

# *Nesticus baeticus* sp. n., a new troglobitic spider species from south-west Europe (Araneae, Nesticidae)

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## Abstract

A new troglobitic species, *Nesticus baeticus* sp. n. (♂♀), inhabiting the karst landscapes of the high part of the Cazorla, Segura and Las Villas Natural Park (NE Jaén, Spain) where it has been found in 8 caves is diagnosed and described, its distribution and habitat are also analyzed. The new species belongs to the Iberian species group that includes *N. luquei*, *N. lusitanicus* and *N. murgis*. Evolutionary relationships of the Iberian *Nesticus* species are discussed on the basis of morphological and molecular data (*cox1* and *rrnL*).

## Keywords

Arachnida, Araneae, taxonomy, description, new species, caves, Iberian Peninsula, Mediterranean basin

## Introduction

The genus *Nesticus* Thorell, 1869 is distributed worldwide except for south-eastern Asia and Australia and comprises 125 species and 8 subspecies (Platnick 2011). In Europe *Nesticus* is represented by 23 species, of which five are known from Iberia. Unlike *N. cellulanus* (Clerck, 1757), a species with a holarctic distribution, the four others are

endemic to Iberia, being cavernicolous species with more or less evident troglomorphic features.

The first species described from Iberia was *N. obcaecatus* Simon, 1907, found only from a single locality: Cueva del Molino de Aso (Huesca), on the southern slopes of the Central Pyrenees (Simon 1907). The species description was originally based on a single female specimen. The male was described several decades later (Ribera 1979), from a series of both sexes collected from the same cave. The second Iberian endemic is *N. lusitanicus* Fage, 1931, a native species of the karst landscape in central Portugal. This species was described on the basis of females (Fage 1931), its male was found half of a century later (Ribera 1988). The third species, which was found in several caves in Asturias and Cantabria (north-western Iberian Peninsula) (Ribera and Guerao, 1995), is *N. luquei* Ribera & Guerao, 1995. Finally, *N. murgis* Ribera & De Mas, 2003 was described from a cave in the province of Almeria (Ribera and De Mas 2003). Overall, ranges of these species are rather small, in two species (*N. obcaecatus* and *N. murgis*) being restricted to a single cave.

This work describes a new cavernicolous species whose distribution includes several karst landscapes of the different mountain formations that make up the Sistema Bético, the ridge in southern Iberian Peninsula.

## Materials and methods

### Taxonomy

Abbreviations used in the text are as follows: PL = prosoma length (from posterior edge of carapace to front edge of clypeus, measured at midline); PW = maximum prosoma width; OL = opisthosoma length (excluding the pedicel); OW = maximum opisthosoma width; MA = median apophysis; Mt = metatarsus; Tb = tibia; TTA = theridioid regular apophysis; TTA p1 = process 1 of TTA; TTA p2 = process 2 of TTA; TL = total length (excluding the pedicel). Eyes: AM = anterior median; AL = anterior lateral; PM = posterior median; PL = posterior lateral; i = immature; sub = subadult; GEV = Grupo de Espeleología de Villacarrillo.

Female vulva was removed and treated with 30% KOH. After observation and drawing, the vulva was washed in distilled water and stored in 70% ethanol. Left male palps were illustrated in all cases. We follow Coyle and McGarity (1992) for describing the paracymbium, and Huber (1993) and Agnarsson et al. (2007) for other parts of male and female copulatory organs. Holotype and paratypes have been deposited in the Arachnida Collection of the CRBA (Centre de Recursos de Biodiversitat Animal) at the University of Barcelona. Catalogue numbers are given in brackets.

A Nikon Coolpix 4500 digital camera attached to a stereomicroscope was used to capture images. Following Coleman (2003) and with the aid of a Trust Scroll Tablet TB-4200 the samples were drawn with repeated reference to the specimen under the microscope. The specimens used for SEM studies were dehydrated with alcohol gradi-

ent dehydration and ultrasonically cleaned. They were then critical-point dried and were mounted and covered with gold and examined using a HITACHI S-2300 Scanning Electron Microscope (SEM) (SCT, Universitat de Barcelona, Spain).

