subcosta. The subcosta is incomplete, not quite reaching the wing margin (Figure 1a). Transverse vein CuA_2 forms distal border of anal cell, convex. Veins R_{4+5} and M_1 are parallel or slightly converging in apical third. Vein A_1+CuA_2 is distinctly bent, not reaching wing margin. Wing is transparent, wholly infuscated, with brownish apical spot or with cross-band on dm-cu and short cross-band anteriorly level of Rs. Greater ampulla is absent. Halter is coloured from whitish to yellow, rarely with blackish-grey knob.

Abdomen is slender, black, sometimes yellow, its colour patterns in some cases (*S. trifasciata* Hennig, 1940) differs in females and males.

The structure of **male genitalia** are known only for several species of *Strongylophthalmyia* (not for *Nartshukia*). Epandrium without surstyli (some authors (Iwasa 1998; Iwasa and Evenhuis 2014) consider that surstyli are completely fused with epandrium). However, genitalia of species of *S. crinita* group have appendages similar to surstyli and attributed to this type of lobe (Shatalkin 1995). The hypandrium is well-developed, bearing two pairs of lobes. The origin of these lobes remains unclear (Steyskal 1987,

Shatalkin 1995, Iwasa 1998, Barber 2006, Lonsdale 2013, Iwasa and Evenhuis 2014). Phallus and phallapodeme are very long; however, several exceptions are known. The male of *S. verrucifera* Shatalkin, 1996 is characterized by a short apodeme of the aedeagus; on the contrary, males of *S. paula* Shatalkin, 1993 have truncated aedeagus.

Female terminalia have ovipositor quite elongate, slender, with unsclerotized cerci. One or two (in *S. pengellyi*) spermathecae present (Iwasa 1998, Barber 2006). Tergite and sternite 7 are fused into syntergosternite.

Ecology. Adult flies can be found on leaves of bushes and trees, on stumps, and fallen logs. Larvae of some Holarctic species are characterised by biramous anterior spiracles, and live under the bark of rotting logs of aspen (*S. ustulata* (Zetterstedt, 1847), *S. pictipes* Frey, 1935, *S. angustipennis* Melander, 1920, *S. pengellyi*) and under bark of rotting logs of birch and elm (*S. stackelbergi* Krivosheina, 1981) (Krivosheina 1981, Barber 2006).

Notes. Neal L. Evenhuis in his review kindly noted that there is apparently a serious mistake in Yang and Wang (1998) with regard to the description and wing illustrations of *S. splendida* and *S. yaoshana*. The descriptions of the wing are either switched or the illustrations are switched. *Strongylophthalmyia splendida* is described as having a hyaline wing, yet the illustration claearly slows bands; whereas *S. yaoshana* is described as having a banded wing, yet the illustration clearly shows it to have a hyaline one. As the holotypes of each species are lost (Wang Xin li, pers. comm.; also N.L. Evenhuis could not find them during Wang's and my search for them when N.L. Evenhuis was in Beijing in 2014; only a paratype of *S. bifasciata* could be found) there is no definitive way to determine which species is which.

Key to species of genus *Strongylophthalmyia* from Vietnam and neighbouring countries (Burma, China, and Thailand)

1 Mesonotum yellow, usually with pair of brown longitudinal stripes, in one case these longitudinal stripes converging before suture **2**

– Mesonotum black, sometimes yellow in anterior part to suture..... **5**

2 Mesonotum with black arcuate concave spot anteromedially; mesonotum with pair of narrow black longitudinal stripes behind suture, these stripes continue onto scutellum. Pedicel black. Body length 4.3. Vietnam (Figure 6) .... ***Strongylophthalmyia orchidanthae*** **sp. n.**

– Mesonotum at most with pair of brown longitudinal stripes. Pedicel yellow.... **3**

3 Abdomen entirely yellow. Head yellow, except ocellar tubercle. Male: first flagellomere dorsally without very short conical process anterior to base of arista; fore femur without spines **4**

– Abdomen black, at most yellow at base. Head partly yellow; frons black, yellow or grey in anterior fourth (female), yellow in anterior third (male). Male: first flagellomere dorsally with very short conical process anterior to base of arista; fore femur without spines. Body length 2.3–3.0. Thailand..... ..... ***Strongylophthalmyia palpalis*** **Papp, 2006**

4 Wing with well-developed apical spot and with crossband on dm-cu vein. Mesonotum with pair of weak brownish stripes on each side posteriorly from dorsocentral setae. Body length 3.5–4.7. Thailand ..... ***Strongylophthalmyia dorsocentralis*** **Papp, 2006**

– Wing with poorly developed greyish spots. Mesonotum with pair of weak brownish stripes from suture to scutellum. Body length 4.7. Indonesia (Java), Thailand ..... ***Strongylophthalmyia lutea*** **(Meijere, 1914)**

5 Mesonotum black in posterior part behind suture; yellow in anterior part, sometimes with brown median strip, going from anterior margin of mesonotum. Body length 3.7–5.0. Burma, Vietnam ..... ***Strongylophthalmyia elegantissima*** **Frey, 1956**

– Mesonotum entirely black **6**

6 Postpronotum, propleuron and often basisternum entirely or partly yellow or yellowish-brown..... **7**

– Postpronotum and propleuron totally black..... **19**

7 Wing transparent or with pale brownish apical spot..... **8**

– Wing with well-expressed brown cross-band on dm-cu vein and with apical spot..... **12**

8 Frons completely black. Veins R_{4+5} and M_{1+2} straight and parallel, not convergent apically..... **9**

– Frons partly yellow. Apical part of vein M_{1+2} strongly curved in direction of vein R_{4+5}..... **11**

9 Abdomen entirely black. Anepisternum without tuft of silvery setulae medially. Legs yellow, mid and hind femora widely darkened. Male fore femur without dorsal row of black spines and with thick medioventral thorn-like tight cluster of setulae basally. Female unknown. Male body length 3.1. Thailand ..... ***Strongylophthalmyia macrocera*** **Papp, 2006**

– Abdomen shining black, basally yellowish. Anepisternum with tuft of silvery setulae medially. Legs yellow, at least hind femur with apical brown ring. Male fore femur with dorsal row of 5–7 short black spines basally (Fig. 10a)..... **10**

10 Male first flagellomere dorsally with long and slender process (see Papp et al. 2006, fig. 56; this paper - Fig. 10d). Hind tibia darkened dorsally and ventrally in basal two thirds. Body length 3.4–3.8. Vietnam, Thailand (Figure 10)..... ***Strongylophthalmyia thaii*** **Papp, 2006**

– Male first flagellomere without dorsal process. Hind tibia darkened dorsally and ventrally in basal half. Female unknown. Male body length 4.0. Burma, Thailand ..... ***Strongylophthalmyia spinosa*** **Frey, 1956**

11 Abdomen entirely black. Frons black, anteriorly yellow. Wing with pale brownish apical spot. Body length 4.5. Burma..... ***Strongylophthalmyia curvinervis*** **Frey, 1956**

– Abdomen black, tergite 6 brownish yellow. Frons yellow with brownish area around ocellar tubercle and on vertex. Wing transparent (Fig. 9a). Body length 3.4. China, Vietnam (Figure 9) ..... ***Strongylophthalmyia splendida*** **Yang & Wang, 1998**

12 Abdomen mostly matte yellow. Head with 2 orbital setae. Female unknown. Male body length 3.4. Vietnam (Figure 4) .... ***Strongylophthalmyia gavryushini* sp. n.**

– Abdomen shining black, at most yellowish basally. Head usually with 3 orbital setae (male and female) **13**

13 Arista with short setulae **14**

– Arista bare **16**

14 Frons black **15**

– Frons black, yellow in anterior third. Basisternum with a pair of yellow spots. Body length 5.5–6.0. Burma.... ***Strongylophthalmyia humeralis* Frey, 1956**

15 Wing: median transverse band between C and R_{2+3} undeveloped; apical spot large, extended from beginning of R_{2+3} vein; short cross-band at level of R_s undeveloped. Face yellow, arista yellow. Body length 5.0 (male), 6.0 (female). China (Zhejiang) ..... ***Strongylophthalmyia bifasciata* Yang & Wang, 1992**

– Wing median transverse band between C and R_{2+3} well-developed, reaching costal margin; apical spot small, its length equal distance from this spot to R_{2+3} vein; short cross-band at a level of R_s in anterior half of wing developed. Face black, arista dark brown. Male unknown. Female body length 5.0. China (Guangxi) ..... ***Strongylophthalmyia yaoshana* Yang & Wang, 1998**

16 Abdomen black, broadly yellow basally, tergite six with lateral yellow spots extending from anterior margin to two thirds of its length. Wing with two brownish marks: short cross-band at a level of R_s undeveloped; median cross-band on a dm-cu vein almost undeveloped between C and R_{2+3}. Male unknown. Female body length 3.6. Vietnam (Figure 8) .... ***Strongylophthalmyia tomentosa* sp. n.**

– Abdomen entirely black, or yellow basally. Wing with three brownish marks: apical spot, cross-band on dm-cu vein and short crossband at level of R_s in anterior half of wing. Median cross-band well developed between C and R_{2+3} **17**

17 At least area around anterior spiracle and postpronotum laterally yellow. Fore legs entirely yellow. Body length 3.8–4.7. Vietnam (Figure 1) .... ***Strongylophthalmyia annulipes* sp. n.**

– Area around anterior spiracle and postpronotum laterally brown. Fore coxa and femur partly black **18**

18 Fore femur black, basally and apically with narrow yellow ring. Fore tibia yellow, brownish in basal third. Two (in males) or three (in females) last segments of tarsi contrastingly black. Hind trochanter of male with posterior round blackish spot; hind femur basally with round posteroventral process, and with small subbasal process bearing patch of yellow setulae situated on the posterior surface of hind femur. Wing with wide median crossband (Fig. 7b). Body length 3.3 (male), 4.8 (female). Vietnam (Figure 7) .... ***Strongylophthalmyia stricta* sp. n.**

– Fore femur yellow with blackish spot on apical third; fore tibia entirely yellow; female with 2 last segments of tarsi black (male unknown). Wing with

narrow median cross-band (Fig. 5b). Body length 4.2–4.6. Vietnam (Figure 5) ..... ***Strongylophthalmyia obtecta* sp. n.**

19 Hind femur entirely yellow or with apical or subapical dark brown ring ... **20**

– Hind femur largely black, including basal half of segment **29**

20 Arista with short distinct setulae. Basisternum with pair of reddish yellow spots (in females) or with one large spot (in males) between fore coxae. Body length 5.5–6.5. Burma, Vietnam ..... ***Strongylophthalmyia angusticollis* Frey, 1956**

– Arista bare. Basisternum with pair of reddish yellow spots or black **21**

21 Wing at least darkened apically **22**

– Wing totally transparent **25**

22 Frons posteriorly black, anteriorly yellow **23**

– Frons entirely black, at most with brownish spot between antenna and eye ... **24**

23 All femora with contrasting apical blackish ring. Thorax with 1 dorsocentral seta. Wing apically, r-m and dm-cu darkened. Body length 4.0–4.4. Burma... ..... ***Strongylophthalmyia punctum* Frey, 1956**

– Fore femur without apical blackish ring. Thorax with 5 dorsocentral setae. Male: wing with median cross-band and apical spot; first flagellomere with long tubular dorsal process, covered with black setulae. Female: wing with median cross-band almost undeveloped. Body length 3.4–3.5. Thailand..... ..... ***Strongylophthalmyia freidbergi* Shatalkin, 1996**

24 Wing darkened apically. Legs yellow, only hind femur with preapical brown ring. Abdomen entirely black, basisternum without yellow spots. Body length 3.6. Thailand ..... ***Strongylophthalmyia pectinigera* Shatalkin, 1996**