## Phylogeny

**Taxonomic sampling.** Taxa analyzed in the present study are listed in Appendix 1. All the Iberian species are included except *N. murgis* due to impossibility to obtain fresh material for DNA analysis. *N. eremita* Simon, 1879 from Croatia and *N. ionescui* Dumitrescu, 1979 from Romania are also included to test the monophyly of Iberian species. Sequence from *Nesticus* sp. from China (Arnedo et al. 2004) was also included in the analysis as a more distantly related *Nesticus* species that was used to root the tree.

**Sample Storage and DNA Extraction.** Specimens were preserved in 95% or absolute ethanol and stored at 4°C. Total genomic DNA was extracted from legs of a single specimen using the QIamp® DNA Mini Kit (QIAGEN) following the manufacturer's protocols. The approximate concentration and purity of the DNA obtained were verified using 1% agarose/TBE gel electrophoresis.

**PCR Amplification and Sequencing.** Two regions of the mitochondrial DNA corresponding to a fragment of the cytochrome oxidase I gene (*cox1*) and 16S rRNA (*rrnL*) were selectively amplified using PCR with the following primer pairs: for *cox1* C1-J-1718 (5' GGAGGATT'TGGAAATTGATTAGTTCC 3') with C1-N-2191 (5' CCCGGTAAAATTTAAAATATAAACTTC 3') (Simon et al. 1994); for *rrnL* LR-N-13398 (5' CGCCTGTTTATCAAAAACAT 3') (Simon et al. 1994) with LR-J-12864 (5' CTCCGGTTTGAAGCTCAGATCA 3') (Arnedo and Gillespie 2006). The PCR reaction mixture contained a final concentration of 0.2 µM of each primer, 0.2 mM of each dNTPs, 0.5 U Taq polymerase (Promega), with the supplied buffer, and 1.5–2.5 mM Mg Cl<sub>2</sub> in a final volume of 25 µL.

A Perkin-ElmerCetus Moldel 480 thermocycler was used to perform 35 iterations of the following cycle: 30s at 95°C, 45s at 45°C, and 1 min at 72°C, beginning with an additional step of 3 min at 95°C, and ending with another step of 10 min at 72°C. PCR results were visualized by means of a 1% agarose/TBE gel. Amplified products were purified using Microcon PCR columns following the manufacturer's specifications. Purified products were directly cycle-sequenced from both strands using ABI BigDye (Applied Biosystems) chemistry, precipitated in DyeEx Spin kit (Qiagen, Chatsworth, CA) columns, and run out on ABI Prism 377 (Applied Biosystems) automated sequencers. Sequencing reactions were performed in our lab with the forward and reverse PCR primers. Resulting product were run and analyzed at the Serveis Científico-Tècnics of the Universitat de Barcelona.

**Alignment.** Raw sequences were compared against chromatograms and complementary contigs built and edited using the Geneious Pro 3.6.2 software (<http://www.genious.com>). Sequences were manipulated and preliminary manual alignments constructed using BioEdit V.7.0.5.3 (Hall 1999). Alignment of the *cox1* gene fragments

was trivial due to the absence of length polymorphism. However, there were some length differences among the *rrnL* fragments, suggesting the occurrence of insertion/deletion events during the evolution of these sequences. Automatic alignment algorithms have been considered as superior to manual protocols due to their objectivity and repeatability (Giribet et al. 2002). Automatic alignments for the *rrnL* data set were constructed with the program MAFFT v 6.240 (Katoh and Toh 2007) The alignment was constructed using the manual strategy option set Q-INS-I, the most accurate multiple sequence alignment, with default options. All analyses were performed by coding gaps as absence/presence character following Simmons and Ochoterena's simple coding method (Simmons and Ochoterena 2000), as implemented in the software GAPCODER (Young and Healy 2002). This method allows the inclusion of gap information in phylogenetic inference, minimizing the effect of increasing the weight of overlapping multiple non-homologous gaps that results from scoring gaps as 5<sup>th</sup> state (Pons and Vogler 2006).