– Wing with median cross-band and apical spot. Mid and hind femora with preapical brown ring. Hind tibia with subbasal brownish ring, occupying one third of tibia. Abdomen black, tergite 4 yellowish laterally, tergite 5 and 6 totally yellowish. Basisternum with a pair of yellow spots (Fig. 2e). Male unknown. Female body length 3.5–3.6. Vietnam (Figure 2) .... ***Strongylophthalmyia basisterna* sp. n.**

25 Face yellow. Frons entirely black. Palpus bicoloured: yellowish, darkened in basal half on anterior margin (Fig. 3c, d). Male unknown. Female body length 4.8. Vietnam (Figure 3) .... ***Strongylophthalmyia dichroa* sp. n.**

– Face black. Palpus monochrom, entirely yellow or entirely black **26**

26 Smaller: Male (female unknown) body length 2.3. Hind tibia yellow, brownish in middle. Male: first flagellomere with short conical process (Shatalkin 1996, Fig. 27). Thailand, Vietnam .... ***Strongylophthalmyia gibbifera* Shatalkin, 1993**

– Larger: Body length 3.5–4.5. Hind tibia yellow or brownish basally. First flagellomere normal in male and female **27**

27 Proximal section of M_{1+2} restricting discal cell before r-m approximately 0.5–0.6 times as short as distal section. Male palpus (Shatalkin 1996, Fig. 23)

with one wide leaf-like scale apically. Body length 3.8. Burma ....***Strongylophthalmyia freyi*** **Shatalkin, 1996**

– Proximal section of M_{1+2} restricting discal cell before r-m approximately 0.7–0.8 times as short as distal section. Male palpus of different form. **28**

28 Hind tibia slightly brownish in basal third. Male palpus yellow, with two or three black leaf-like scale apically (Shatalkin 1996, Fig. 22). Genitalia with aedeagal apodeme very long, more than two times as long as epandrium (Shatalkin 1996, Fig. 16). Body length 3.5–4.5. Taiwan; Burma, Vietnam, Japan, Russian Far East ..... ***Strongylophthalmyia crinita*** **Hennig, 1940**

– Hind tibia totally yellow. Male palpus dark, normal without black leaf-like scale. Genitalia with aedeagal apodeme very short and completely closed by epandrium (Shatalkin 1996, Fig. 21). Body length 3.5–3.7. Vietnam..... .....***Strongylophthalmyia verrucifera*** **Shatalkin, 1996**

29 Wing smoky brown darkened. All coxae yellow. Body length 4.0. Indonesia (Java); Philippines, Thailand ....***Strongylophthalmyia brunneipennis*** **(Meijere, 1914)**

– Wing transparent, with or without median brownish cross-band on level of dm-cu and apical brownish spot **30**

30 Wing clear, or brownish apically, without brownish cross-band on level of dm-cu **31**

– Wing with median cross-band and with apical spot **32**

31 All coxae blackish. Face light brown to yellow. Frons with narrow yellow band in male and usually totally black in female. Fore femur black, yellowish apically; mid and hind femora totally black. Male first flagellomere without dorsal process. Male fore femur with large black setae on anterior surface and cercus extremely long, slender. Body length 2.3–2.6. Indonesia (Java, Sumatra), Thailand..... ***Strongylophthalmyia nigricoxa*** **(Meijere, 1914)**

– All coxae yellow. Head black. Fore leg yellow, mid and hind femora blackish, yellow in basal third. Male first flagellomere with dorsal process long, S-shaped; arista long, as long as first flagellomere. Wing length 3.7; Head length (without antenna) 0.87. Taiwan, Thailand ....***Strongylophthalmyia punctata*** **Hennig, 1940**

32 Fore tibia entirely blackish. Fore tarsus with segment 1 yellow basally and brown apically, segments 2–5 blackish brown. Body length 4.0. Indonesia (Sumatra), Thailand, Vietnam.....***Strongylophthalmyia metatarsata*** **Meijere, 1919**

– Fore tibia entirely or mostly yellow. Two or three basal segments of fore tarsus yellow **33**

33 Vein r-m divides discal cell in half. Body length 4.0. Indonesia (Java, Sumatra), Thailand.....***Strongylophthalmyia polita*** **Meijere, 1914**

– Vein r-m divides discal cell in relation from 1:2.5 to 1:4.0 .....**34**

34 Mesonotum shining. Anepisternum covered with whitish setulae. Face yellowish. Palpus yellow. Mid tibia yellow in apical half. Body length 3.8–4.0. Philip-

pines, Burma, Vietnam ..... ***Strongylophthalmyia fascipennis* Frey, 1928**

– Mesonotum matte. Anepisternum bare, with blackish short setae on posterior margin. Face dark brown, matte. Palpus black. Mid tibia entirely blackish **35**

35 Fore femur black, narrowly yellow basally and apically, fore tibia yellow, brown in basal third; female with 3 distal segments of tarsi black (see also couplet 18) (Figure 7) ..... ***Strongylophthalmyia stricta* sp. n.**

– Fore femur yellow with black ring in apical third; fore tibia entirely yellow; female with 2 distal segments of tarsi black (see also couplet 18) (Figure 5) .... ***Strongylophthalmyia obtecta* sp. n.**

Descriptions of new species

***Strongylophthalmyia annulipes* sp. n.**
http://zoobank.org/6F1961E2-9838-4C98-A1BE-4B348AD527F5
Figure 1

Type material. Holotype: 1 male, Vietnam, Lai Châu Province, Hoáng Liên (22.34997°N, 103.76818°E), 1947 m, 11.IV.2012 (D. Gavryushin). Paratypes: 2 female, Vietnam, Lai Châu Province, Hoáng Liên (22.347948°N, 103.769714°E), 1900 m, 16.IV and 19.IV.2012 (A.L. Ozerov); 1 male, 1 female, Vietnam, Lai Châu Province, Hoáng Liên (22.33788°N, 103.77922°E), 2068 m, 21.IV. and 7.V.2013 (T.V.Galinskaya). ZMUM.

Diagnosis. This new species belongs to a group of species characterized by a yellow postpronotum, propleuron and basisternum. Three species from this group, *S. humeralis*, *S. bifasciata* and *S. yaoshana* are otherwise characterized by an arista with very short setulae and easily differentiated. Other species of this group, *S. spinosa*, *S. thaii*, *S. coarctata* Hendel, 1913 and *S. macrocera* differ from *S. annulipes* by transparent wings. *Strongylophthalmyia annulipes* sp. n. is close to *S. tomentosa* sp. n. and differs from it by the presence of a preapical black ring on the mid and hind femora and by black ring on the mid and hind tibia.

Description. Male. *Head* black, shining, slightly longer than its height; frons entirely black, covered with grey tomentose of by very short setulae (0.01 mm). Occiput slightly convex (in dorsal view). Face black, matte, with row of short setulae along suture; parafacial black, covered with silvery grey tomentum, length of its setulae: 0.021–0.028 mm. Gena with brownish short stripe directly below parafacial. Antenna brownish yellow. First flagellomere rounded, its length almost equal to height; dark brown, narrowly yellow at base, with short yellow setulae dorsally. Arista dark brown, bare. Palpi brownish yellow. Chaetotaxy: three reclinate to lateroclinate orbital setae (the middle seta 2.5 times longer than others), 1 ocellar seta, 1 postocellar seta, 1 inner vertical seta, 1 outer vertical seta, 2 hair-like short frontal setae.

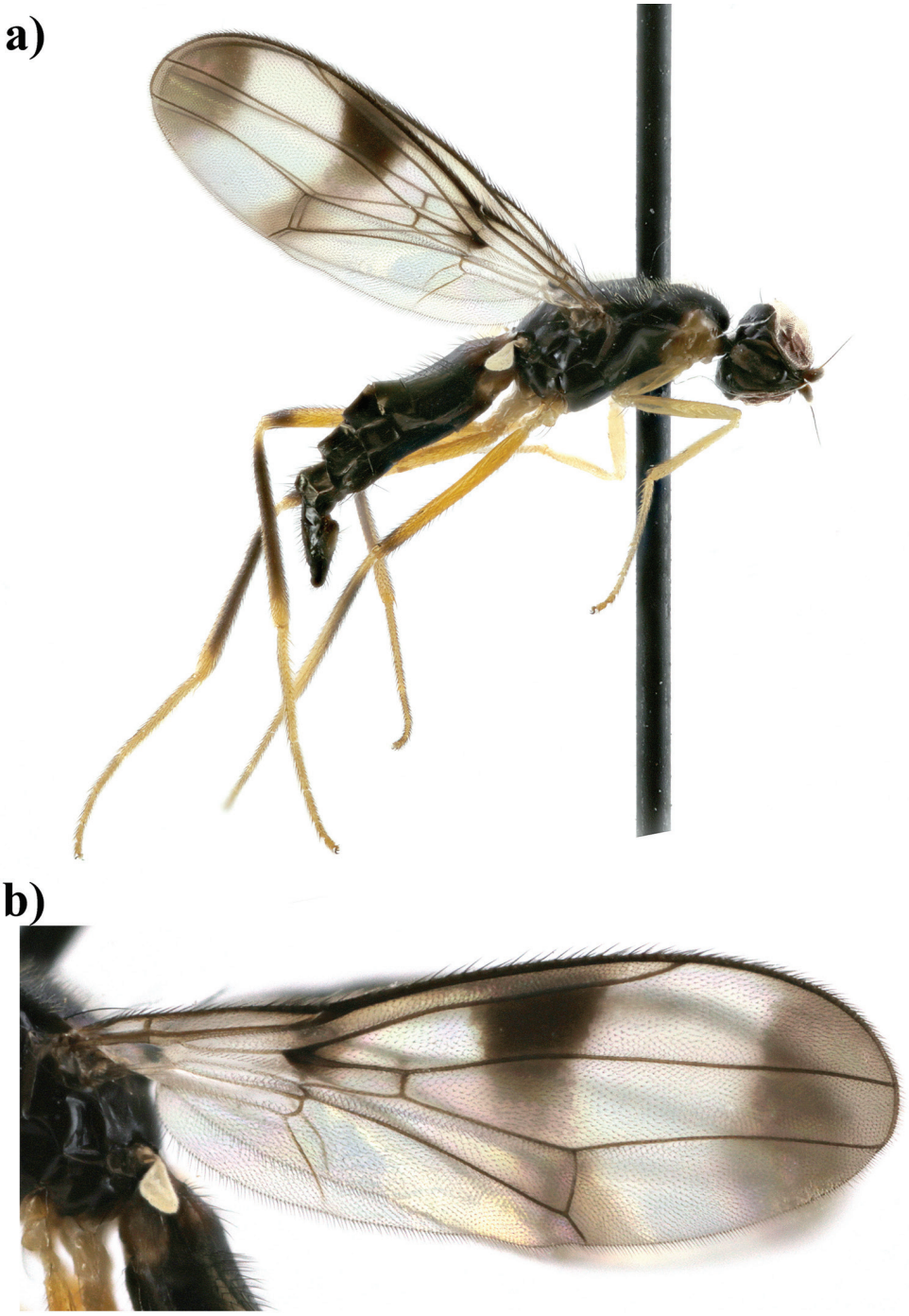

Figure 1. *Strongylophthalmyia annulipes* sp. n. **a** habitus, lateral view **b** wing.

Thorax black. Postpronotum laterally, propleuron, basisternum, and anepisternum around spiracle yellow. Mesonotum matte, sparsely covered with short pale setulae; pleuron shiny; scutellum bare, matte. *Legs* yellow, mid and hind femora with dark brown preapical ring; mid tibia yellow basally and in apical half, and darkened between these yellow areas; hind tibia darkened, yellowish basally and in apical quarter. *Wings* with apical spot, with median transverse band on level of dm-cu vein and with weak darkening anteriorly at level of vein R_s. R_{2+3} long: section of C between R_1 and R_{2+3} 1.5 times longer than following section (between R_{2+3} and R_{4+5}). R_{4+5} and M_{1+2} almost parallel apically. Section of M_{1+2} between r-m and dm-cu approximately 1.8 times longer than proximal section and 0.6 times shorter than distal section. Cell bm is 0.5 times shorter than discal cell. Calypter light grey with fan of very long light setulae on its margin. Halter with yellow stem and whitish knob. Chaetotaxy: one small postpronotal seta, one dorsocentral seta, two notopleural setae, one supraalar seta, one postalar seta, one anepisternal seta, one stout apical scutellar seta. All setae black.

Abdomen shiny black, narrowly yellow basally.

Body length 4.2 mm. Wing length 3.8 mm.

Female differs from male in following characters. Frontal setulae very short, hardly visible. Palpi dark brown. Mesonotum covered with short setulae. Mid tibia widely darkened, yellowish basally and in apical quarter. Body length 3.8–4.7 mm; wing length 3.5–4.2 mm.

Etymology. The specific name refers to black ring on mid and hind femora.

***Strongylophthalmyia basisterna* sp. n.**
http://zoobank.org/8A8E266B-04BC-4B10-A229-5C37EBFC6ECF
Figure 2

Type material. Holotype: 1 female, Vietnam, Lai Châu Province, Hoáng Liên (22.347948°N, 103.769714°E), 1700 m, 22.V.2014 (A.L. Ozerov). Paratype: 1 female, Vietnam, Lai Châu Province, Hoáng Liên (22.347948°N, 103.769714°E), 1900 m, 22.V.2014 (A.L. Ozerov); 1 female, Vietnam, Lai Châu Province, Sa Pa env. (22.3872°N, 103.7867°E), 1682 m 23.V.2014 (D. Gavryushin) ZMUM.

Diagnosis. This new species strongly differs from all species from Vietnam and neighbour countries. It is morphologically close to *S. puncticollis* Frey, 1928 (from Philippines and Papua New Guinea) and to *S. fasciolata* Meijere, 1919 (from Sumatra). *Strongylophthalmyia. puncticollis* differs from the new species by having the abdomen entirely black; all femora have a brown preapical ring, the hind tibia is black basally and apically and yellow in the median third. *Strongylophthalmyia fasciolata* differs from the new species by an entirely black abdomen, black matte mesonotum, dark legs, and halteres with a brownish stem. In the key by Steyskal (1971) *S. fasciolata* is close to *S. angusticollis* Frey, 1956 (from Burma). The last species is characterized by the arista covered with short setulae.

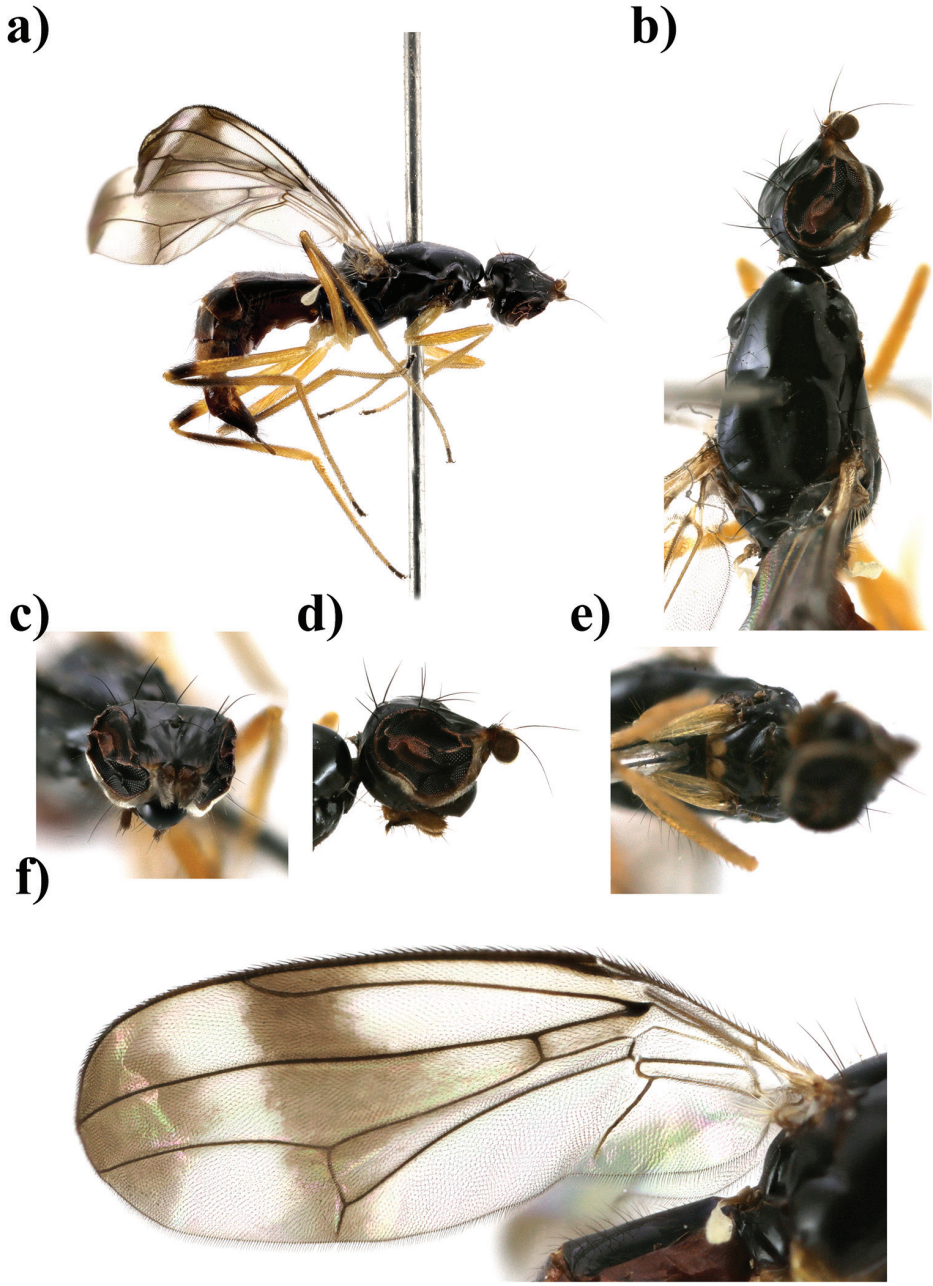

Figure 2. *Strongylophthalmyia basisterna* sp. n. **a** habitus, lateral view **b** head and thorax, dorsal view **c** head, anterior view **d** head, lateral view **e** basisternum **f** wing.

Description. Female. *Head* black, 1.3 times longer than height. Frons matte with yellowish brown spot between antenna and eye. Ocellar tubercle slightly shifted anteriorly: the ratio between height of the frons from its anterior margin to hind ocelli and

from the hind ocelli to the vertex or vti equal to 1.3. Occiput poorly convex (in dorsal view). Face dark brown, matte, with row of short setulae along suture. Parafacial yellow, covered with whitish tomentum, setulae on parafacial around 0.5 times shorter than setulae along facial suture. Antenna dark brown. First flagellomere 1.1 times longer than high, dark brown with long yellowish marginal setulae. Arista dark brown, bare. Mouthparts dark, palpi darkish brown. Chaetotaxy: two reclinate to lateroclinate orbital setae (posterior seta 2.0 times longer than anterior), 1 oc, 1 poc, 1 vti, 1 vte, frontal setae absent.

Thorax black, shining. Basisternum with two bright yellow spots between fore coxae; yellowish brown stripe extended from postpronotum over anterior spiracle to coxa. Mesonotum shining, with 4 rows of short yellow setae along dc and ac rows. Scutellum matte. *Legs* yellow, mid and hind femur with preapical brown ring (this ring narrower on mid femur); mid and hind tibiae with subbasal brownish ring; two last tarsal segments black. *Wings* with apical spot, median transverse band through dm-cu and with weak darkening in anterior part of R_s. Right border of median band situated slightly laterally to R_{2+3} vein. R_{2+3} long: section of C between R_1 and R_{2+3} 1.3 times longer than following section (between R_{2+3} and R_{4+5}). R_{4+5} and M_{1+2} almost parallel apically. Section of M_{1+2} between r-m and dm-cu slightly concave, 3 times longer than proximal section and 0.9 times shorter than distal section. Cell bm 0.5 times shorter than discal cell. Calypter brownish yellow with fan of very long yellowish setulae on its margin. Halter with yellow stem and whitish knob. Chaetotaxy: one small pprn, one pprn, one dc, ac in two rows, two npl, one sa, one pa, one anepst, scutellum with a pair of stout setae apically. All setae black.

Abdomen black, shiny; tergite 4 laterally, tergite 5, 6 totally yellow.

Body length 5.6 mm (5.5 and 6.5 in paratypes); wing length 5.2 mm (5.2 and 5.3 in paratypes).

Male unknown.

Etymology. The specific name refers to the two bright yellow spots on basisternum.

***Strongylophthalmyia dichroa* sp. n.**

http://zoobank.org/41F487F6-4F57-4BE6-B9AE-506B4A0DFBA0
Figure 3

Type material. Holotype: 1 female, Vietnam, Lai Châu Province, Hoáng Liên (22.33788°N, 103.77922°E), 2068 m, 7.V. 2013 (T.V. Galinskaya). ZMUM.

Diagnosis. This new species belongs to the *S. crinita* species group. Species of this group are characterized by transparent wing, bare arista, yellow legs, mid and hind femora with apical dark brown ring. Within this group the new species is close to species with yellow face and totally black frons. The new species differ from all species of *S. crinita* species group by palpus with character coloration (yellow, darkened in basal half on anterior margin, with some black setulae apically). Among oriental species only *S. stylocera* from Philippines has these characters, but it is a much smaller species, 2.7 mm (4.8 mm in *S. dichroa*).