**Phylogenetic analyses.** Parsimony analyses of the combined data matrices were conducted with the program Winclada v.1.00.08 (Nixon 2002) using the following heuristic tree search strategy: 1000 iterations of 10 Wagner trees constructed with random addition taxa and subsequent TBR branch swapping, holding a total maximum of 10000 trees. This program facilitated combination of the different gene fragments in a single data set for simultaneous analyses and also provided additional statistics for those trees (CI and RI values). Clade support was assessed via Bootstrap (Felsenstein 1985) as implemented in Winclada, based on 1000 bootstrap replicates with 20 iterations and 10 starting trees per replica. Uncorrected genetic distances between taxa of *cox 1* gene from terminal taxa were assessed with the program MEGA v.3.0 (Kumar et al. 2004).

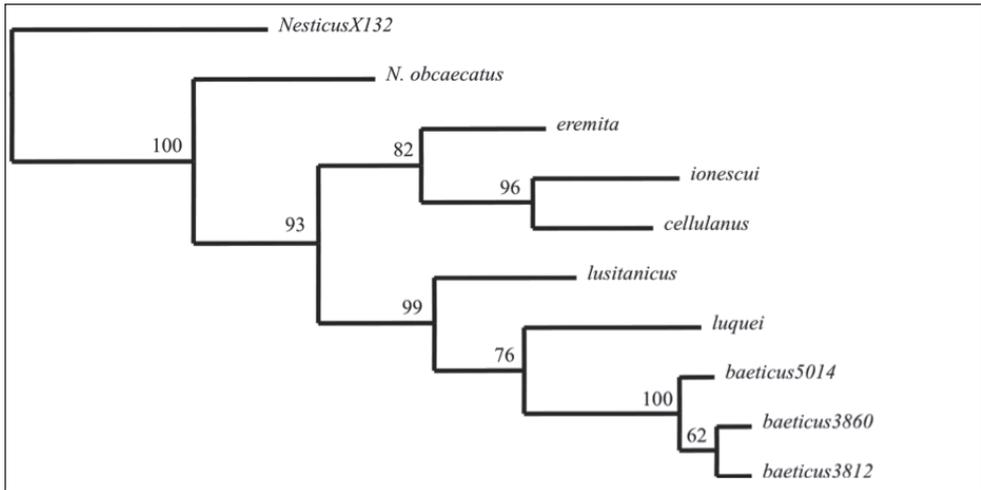
## Description

### *Nesticus baeticus* sp. n.

urn:lsid:zoobank.org:act:51EE521C-466B-47F7-81F6-244EF9FA8547

Figs 1–17

**Material examined. Holotype:** ♂ (1619-A25) Cueva de la Murcielaguina, Hornos, Jaén, Spain, 5.11.2006, GEV leg. **Paratypes:** 2♀♀ (1619-A25) same locality and data; 1♀ (1720-A29) same locality, 30.12.2007, GEV leg.; 1♀ (1530-A22) Sima HO-55, Hornos, Jaén, 14.8.2006, GEV leg. (drawings and description of the female are based on this specimen); 1♂ (1524-A21), 1♂sub., 1♀, 7i (1525-A22) same locality and data, GEV Leg.; 1♀ (3811–150) Sima de los Alhaurinos, Hornos, Jaén, 12.05.2002, GEV leg.; 1♀ (3812–150) same locality and data; 2i (3860-151) Sima del Campamento, Hornos, Jaén, 02.03.2003, GEV leg.; 2i, (5023-189) same locality, 27.08.2004, GEV leg.; 2♂sub., 1f, 5i (1157-A07) Sima del Laberinto, Hornos, Jaén, 04.02.2006 López, A. & Pérez, A. leg.; 1♀, 1♀sub. (1343-A14) Cueva SE-20, Santiago de la Es-