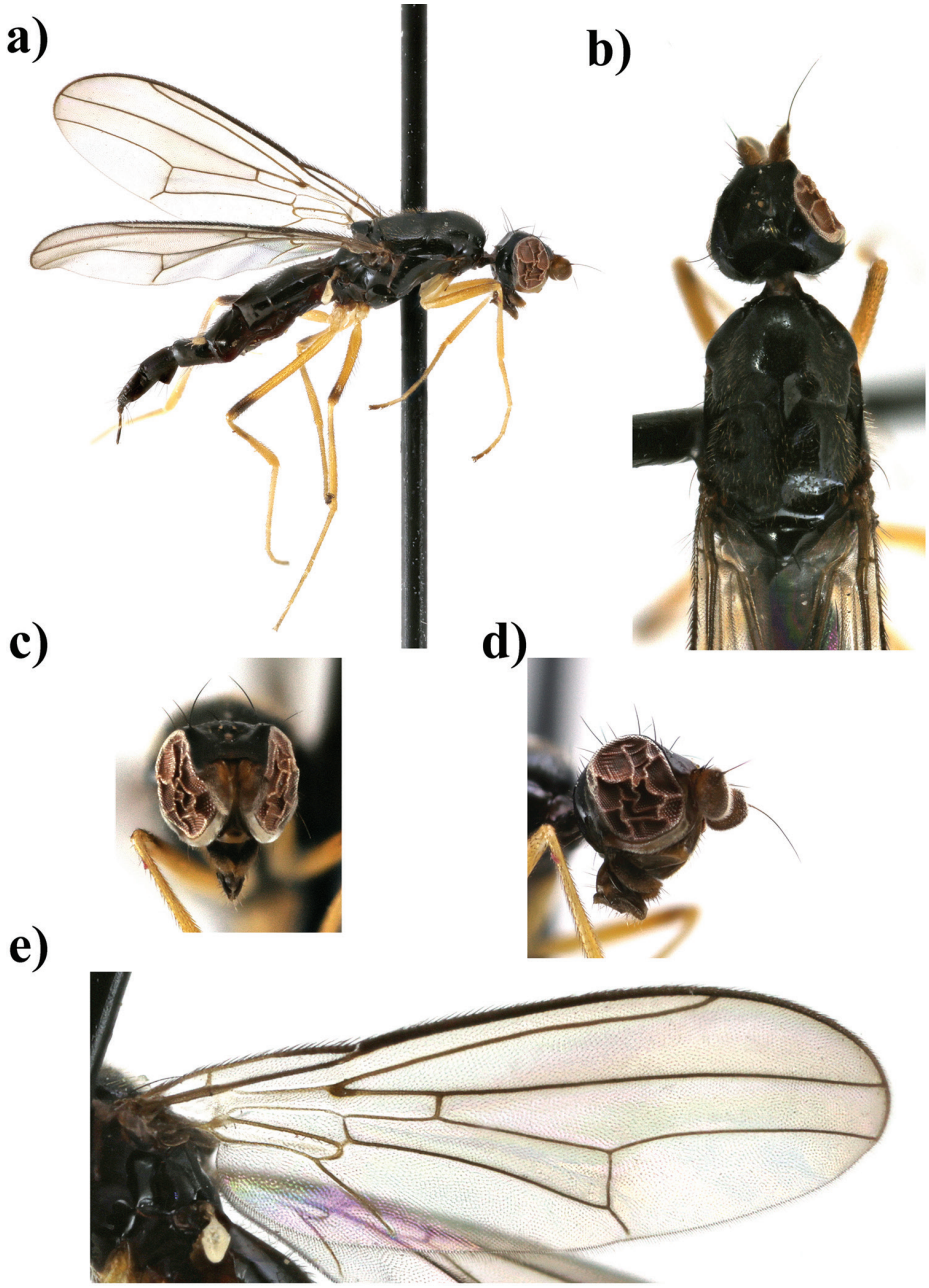

Figure 3. *Strongylophthalmyia dichroa* sp. n. **a** habitus, lateral view **b** head and thorax, dorsal view **c** head, anterior view **d** head, lateral view **e** basisternum **f** wing.

Description. Female. *Head* black, shining, slightly shorter than height. Frons entirely black, shining; ocellar tubercle slightly shifted towards anterior: the ratio between height of the frons from its anterior margin to hind ocelli and from hind ocelli to vertex or in-

ner vertical seta equal to 1.1. Occiput black shining, slightly convex. Face very narrow, yellowish, with triangular black spot in lower part, and consequently it seems dark; face with row of short setulae along suture. Parafacial yellow, covered with white tomentum. Basal antennal segments yellow, first flagellomere darkened, rounded, with short yellow dorsal setulae. Arista brown, bare. Mouthparts brown; palpus yellow, darkened in basal half on anterior margin, with some black setulae apically. Chaetotaxy. Three orbital setae, 1 ocellar seta, 1 postocellar seta, 1 inner vertical seta, 1 outer vertical seta, 1 frontal seta.

Thorax black. Basisternum without a pair of yellow spots. Mesonotum slightly matte, clothed with short yellow dense setulae; postpronotum shining, practically bare; pleuron, including region surrounding anterior spiracle black shining, anepisternum with well-developed light or yellowish setulae ventrally near the mid-coxa and posteriorly near the pleural suture; scutellum bare, matte. *Legs* yellow, mid and hind femora with dark brown preapical ring; hind tibia with traces of darkening in the basal half; last two tarsal segments slightly brownish. *Wings* transparent; cell r_{4+5} and posterior border of cell r_{2+3} slightly smoke-coloured. Vein R_{2+3} long, its end far beyond the level of dm-cu: section of C between R_1 and R_{2+3} in 1.9 times longer than a projection of a following section (between R_{2+3} and R_{4+5}). R_{4+5} and M_{1+2} nearly parallel apically. Vein M_{1+2} between r-m and dm-cu approximately 2 times longer than previous one and nearly 1.4 times shorter than ultimate one. Cell bm approximately 0.45 times shorter than discal cell. Calypter dark grey with fan of very long light setulae on margin. Halter with yellowish stem and whitish knob. Chaetotaxy: one very small postpronotal seta, one dorsocentral seta, two notopleural setae, one supraalar seta, one postalar seta, one anepisternal seta, one stout apical scutellar seta. All setae black.

Abdomen shiny black.

Body length 4.8 mm; wing length 4.0 mm.

Male unknown.

Etymology. The specific name refers to a bi-coloured palpus.

***Strongylophthalmyia gavryushini* sp. n.**

http://zoobank.org/B5F8CFA3-5B49-4068-A239-C8FD3D689163

Figure 4

Type material. Holotype: 1 male, Vietnam, Lai Châu Province, Sa Pa env. (22.3872°N, 103.7867°E), 1682 m, 25.V.2014 (D. Gavryushin) ZMUM

Diagnosis. Species of *Strongylophthalmyia* are characterized by a slight shift of the ocellar tubercle anteriorly and in some species, the ocellar tubercle can be situated in the middle of the frons. *Strongylophthalmyia gavryushini* sp. n., however, has the ocellar tubercle positioned nearly on the edge of the vertex. The value of frontal index (the ratio between height of the frons from its anterior margin to hind ocelli and from hind ocelli to vertex or vti) is high, equal to 4.3. The frontal index of the other new species of *Strongylophthalmyia* described in this paper vary from 1 up to 1.7. Only *S. tomentosa* sp. n. has the frontal index equal to 2.7.

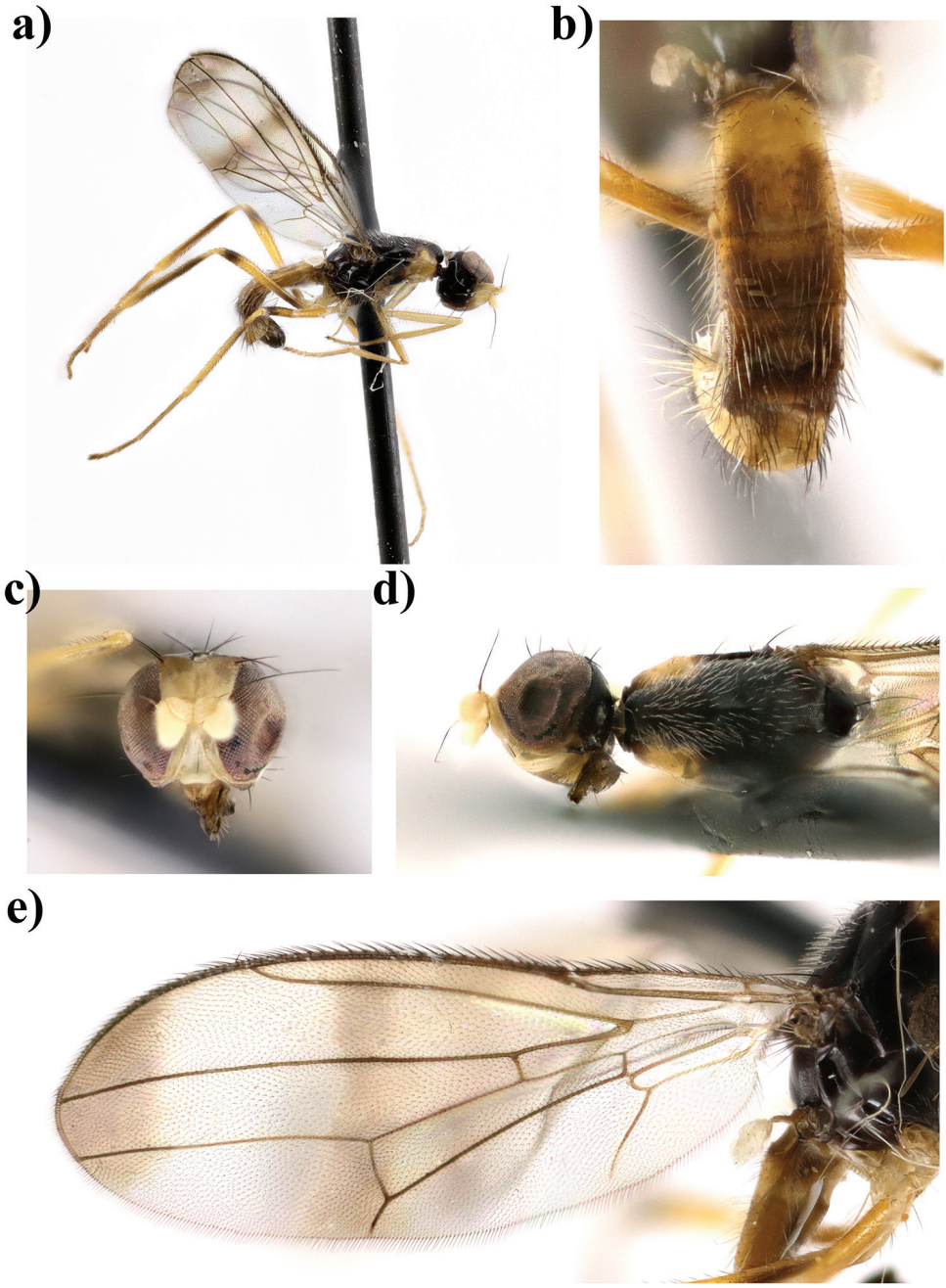

Figure 4. *Strongylophthalmyia gavryushini* sp. n. **a** habitus, lateral view **b** abdomen, dorsal view **c** head, anterior view **d** head and thorax, dorsal view **e** wing.

The new species is characterised by fore femur with row of ten black setae dorsally and with row of long dense yellowish setulae bent lateroapically aside of femoral apex, proximally these setulae 1.3 longer than distally. Tergite six with long black setulae dorsally and 6–7 long yellowish setulae laterally (Figure 4b). The new species is close to *S. trifasciata* Hennig. Males of these species are similar by abdomen partially yellow. *Strongylophthalmyia trifasciata* differs by wing with a short cross-band in anterior part at a level of R_s. At least last abdominal tergites of male are yellow, with black transverse band, tergite six with patch of thick black setae laterally (fig. 20 in Hennig 1941).

Description. Male. *Head* yellow, length almost equal to its height. Frons yellow, black shining posteriorly to hind margin of ocellar tubercle. Frontal index 4.3. Occiput black, slightly convex. Gena yellow. Face yellow, with row of short setulae along suture. Parafacial covered with silvery grey tomentum. Antenna light yellow. First flagellomere 1.6 times longer than width, with short yellow dorsal marginal setulae. Arista dark brown, basally yellow, bare. Palpus yellow. Clypeus dark brown. Frons between upper orbital and outer vertical setae with one short setula. Chaetotaxy: Two orbital setae (anterior – 0.18 mm, posterior – 0.24 mm); 1 ocellar seta, 1 postocellar seta, 1 inner vertical seta, 1 outer vertical seta, 1 hair-like very short frontal seta.

Thorax black. Postpronotum laterally and dorsally, propleuron, anepisternum around spiracle yellow. Mesonotum matte, sparsely covered with short yellow setulae; pleuron shiny; scutellum bare, matte. *Legs* yellow, mid and hind femora with dark brown preapical ring, hind femur brownish basally; mid tibia with brownish spot in basal quarter; hind tibia brown, yellow basally and apically. Fore femur with prominent row of ten black setae dorsally and with row of long dense yellowish setulae bent lateroapically aside of femoral apex, proximally these setulae almost as long as femor width. Mid tibia with two spurs, yellow and black, both 0.1 mm. *Wings* with apical spot, median transverse band at level of dm-cu and with weak darkening in anterior part at level of R_s. Vein R_{2+3} long, merged with C vein far from the level of dm-cu: section of C between R_1 and R_{2+3} 1.6 times longer than section between R_{2+3} and R_{4+5}. Veins R_{4+5} and M_{1+2} almost parallel apically. Section of M_{1+2} between r-m and dm-cu nearly 1.5 times longer than proximal section and around 0.6 times shorter than distal section. Cell bm approximately 0.4 times shorter than discal cell. Calypter brownish grey with fan of very long dark setulae on margin. Halter with yellow stem and whitish knob. Chaetotaxy: two small postpronotal seta, one dorsocentral seta, two notopleural setae, one supraalar seta, one postalar seta, one anepisternal seta, one stout apical scutellar seta (0.42 mm). All setae black.

Abdomen yellow, matte, with light brownish spots and strips (Figure 4b). Tergites with long dark brownish setulae, length of these setulae increased posteriorly; tergite six with 6–7 long yellowish setulae laterally.

Body length 3.4 mm; wing length 3.2 mm.

Female unknown

Etymology. The species is named after our colleague Dr. D.I. Gavryushin.

***Strongylophthalmyia obtecta* sp. n.**
http://zoobank.org/6BB4B76A-CC6A-4FF0-A283-3F9382A3F6F5
Figure 5

Type material. Holotype: 1 female, Vietnam, Lai Châu Province, Hoáng Liên (22.347948°N, 103.769714°E), 1900 m, 18.IV.2012 (A.L. Ozerov). Paratype: 1 female, Vietnam, Lai Châu Province, (22.347948°N, 103.769714°E), 1947 m, 22.V.2014 (D. Gavryushin) ZMUM.

Diagnosis. *Strongylophthalmyia obtecta* sp. n. and *S. stricta* sp. n. have the thorax with 2 dc and two rows of black setulae on the line of dc with one large setula before transverse suture, 2 rows of long black ac. Based on these characters both species are close to *S. raricornis* Shatalkin, 1981; *S. raricornis* differs from two new species by first flagellomere bilobate. *Strongylophthalmyia obtecta* sp. n. differs from *S. stricta* sp. n. in having fore femur and tibia yellow; and by having the two distal segments of the tarsus black (distal three segments black in female of *S. stricta* sp. n.)

Description. Female. *Head* entirely black, 0.9 times shorter than height; frons shining, entirely black, upper occiput slightly convex. Gena narrow; postgena broad, approximately 0.5 times shorter than eye height. Face dark brown, matte, with row of short setulae along suture. Parafacial with silvery grey tomentum, these setulae 0.25 times shorter than setulae along facial suture. Scape and pedicel yellowish brown, first flagellomere length almost equal to height. First flagellomere dark brown, with long pale marginal setulae, nearly 3 times less than flagellomere width. Arista dark brown, bare. Mouthparts and palpus black. Chaetotaxy: three orbital setae (medial seta 1.8 times longer than others); 1 ocellar seta, 1 postocellar seta, 1 inner vertical seta, 1 outer vertical seta; hair-like short frontal setae present.

Thorax black. Postpronotum laterally, proepisternum, anepisternum behind anterior spiracle yellowish brown, shining. Mesonotum matte, with rows of black setulae; dc row of setae includes 1+2 large dc; pleuron shining; scutellum slightly shiny. *Legs*. Fore coxa yellow with blackish stripe on anterior surface basally, mid and hind coxae yellow. Fore femur yellow, with blackish ring in apical third; mid and hind femora black, narrowly yellowish basally; fore tibia yellow, mid and hind tibia black, tarsi yellow, segment 3 brown, segments 4–5 dark brown to black. *Wings* with apical spot, median transverse band on level of dm-cu and with light brown spot anteriorly on level of R_s. Distal border of median band nearly reaching apex of vein R_{2+3}. Vein R_{2+3} long: section of C between R_1 and R_{2+3} 1.5 times longer than section between R_{2+3} and R_{4+5}. Veins R_{4+5} and M_{1+2} almost parallel apically. Section of M_{1+2} between r-m and dm-cu slightly concave, 2.5 times longer than proximal section and 0.7 times shorter than distal section. Cell bm 0.4 times shorter than discal cell. Calypter brownish grey with fan of very long yellowish setulae on margin. Halter with brownish stem and whitish knob. Chaetotaxy: one short postpronotal seta, two dorsocentral seta and two rows of black setulae on the line of dc with one large setula before transverse suture, two rows of long black acrostichal setulae, two notopleural setae, one supraalar seta, one postalar seta, one anepisternal seta, one stout apical scutellar seta and one short discal scutellar seta in front of apical ones. All setae black.

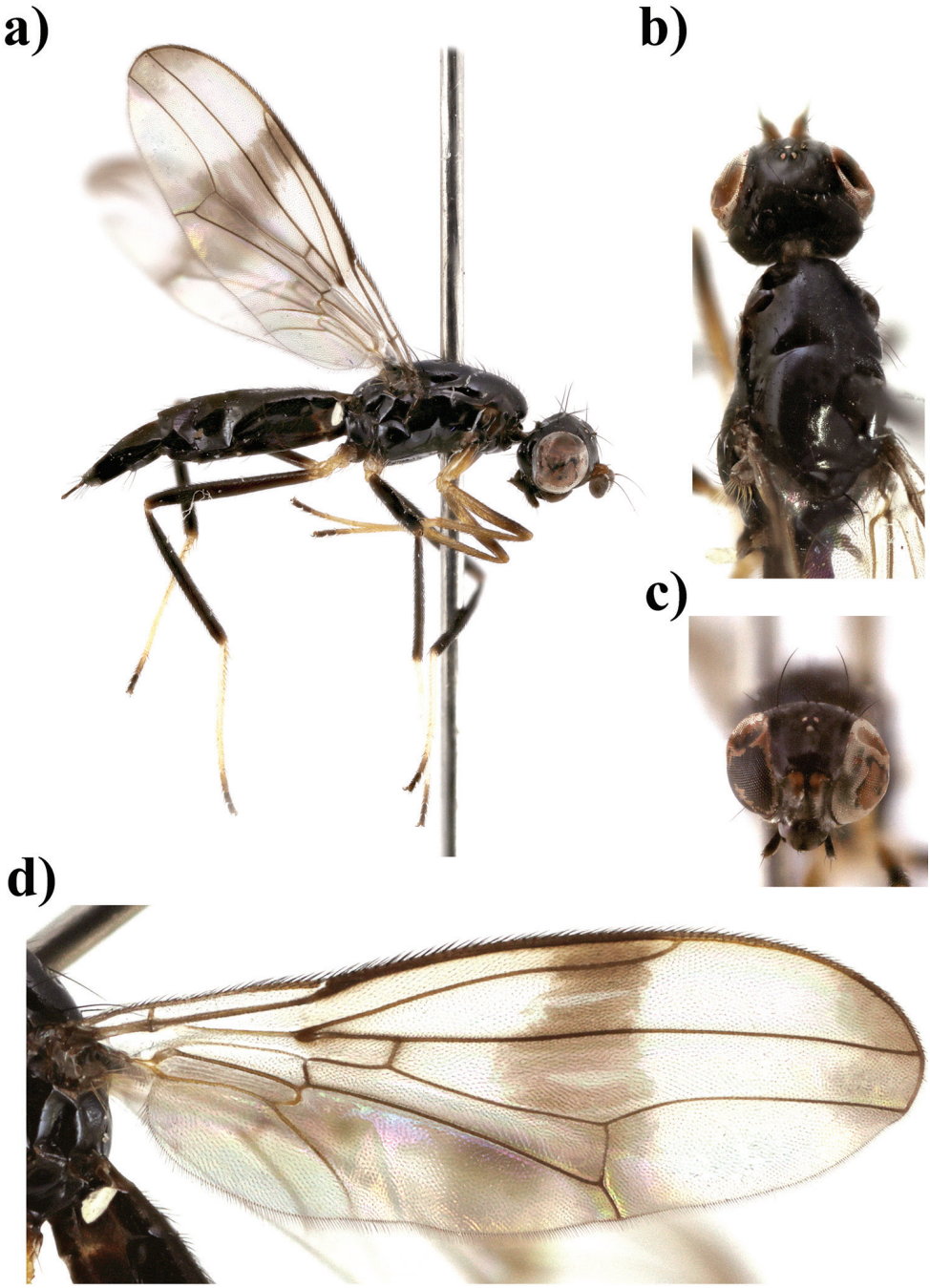

Figure 5. *Strongylophthalmyia obtecta* sp. n. **a** habitus, lateral view **b** head and thorax, dorsal view **c** head, anterior view **d** wing.

Abdomen shiny black, with brownish tinge on anterior margin of tergite 1.

Body length 4.2 mm (4.6 in paratype); wing length 3.7 mm (4.1 in paratype).

Male unknown.

Etymology. Obtectus (Latin) = matted. In this case it refers to the setulae covering the mesonotum.

Strongylophthalmyia orchidanthae **sp. n.**

http://zoobank.org/DDCAAC2F-4088-4CAF-A04E-C3F2FF18D565

Figure 6

Type material. Holotype: 1 male, Vietnam, Phu Tho province, Thanh Son district, Xuan Son National Park, (21°6'45"N, 104°57'25"E.), 23.X.2014 (T.V. Galinskaya). ZMUM.

Diagnosis. *Strongylophthalmyia orchidanthae* sp. n. is superficially similar to the *S. lutea* species group in having a yellow thorax. *S. lutea* species group is characterized by vein R_{2+3} short and section of C between R_1 and R_{2+3} 0.7 times shorter than distal section between R_{2+3} and R_{4+5}. The new species has section of C between R_1 and R_{2+3} approximately equal to distal section. This new species is similar to *S. immaculata* Hennig, 1940 from Formosa, which is included in the *S. lutea* group, i.e. characterized by short vein R_{2+3}. but *S. immaculata* differs from S. *orchidanthae* by armed fore femur, abdomen entirely black, mesonotum without pair of brown longitudinal stripes, wing transparent. *Strongylophthalmyia nigriventris* Frey, 1928 from Philippines, Malaysia and Papua New Guinea is characterized by a pair of brown stripes coalesced in anterior part of mesonotum, as in this new species. It differs from *S. orchidanthae* sp. n. by abdomen entirely black, legs entirely yellow and pedicel yellow, first flagellomere brownish.

Description. Male. *Head* yellow, 1.3 times longer than height, ocellar tubercle small, black; frons slightly widened towards vertex, frontal index equal to 1.3. Occiput slightly convex. Face yellow with row of yellow setulae along suture. Parafacial covered with silvery grey tomentum. Antenna yellow, pedicel black. First flagellomere slightly brownish basally, its length around 0.9 times shorter than high. Arista dark yellow, bare. Mouthparts and palpus yellow. Chaetotaxy: three orbital setae, 1 ocellar seta, 1 postocellar seta, 1 inner vertical seta, 1 outer vertical seta.

Thorax yellow with anterior 3/4 of presutural scutum black with one pair of black lines continuing along dorsocentral rows onto sides of scutellum. A pair of postsutural longitudinal stripes not merged with anterior arcuate area; in posterior part of mesonotum these stripes continued on scutellum. Scutellum yellow with black border laterally. Mediotergite blackish brown. *Legs* yellow, with coxae and tarsi white, two distal tarsal segments brown. Mid femur with light brownish preapical ring, mid tibia with brown subbasal band. Hind femur with light brown subapical ring; hind tibia with brown subbasal band; basal segment of hind tarsus darkened. *Wings* with brown apical spot and cross-band at level of vein dm-cu. Vein R_{2+3} long: section of C between R_1 and R_{2+3} nearly 1.1 times longer than section between R_{2+3} and R_{4+5}. Section of M_{1+2} between r-m and dm-cu nearly 2.3 times longer than proximal section and 0.7 times

Figure 6. *Strongylophthalmyia orchidanthae* sp. n. Habitus, lateral view.

shorter than distal section. Posterior basal cell approximately 0.6 times shorter than discal cell. Calypter yellowish with fan of very long yellow setulae on margin. Halter with yellow stem and whitish knob. Chaetotaxy: one very small postpronotal seta, one dorsocentral seta, two notopleural setae, one supraalar seta, one postalar seta, one anepisternal seta, one stout apical scutellar seta.