**Figure 1.** Single most parsimonious tree ( $L = 3454$ ,  $CI = 0.447$ ,  $RI = 0.769$ ) found by MP analysis of the combined data set ( $cox1=472$  bp,  $rrnL=456$  bp and 22 gap characters) of Iberian *Nesticus* species (except *N. murgis*), *N. ionescui* from Romania and *N. eremita* from Croatia. Numbers on nodes represent bootstrap support values. The outgroup *Nesticus* X130 is from China. Numbers on terminals refers to different localities (see material examined)

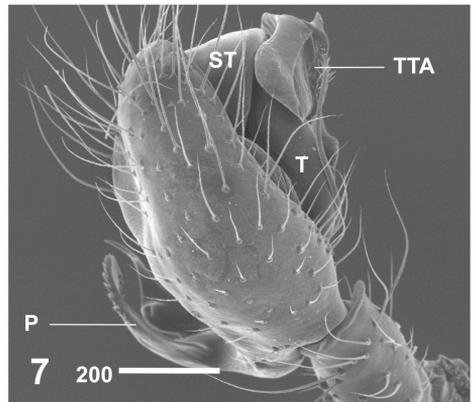
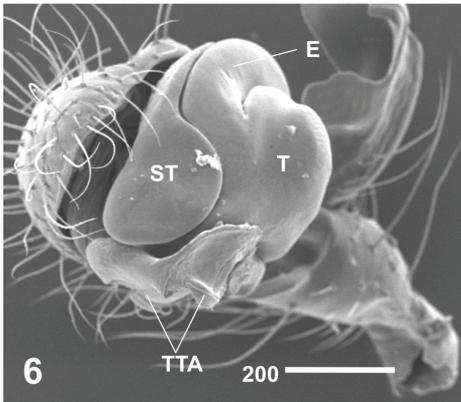
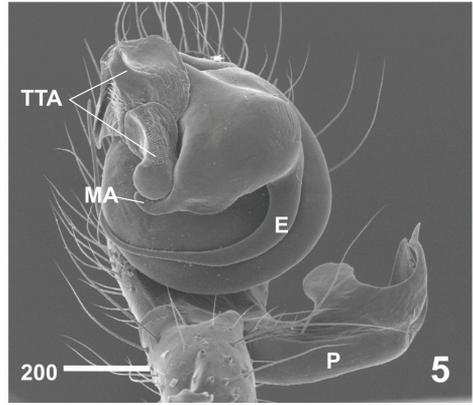
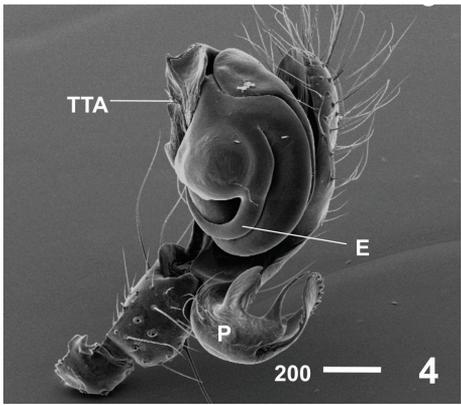
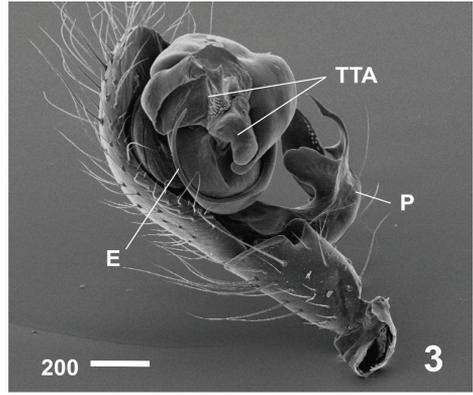
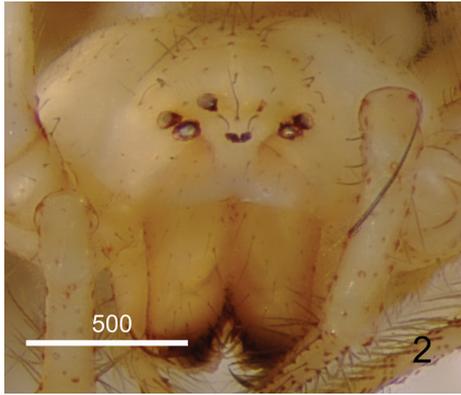
pada - Pontones, Jaén, 30.04.2006, GEV leg.; 1i (1539-A22) Sima del Órgano (HO-25), Hornos, Jaén, 14.08.2006, GEV leg.; 1i (5014-189) Sima Irene, Hornos, Jaén, 15.02.2004, GEV leg.