Abdomen yellow, tergite 1–2 with pair of black longitudinal stripes, tergite 3 with pair of light brown median spots, tergite 4 with large black triangular spot, tergite 5 with narrow dorsal band on anterior ¾. Sternite 8 large, situated on dorsal side, with large black spot. Epandrium yellow apically, brown basally.

Body length 4.3 mm; wing length 3.1 mm.

Female unknown

Etymology. The new species was collected on *Orchidantha* (Zingiberaceae).

***Strongylophthalmyia stricta* sp. n.**
http://zoobank.org/6EDEDB8E-FE8D-4F51-AA68-BB1396B66110
Figure 7

Type material. Holotype: 1 male, Vietnam, Lai Châu Province, Sa Pa env. (22.330396°N, 103.82418°E), 1284 m, 12.IV.2012 (A.L. Ozerov) ZMUM. Paratypes: 1 male, Vietnam, Lai Châu Province, Sa Pa env. (22.1454°N, 103.8053°E), 1448 m 21.V.2014 (D. Gavryushin); 1 female, Vietnam, Lai Châu Province, Hoáng Liên (22.347948°N, 103.769714°E), 1900 m, 22.V.2014 (A.L. Ozerov).

Diagnosis. *Strongylophthalmyia stricta* sp. n. and *Strongylophthalmyia obtecta* sp. n. have the thorax with 2 dc and two rows of black setulae on the line of dc with one large setula before transverse suture, 2 rows of long black ac. Based on these characters both species are close to *S. raricornis* Shatalkin, 1981; *S. raricornis* differs from two new species by first flagellomere bilobate. *Strongylophthalmyia obtecta* sp. n. differs from *S. stricta* sp. n. by fore femur and tibia yellow; two distal segments of tarsi black (instead of three distal segments in female of *S. stricta* sp. n.).

Description. *Head* entirely black, its length equal to its height. Frons shining, entirely black. Occiput slightly convex. Face brownish, with row of short setulae along suture. Parafacial covered with short silvery grey tomentum. Antenna yellowish brown, first flagellomere 1.5 times longer than high. First flagellomere yellow with dorsum dark brown, with long pale marginal setulae. Arista dark brown, bare. Mouthparts and palpus black. Chaetotaxy: Three orbital setae, 1 ocellar seta, 1 postocellar seta, 1 inner vertical seta, 1 outer vertical seta, 3 hair-like short frontal setae.

Thorax black. Postpronotum laterally, proepisternum, anepisternum behind anterior spiracle yellowish brown, shining. Mesonotum matte, with rows of black setulae; 1+3 large dorsocentral setae; pleuron shining; scutellum matte. *Legs*. Fore coxa black with anterolateral margin of fore coxa yellowish and mid and hind coxae yellowish distally, fore femur black, narrowly yellowish basally and apically; mid and hind femora black; fore tibia yellow with brownish ring in basal third; mid and hind tibia black, tarsi yellow, two distal segments black. Hind trochanter with a posterior round blackish spot; hind femur basally with round posteroventral process, without 3–4 ventral setulae (as in male of *S. papuana*), and with small subbasal black posterior process with two yellow setulae on it and with two yellow setae on trochanter distally to this process. *Wings* with apical spot, median transverse band at level of dm-cu and light brown spot anteriorly at level of R_s. Distal border of median band reaching apex of vein R_{2+3}. Vein R_{2+3} long: section of C between R_1 and R_{2+3} 1.3 times longer than penultimate section (between R_{2+3} and R_{4+5}). Veins R_{4+5} and M_{1+2} almost parallel apically. Proximal section of M_{1+2} between r-m and dm-cu 2.7 times longer than proximal one and 0.7 times shorter than distal one. Cell bm 0.4 times shorter than discal cell. Calypter brownish grey, with fan of very long light setulae on margin. Halter with brownish stem and whitish knob. Chaetotaxy: one small postpronotal seta, 1+3 dorsocentral setae, two rows of acrostichal setulae, two notopleural setae, one supraalar seta, one postalar seta, one anepisternal seta, one stout apical scutellar seta and one short discal scutellar seta in front of apical seta. All setae black.

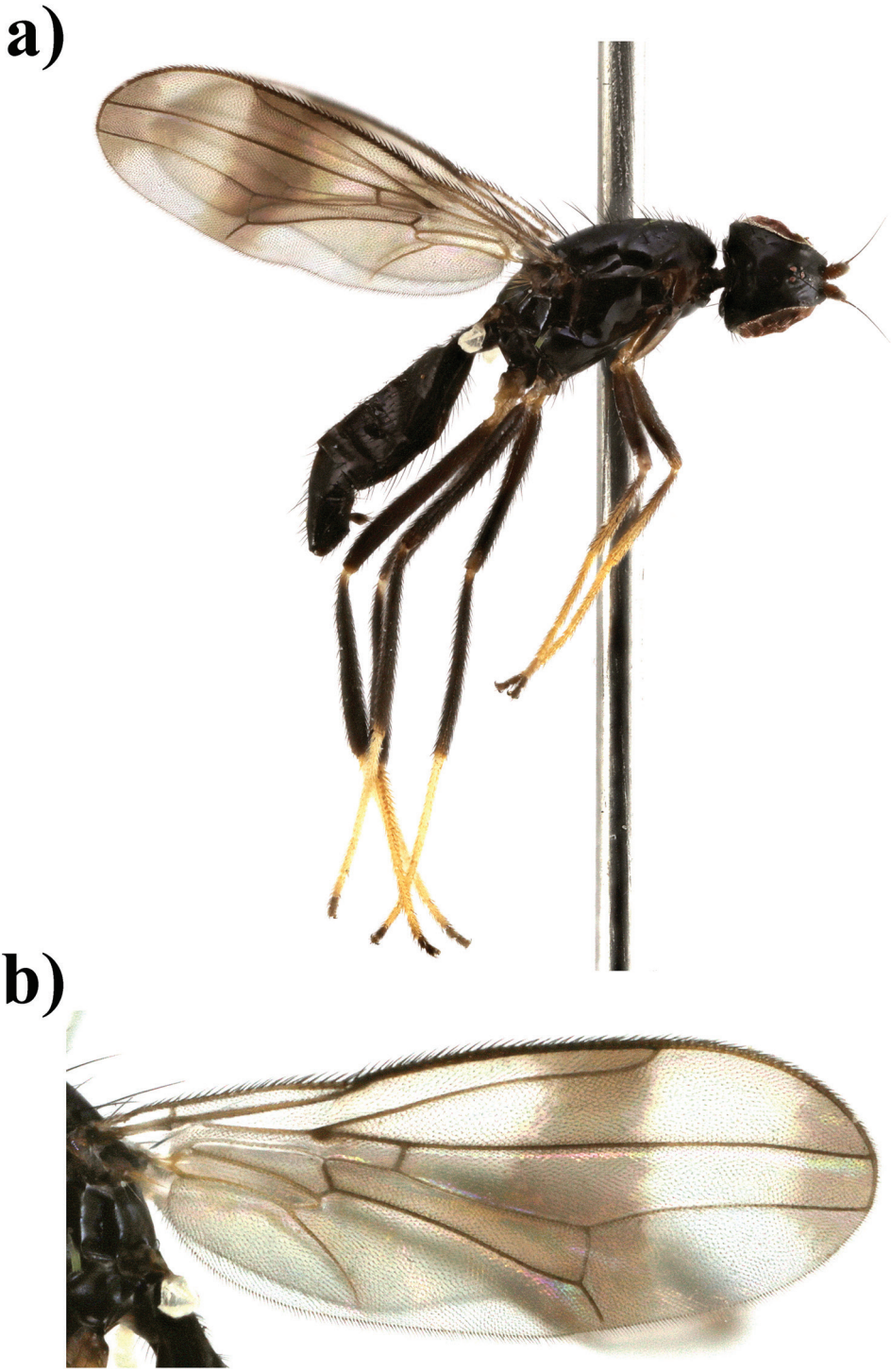

Figure 7. *Strongylophthalmyia stricta* sp. n. **a** habitus, lateral view **b** wing.

Abdomen black, shining, with two yellowish spots dorsally on first tergite.

Body length 3.3 mm; wing length 2.9 mm.

Female differs by having three black distal tarsal segments; abdomen totally black; trochanter without processes.

Body length 4.8 mm; wing length 4.2 mm.

Etymology. *Strongylophthalmyia stricta* is characterized by a dense field of adjoining setulae on the scutum that are sticking out or protrusive (*strictus* in Latin), and rarely ordered in regular lines.

Strongylophthalmyia tomentosa sp. n.

http://zoobank.org/D592F9BC-9BC5-42EE-AF71-E4F9960805DA
Figure 8

Type material. Holotype: 1 female, Vietnam, Lai Châu Province, Tam Duong Distr. (22.37017°N, 103.75793°E), 1745 m, 26.X.2015 (D.Gavryushin). Paratype: 1 female, Vietnam, Lai Châu Province, Hoáng Liên (22.347948°N, 103.769714°E), 1900 m, 11.IV.2012 (A.L.Ozerov).

Diagnosis. The new species belongs to a large and varied group of species with a yellow postpronotum. Within this group, *Strongylophthalmyia tomentosa* sp. n. is close to *S. bifasciata* Wang & Yang from China (Zhejiang) on the basis of a longitudinal band running along R2+3 from its base to the medial transverse band. *Strongylophthalmyia dorsocentralis* has a similar wing band, but this species differs from first two species by many characters, including the coloration of the thorax and by the absence of microsetulae across the mesonotum. *Strongylophthalmyia tomentosa* sp. n. differs from *S. bifasciata* by the shorter vein R_{2+3}, smaller size of apical spot and by bare arista. *Strongylophthalmyia tomentosa* sp. n. is also similar to *S. annulipes* sp. n. (see the diagnosis of *S. annulipes*).

Description. Female. *Head* slightly shorter than height. Frons matte black, narrowly yellow anteriorly. The ratio between height of the frons from its anterior margin to hind ocelli to the distance from the hind ocelli to vertex or vti is equal to 2.7. Occiput black, shining, slightly convex. Face yellow with row of short setulae along suture, clypeus yellow shining; parafacial yellow with silver grey tomentum. Gena black with small brownish spot between lateral margin of mouth and parafacial. Antenna yellow, first flagellomere rounded, 0.9 times shorter than height, dark brown on inner surface, narrowly yellow basally, widely dark brown apically and on dorsal margin externally, with short yellow dorsal setulae. Arista dark brown, bare. Mouthparts dark brown; palpus yellow, with short and thick black setulae apically. Chaetotaxy: Two orbital setae (posterior seta 2.6 times longer than anterior seta), 1 ocellar seta, 1 postocellar seta, 1 inner vertical seta, 1 outer vertical seta, frontal setae absent.