**Etymology.** The Latin name ‘*baeticus*’ means ‘from *Baetica*’ (the south of Spain) and refers to the ‘Sistema Bético’, the ridge containing the karst landscapes from where the new species was collected.

**Diagnosis.** Males clearly differ from those of other *Nesticus* species in the shape of paracymbium (Figs 4–5, 8–10) and in the TTA structure (Figs 3–5, 11–12). In females, the development of the median septum of the vulva (Figs 14–16), the shape of the spermathecae and adjoining structures are also diagnostic (Fig. 17). The degree of ocular reduction of the AM eyes (Fig. 2) is also characteristic compared to other Iberian species.

**Comments.** On the basis of morphology, *N. baeticus* sp. n. lies within the group including *N. murgis* (known from Almería) and *N. luquei* (an endemic to northwestern Spain). The shape and arrangement of the median apophysis (Figs 5, 11–12), the embolus (Figs 3–7, 11–12) and the paracymbial processes of male palp (Figs 8–10), plus the location and structure of the spermathecae and vulval glands of the female (Fig 17) are similar in all three species. *N. baeticus* sp. n. differs more significantly from *N. lusitanicus*, both in the morphology of the copulatory organs of both sexes.

The new species cannot be assigned to *Carpathonesticus*, *Typhlonesticus* or *Canarionesticus*, and differs from their representatives in having a different shape, ramification and modifications associated to the paracymbium, the general structure and arrangement of the embolous, as well as of the p1 and p2 TTA processes. Yet, the shape and disposition of the spermathecas and vulval glands shows markedly differences.



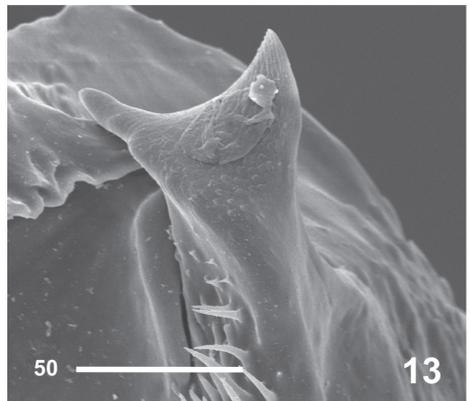
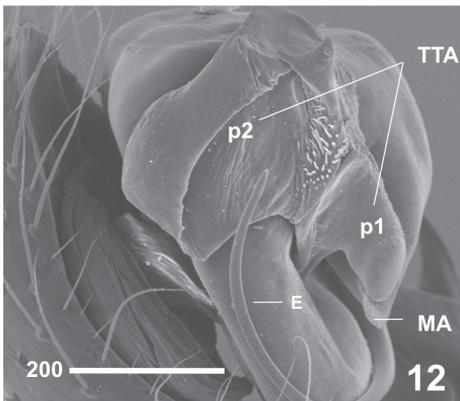
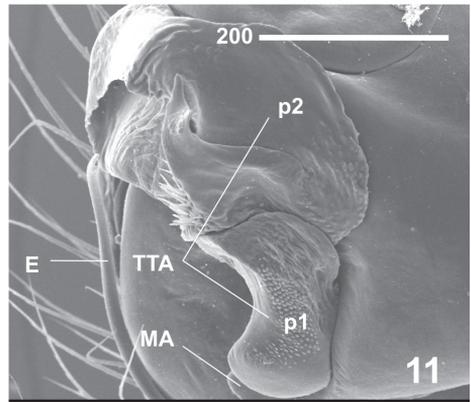
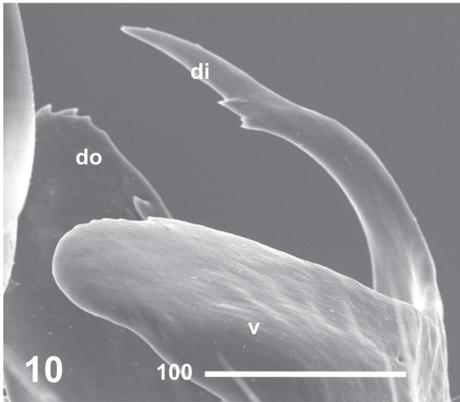
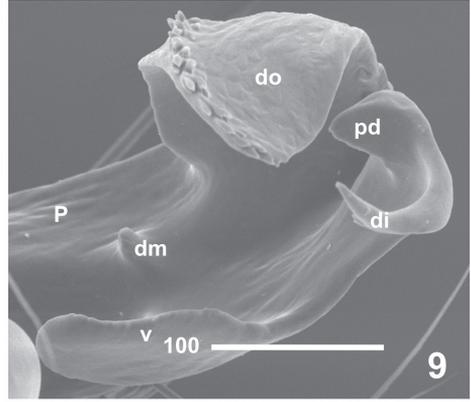
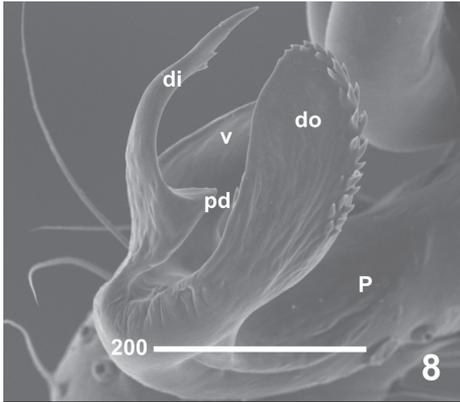
**Figures 2–7.** *N. baeticus* sp. n.: **2** female (1525) frontal view **3–6** male palp (Holotype). **3** retrolateral view **4** prolateral view **5** ventral view **6** frontal view **7** dorsal view. Abbreviations: **E** embolus **MA** median apophysis **P** paracymbium **ST** subtegulum **T** tegulum **TTA** theridioid tegular apophysis. Scale bar in  $\mu\text{m}$ .

**Description of the male** (Holotype). *Coloration*: carapace uniform yellowish. Opisthosoma grayish, with some clearly-marked darker patches. Appendages of the same colour as the carapace, slightly darker around distal segments. Sternum yellowish, slightly paler than the carapace. *Carapace*: approximately circular in dorsal view. Cephalic region not raised but differentiated from the rest of the prosoma. Fovea and thoracic grooves clearly visible. Significantly reduced eyes, more evident in the AM (Fig. 1). Eye size and interocular distances: AM = 0.03; AL = 0.07; PM = 0.06; PL = 0.07; AM - AL = 0.14 AL; AM - AM = 0.03; PM - PL = 0.14; PM - PM = 0.18 PM; PL - AL almost touching. *Opisthosoma*: sub-elliptical in dorsal view. *Appendages*: prolateral margin of the chelicerae with 3 teeth, the two distal ones larger. *Male palp* (Figs 3–7). Paracymbium large (Figs 3–5) with well-developed dorsal and ventral processes (Figs 8–10). Broad, translucent dorsal process with a saw-toothed upper edge (Figs 8–10). Dorsomedial apophysis small and pointed (Fig. 9). Ventral region apically notched (Fig. 10). Short paradistal region, almost conical (Figs 8–9). Distal apophysis long, acuminate and slightly curved (Figs 8–10). Poorly developed MA, reduced to a small fingerlike process fused to the tegulum (Figs 5, 11–12). Conductor absent. TTA with two processes, TTA p1 and TTA p2 (Figs 11–12) (homologous to p1-p6 processes of conductor complex in Huber 1993). TTA p1 is saddle-shaped, longer than wide, slightly curved and serrated in the central area (figs 11, 12). TTA p2 is located in apical position and serves as a conductor of embolus (figs 11–13). Embolus filamanteous with a semicircular course progressively acuminate towards the apex and partially bordering the tegulum (Figs 3–5, 12). *Measurements*: PL: 3.3; PW: 3.1; OL: 3.1; OW: 2.4; total body length = 6.4. Leg I > leg IV > leg II > leg III.