Thorax black. Postpronotum, proepisternum, basisternum, anepisternum around of spiracle yellow, and yellow coloration on postpronotum slightly extending to mesonotum (Figure 8a,b). Mesonotum matte, sparsely covered with short pale setulae; pleuron

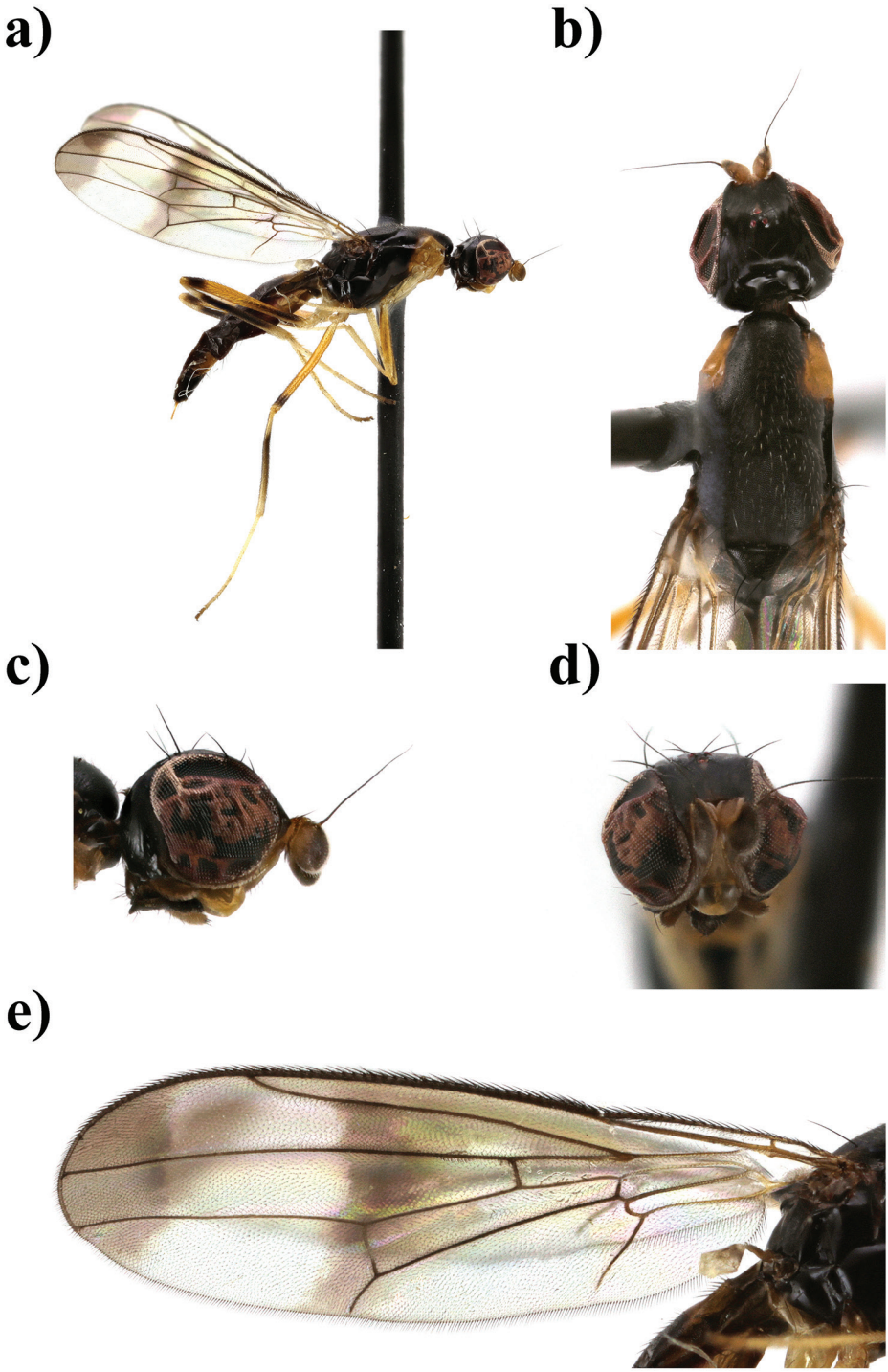

Figure 8. *Strongylophthalmyia tomentosa* sp. n. **a** habitus, lateral view **b** head and thorax, dorsal view **c** head, lateral view **d** head, anterior view **e** wing.

shining; scutellum bare, matte. *Legs*. Fore legs yellow, fore femur externally with pale brownish spot basally and apically. Mid legs yellow, mid femur with dark brown preapical ring, mid tibia: yellow, dark brown medially. Hind legs yellow, hind femora with dark brown preapical ring and with brown spot basally, hind tibia blackish, yellow basally and in apical quarter; fore tarsi yellow, mid tarsi yellow with brownish apical segment, hind tarsi yellow with three apical segments brownish. *Wings* with strong apical band, median transverse band on level of dm-cu (section of this vein between C and R_{2+3} undeveloped). R_{2+3} long: section of C between R_1 and R_{2+3} 1.5 times longer than distal section (between R_{2+3} and R_{4+5}). R_{4+5} and M_{1+2} almost parallel apically. Section of M_{1+2} between r-m and dm-cu 1.8 times longer than proximal section and 0.6 times shorter than distal section. Cell bm 0.6 times shorter than discal cell. Calypter light grey with fan of very long light setulae on margin. Halter with yellow stem and whitish knob. Chaetotaxy: one very small postpronotal seta, one dorsocentral seta, two notopleural setae, one supraalar seta, one postalar seta, one anepisternal seta, one stout apical scutellar seta. All setae black.

Abdomen black, shining, tergite 1 and basal half of tergite 2 yellow, and one pair of lateral yellow spots on anterior margin of tergite 6 extending to two thirds of its length.

Body length 3.6 mm; wing length 3.7 mm.

Male unknown.

Etymology. The mesonotum of the new species is covered with short setulae, giving it the appaerance of a felt surface.

Species new to the fauna of Vietnam

Strongylophthalmyia angusticollis Frey

Strongylophthalmyia angusticollis Frey, 1956: 132 (male, female)

Material. 1 male, Vietnam, Lai Châu Province, Hoáng Liên (22.347948°N, 103.769714°E), 1900 m, 16.IV.2012 (A.L. Ozerov); 1 female, same locality, 18.IV.2012 (A.L. Ozerov). ZMUM.

Diagnosis. *Strongylophthalmyia angusticollis* is characterized by the following combination of characters. Frons black, shining, yellow in anterior one third or quarter. Face yellow. Arista dark brown, pubescent. Thorax black, basisternum yellow in male or with a pair of yellow spots in female (original description lack these characters). Mesonotum matte, sparsely covered with short yellowish setulae. 1 dc. Legs yellow, mid and hind femora with preapical brown ring; mid and hind tibiae with wide subbasal brownish ring. Wings with apical spot, median transverse band at level of dm-cu and with light darkening in anterior part at level of R_s. Wing median band expanded along cell r_{4+5} in the direction of vein r-m. Abdomen entirely black, shining.

Strongylophthalmyia fascipennis **Frey**

Strongylophthalmyia fascipennis Frey, 1928: 102 (male)

Material. 1 male, Vietnam, Lai Châu Province, Hoáng Liên (22.33788°N, 103.77922°E), 2068 m, 1 and 7.V. 2013 (T.V. Galinskaya). ZMUM.

Strongylophthalmyia metatarsata **Meijere**

Strongylophthalmyia metatarsata Meijere, 1919: 35 (female)

Material. 1 male, Vietnam, Lai Châu Province, (22.347948°N, 103.769714°E), 1947 m, 22.V.2014 (D. Gavryushin). ZMUM.

Strongylophthalmyia splendida **Yang & Wang**
Figure 9

Strongylophthalmyia splendida Yang & Wang, 1998 (1996): 459 (female).

Material. 1 male, Vietnam, Lai Châu Province, Hoáng Liên (22.347948°N, 103.769714°E), 1700 m, 22.V.2014 (A.L. Ozerov); 1 female, Vietnam, Lai Cai Province, So Pa (22.34147°N, 103.85818°E), 1490 m, 24.X.2015 (D. Gavryushin).

Diagnosis. *Strongylophthalmyia splendida* was described based on single female from Tibet (China), caught at a height of 2050 m. This species is characterized by some interesting and unique characters, including a head that is nearly 1.4 times longer than high (which is caused mainly by lengthening of the occiput). In other *Strongylophthalmyia*, the head is globular (approximately as high as long) and the occiput part is short, only 0.1–0.2 times shorter than length of the eye. Furthermore, the outer vertical seta is absent, the frontal setae are absent and only one short hair-like orbital seta is developed. We also note yellow coloration of frons with a brown round spot between the ocellar tubercle and the vertex.

Other interesting character of the species is the presence of a large seta situated on the anterior part of the mesonotum near the postpronotum in front of posthumeral line (Fig. 9b). Vein R_{4+5} is slightly arcuate, terminating behind the wing apex (Fig. 9a); vein CuA_2 is more or less straight; cell bm is rather long, terminating approximately at the level of the costal break; cell bm is shorter than the discal cell by approximately in 0.8 times.

Description of male. *Head* yellow, shining in black area, 1.4 times longer than height. frons with brownish to blackish (in view from different angles) rounded spot not

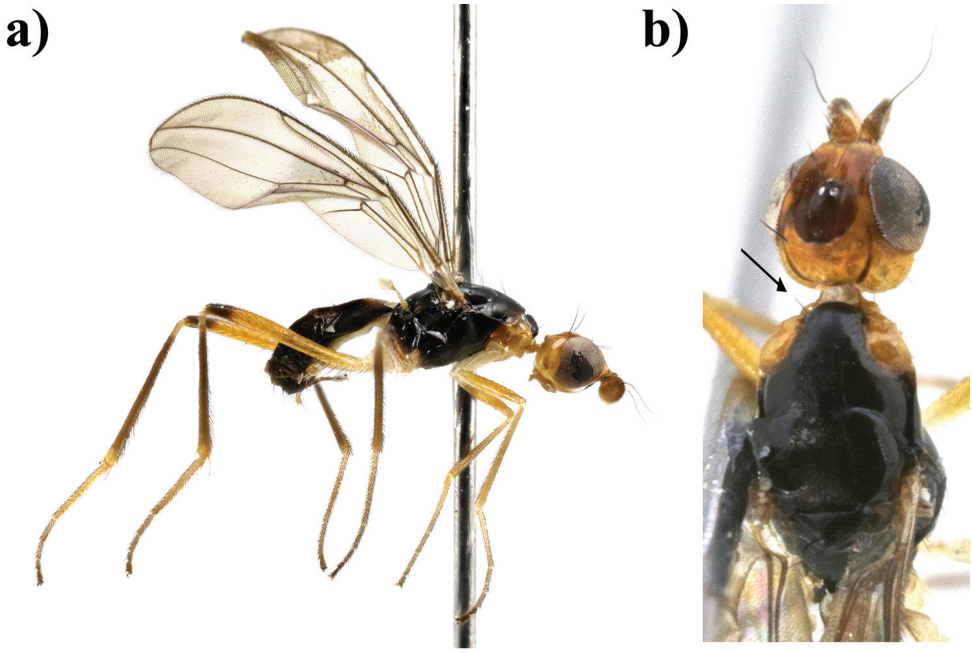

Figure 9. *Strongylophthalmyia splendida* Yang & Wang, 1996. **a** habitus, lateral view **b** head and thorax, dorsal view.

reaching eye that extends from level of hind ocelli to postocellar setae. Ocellar tubercle slightly shifted anteriorly: ratio between height of frons from its anterior margin to hind ocelli and from hind ocelli to vertex or vti equal to 1.4. Occiput convex. Face yellow, with row of short setulae along suture. Parafacial covered with silvery grey tomentum. Antenna yellow. First flagellomere rounded, length almost equal to height, very large, 1.9 times less than eye length, with short yellow dorsal setulae. Arista dark brown, basally yellow, bare. Palpus yellow. Chaetotaxy: One short orbital seta, 1 ocellar seta, 1 postocellar seta, 1 inner vertical seta, outer vertical seta absent; frontal setae absent.