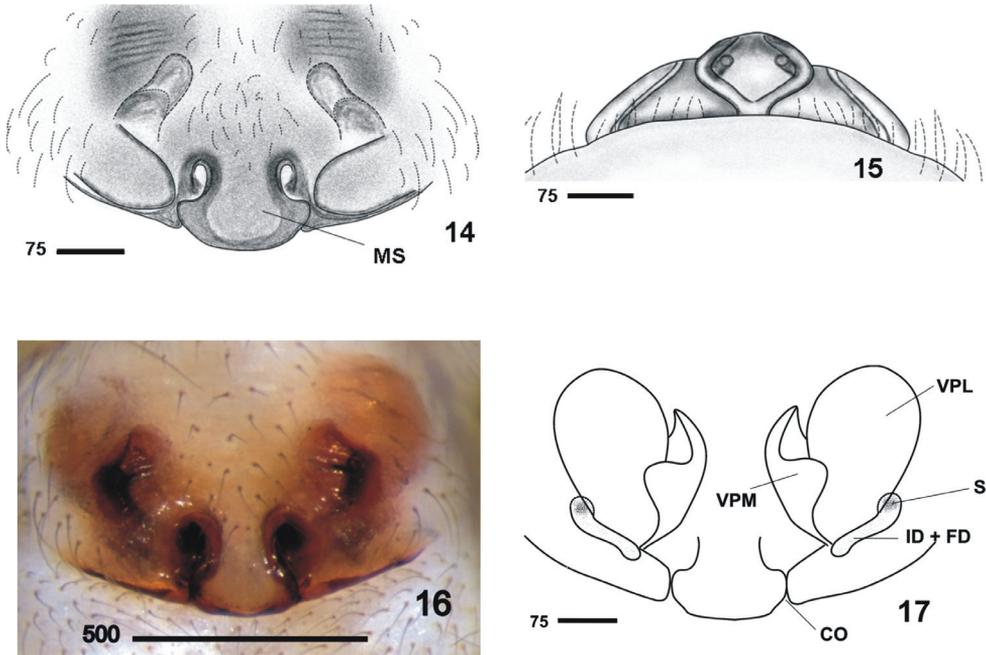
Leg	coxa	troc.	femur	patella	tibia	meta.	tarsus	total
I	1.1	0.7	9.4	1.4	9.1	9.7	2.9	34.3
II	1.0	0.4	6.9	1.4	6.6	6.9	2.6	25.8
III	0.9	0.6	5.1	1.1	4.0	4.6	1.9	18.2
IV	1.1	0.6	8.3	1.4	6.3	6.6	2.3	26.6

**Description of the female.** All characters as in male except: cephalic region scarcely differentiated, much less marked than in male. Fovea visible and thoracic grooves not clearly marked. *Epigynum and vulva.* Epigynum wide and convex (Figs 14–16). Median septum wide and prominent, caudally projected by a bell-shaped flap. Vulva (Fig. 17, drawing is based on specimen 3812-150) with well-developed lateral pockets divided by a ventral fold in two, the lateral and medial part. *Measurements*: PL: 2.9; PW: 2.9; OL: 3.4; OW: 2.6; total length = 6.3. Leg I > leg IV > leg II > leg III.