Thorax black. Postpronotum laterally and dorsally, propleuron, anepisternum around spiracle, basisternum yellow. Mesonotum matte, covered with short yellowish setulae; pleuron shining. Scutellum bare matte. ***Legs*** yellow, mid and hind femora with narrow dark brown apical ring; mid tibia with brownish stroke in basic quarter; hind tibia darkened, yellowish basally and apically. Fore femur dorsally with row of 10 black setae. Fore femur laterally with row of long dense yellowish setulae situated apically aside of femoral apex; basally these setulae 1.3 times longer than those occurring apically. *Wings* greyish, without apical spot and without median transverse band. Vein R_{2+3} short, section of C between R_1 and R_{2+3} equal to following section (between R_{2+3} and R_{4+5}). R_{4+5} and M_{1+2} convergent and almost parallel apically. Section of M_{1+2} between r-m and dm-cu 2.2 times longer than proximal section and 0.45 times shorter than distal section. Calypter brownish grey with 4 long dark setulae on margin. Halter

with yellow stem and whitish knob. Chaetotaxy: postpronotal seta absent, 1 + 2 dorsocentral setae, two notopleural setae, one supraalar seta, one postalar seta, one large anepisternal seta, one stout apical scutellar seta. All setae black. Mesonotum with large seta anteriorly, near postpronotum (Fig. 9b: marked by arrow), which we consider as sublateral (Hennig 1973: 184, fig. 109).

Abdominal tergites black, shining; tergite 6 brownish yellow; sternites yellow.

Body length 3.4 mm; wing length 3.7 mm.

Strongylophthalmyia thaii **L. Papp**

Figure 10

Strongylophthalmyia thaii L. Papp, 2006: 171 (male)

Material. 2 females, Vietnam, Lai Cai Province, So Pa (22.34147°N, 103.85818°E), 1490 m, 28.X.2015 (D. Gavryushin); 1 male, Thailand, Chang Mai, Sop Poeng (19.122°N, 98.805°E), 13–17. XI. 2009 (N. Vikhrev).

Description of female. *Head* rounded, its length equal to its height. Frons shiny black, with yellowish spot between antenna and eye. Face yellow with row of short blackish setulae along suture. Clypeus black, shining. Parafacial yellowish, covered with silvery grey tomentum. Antenna yellow, first flagellomere transversal, its length 0.7 times shorter than its height, yellowish-orange, widely dark brown on apical margin on outer surface, with short yellow dorsal setulae. Arista dark brown, bare. Mouthparts dark brown; palpus black, yellowish on basal fourth, with short thick black setulae apically. 3 orbital setae, 1 ocellar seta, 1 postocellar seta, 1 inner vertical seta, 1 outer vertical seta; 2 short frontal setae.

Thorax. Postpronotum laterally, propleuron, anepisternum around of spiracle and basisternum yellow. Mesonotum black shining, sparsely clothed with short yellowish setulae; pleuron shiny, anepisternum medially without fan of 14–15 very long yellow setulae peculiar to male; scutellum shiny black. *Legs* yellow, hind femora with dark brown narrow apical ring; mid tibia darkened slightly in basal half; hind tibia darkened dorsally and ventrally in basal two thirds. *Wings* transparent. R_{2+3} long: section of C between R_1 and R_{2+3} 1.6 times longer than a projection of the following section (between R_{2+3} and R_{4+5}). R_{4+5} and M_{1+2} nearly parallel apically. Basal section of M_{1+2} between r-m and dm-cu equal to previous section and nearly 2.0 times less than ultimate section. Cell bm approximately 0.5 times shorter than discal cell. Calypter light grey with fan of very long light setulae on margin. Halteres with yellow stem and whitish knob. Chaetotaxy: two small postpronotal seta, two dorsocentral seta, two notopleural setae, one supraalar seta, one postalar seta, one anepisternal seta, one stout apical scutellar seta and one short setae in front of apical ones. All setae black.

Abdomen shiny black, tergite 1 and partly 2 yellowish.

Body length 3.9 mm; wing length 3.5 mm.

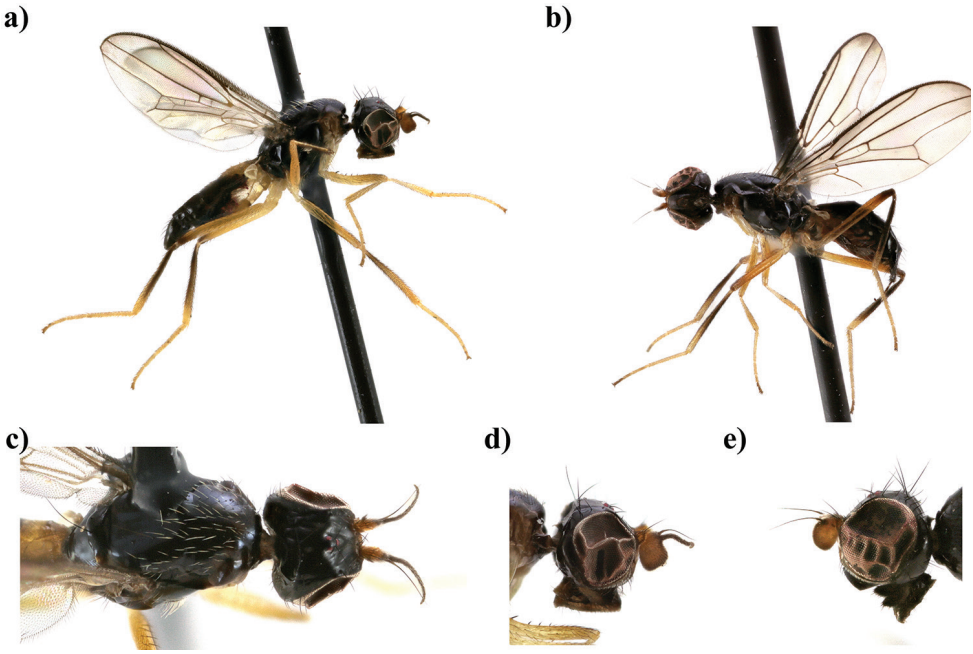

Figure 10. *Strongylophthalmyia thaii* L. Papp, 2006 **a** habitus male, lateral view **b** habitus female, lateral view **c** head and thorax, male, dorsal view; head, anterior view **d** head, male, lateral view **e** head, female, lateral view.

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References

Barber KN (2006) *Strongylophthalmyia pengellyi* n. sp., a second species of Nearctic Strongylophthalmyiidae (Diptera). Journal of the Entomological Society of Ontario 137: 81–109.

Barraclough DA (2000) The identity of *Strongylophthalmyia* Heller species (Diptera: Schizophora: Strongylophthalmyiidae) described from the Afrotropical Region, and their transfer to the family Clusiidae. Annals of the Natal Museum 41: 103–106.

Cumming JM, Wood DM (2009) Chapter 2: Adult morphology and terminology. In: Brown BV, Borkent A, Cumming JM, Wood DM, Woodley NE, Zumbado MA (Eds) Manual of Central American Diptera. Volume 1, National Research Council of Canada, Ottawa, 9–50.

Frey R (1928) Philippinische Dipteren – VI. Fam. Sciomyzidae, Psilidae, Megamerinidae, Sepsidae, Piophilidae, Clusiidae. Notulae Entomologicae 8: 100–108.

Frey R (1956) Studien uber ostasiatische Dipteren. V. Psilidae, Megamerinidae. Notulae Entomologicae 35: 122–137.

Heller KM (1902) *Strongylophthalmyia* nom. nov. für *Strongylophthalmus* Hendel. Wiener Entomologische Zeitung 21: 226.

Hendel F (1913) H. Sauter's Formosa-Ausbeute. Acalyptrate Musciden (Dipt.). II. Supplementa Entomologica 2: 77–112

Hennig W (1940) Aussereuropaische Psiliden und Platystomiden im Deutschen Entomologischen Institut (Diptera). Arbeiten über Morphologische und Taxonomische Entomologie aus Berlin-Dahlem 7: 304–318.

Hennig W (1973) Ordnung Diptera (Zweiflfiger). In: Beier M (Ed.) Handbuch der Zoologie 4(2): 1–337.

Iwasa M (1998) Family Strongylophthalmyiidae. In: Papp L, Darvas B (Eds) Contributions to A Manual of Palaearctic Diptera, 3. Science Herald, Budapest, 173–175. doi: 10.1111/ens.12033

Iwasa M, Evenhuis NL (2014) The Strongylophthalmyiidae (Diptera) of Papua New Guinea, with descriptions of five new species and a world checklist. Entomological Science 17: 96–105.

Krivosheina NP (1981) New Palearctic species of Diptera of the genus *Strongylophthalmyia* Hell. (Diptera, Strongylophthalmyiidae). Entomological Review 60: 162–165.

Krivosheina NP (1984) Family Strongylophthalmyiidae. In: Soos A, Papp L (Eds) Catalogue of Palaearctic Diptera. 9. Micropezidae–Agromyzidae. Akademiai Kiado, Budapest, 27–28.

Lonsdale O (2013) Review of the Families Tanypezidae and Strongylophthalmyiidae, with a Revision of Neotanypeza Hendel (Diptera: Schizophora). Smithsonian Contributions to Zoology 641: 1–60. doi: 10.5479/si.19436696.641.1

Meijere JCH de (1914) Studien uber sudostasiatische Dipteren IX. Tijdschrift voor Entomologie 57: 137–168.

de Meijere JCH (1919) Beitrag zur Kenntnis der sumatranischen Dipteren. Beitrage tot de Dierkunde 21: 13–40.

Papp L (2006) Strongylophthalmyiidae. In: Papp L, Merz B, Földvári M (Eds) Diptera of Thailand – A summary of the families and genera with references to the species representations. Acta Zoologica Academiae Scientiarum Hungaricae 52(2): 165–172.

Shatalkin AI (1981) *Claraeola crassula* sp. n. (Pipunculidae) and *Strongylophthalmyia raricornis* sp. n. (Strongylophthalmyiidae), new peculiar Diptera from the USSR. Zoologicheskiy Zhurnal 60: 791–793. [In Russian]

Shatalkin AI (1993) On the taxonomy of the flies of the family Strongylophthalmyiidae (Diptera). Zoologicheskiy Zhurnal 72: 124–131. [In Russian]

Shatalkin AI (1995) Palaearctic species of Pseudopomyzidae (Diptera). Russian Entomological Journal 3: 129–145.

Shatalkin AI (1996) New and little known species of flies of Lauxaniidae and Strongylophthalmyiidae (Diptera). Russian Entomological Journal 4: 145–157.

Steyskal GC (1971) Notes on the genus *Strongylophthalmyia* Heller, with a revised key to the species (Diptera: Strongylophthalmyiidae). Annals of the Entomological Society of America 64: 141–144. doi: 10.1093/aesa/64.1.141

Steyskal GC (1987) 59. Strongylophthalmyiidae. In: McAlpine JF, Peterson BV, Shewell GE, Teskey HJ, Vockeroth JR, Wood DM (Eds) Manual of Nearctic Diptera. 2. Research Branch, Agriculture Canada, Ottawa, 777–780.

Verbeke J (1963) Note sur quelques Psilidae et Micropezidae éthiopiens et malgaches (Diptera, Acalyptera). Revue de Zoologie et de Botanique Africaines 67: 163–175.

Verbeke J (1968) Psilidae (Diptera, Acalyptera). Parc National Garamba. Mission H de Saeger. Bruxelles 53: 65–78.

Yang C, Wang X (1992) A preliminary study of the Psilidae and Strongylophthalmyiidae of Mt. Mogan with two new species (Diptera: Acalyptratae). Journal of the Zhejiang Forestry College 9: 446–449. [In Chinese]

Yang C, Wang X (1998) Strongylophthalmyiidae. In: Xue W, Chao C (Eds) Flies of China 1, Liaoning Science and Technology Press, Shenyang, 457–463. [In Chinese with English summary]