Leg	coxa	troc.	femur	patella	tibia	meta.	tarsus	total
I	1.1	0.7	8.9	1.1	8.9	8.6	3.4	32.7
II	1.0	0.6	8.0	1.1	6.0	6.3	1.4	24.4
III	0.9	0.4	6.3	1.0	3.4	4.0	1.7	17.7
IV	1.3	0.9	7.4	1.0	6.0	6.3	1.7	24.6



**Figures 8–13.** *N. baeticus* sp. n., male (holotype). **8–10** paracymbium **8** lateral view **9** frontal view **10** ventral view **11** median apophysis and theridioid tegular apophysis, ventral view **12** ditto, retrolateral view **13** apical protuberance of TTA p2. Abbreviations: **di** distal process **dm** dorsomedial apophysis **do** dorsal process **E** embolus **P** paracymbium **pd** paradistal apophysis **MA** median apophysis **TTA** theridioid tegular apophysis **TTA p1** process 1 of TTA **TTA p2** process 2 of TTA **v** ventral process. Scale bar in  $\mu\text{m}$ .



**Figures 14–17.** *N. baeticus* sp. n., female. **14** epigynum (1530) ventral view **15** ditto, caudal view **16** epigynum (1619) ventral view **17** vulva ventral view. Abbreviations: **CO** copulatory orifice **ID + FD** insemination duct + fertilization duct **MS** median septum **S** spermathecae **VPL** vulval pocket lateral **VPM** vulval pocket medial. Scale bar in  $\mu\text{m}$ .

**Distribution.** *Nesticus baeticus* sp. n. inhabits the karst landscapes of the high part of the Cazorla, Segura and Las Villas Natural Park (NE Jaén, Spain) where it has been found in 8 caves. Most of the material studied comes from the area surrounding the Tranco's Reservoir, in Hornos, Jaén. The area is calcareous, lush and quite humid, with numerous, medium-sized caves, both horizontal and vertical. The specimens were generally located within the first few meters of the dark zone, their presence reaching towards the cave interiors, which were sampled more intensively.

## Discussion

Specimens and sequences, with corresponding Genbank accession numbers, analyzed in the present study, are listed in Appendix 1. Alignments of two mitochondrial genes and gap scores as presence/absence were merged resulting in a combined data matrix of 950 characters (*cox1*=472, *rrnL*=456 and 22 gap characters). Uncorrected *cox1* genetic divergences among terminal taxa are summarized in Appendix 2. Parsimony analyses of the combined data matrix yielded a single most-parsimonious tree of 705 steps (CI = 73 and RI = 62) (Fig. 1).

The results show that the Iberian species do not constitute a monophyletic group. *N. luquei*, *N. lusitanicus* and *N. baeticus* sp. n. form a clade with high bootstrap support (99%), while *N. cellulanus* is nested within a clade that also includes *N. ionescui*, from Romania, and *N. eremita*, from Croatia. *N. obcaecatus* is the sister group of the remaining species of the ingroup. This topology, along the high genetic divergences observed between *N. obcaecatus*, *N. cellulanus* and the remaining Iberian species suggest the existence of tree independent colonization to the Iberian Peninsula. Preliminary results of a more extensive phylogenetic analysis, including almost all the Mediterranean species of Nesticidae (our work in progress), support this hypothesis.

The morphology of both the male and female copulatory organs of this Iberian group of species (*N. luquei*, *N. lusitanicus*, *N. murgis* and *N. baeticus* sp. n.) shows important differences as compared to *N. cellulanus*, the type species of the genus *Nesticus*, as well as to the *Carpathonesticus* species. Thus, the absence of paracymbial ramification, the shape and size of the median apophysis, plus clear differences in size and arrangement of the TTA processes constitute the major differences in the males. The number of spermathecae is the most noticeable character in the females.

With regards to *N. obcaecatus*, significant differences in the shape and structure of the paracymbium, the median apophysis and the TTA (see Ribera 1988), as well as in the shape of the epigynum and the arrangement of the spermathecae (see Fage 1931), indicate that this species is most distantly-related to all the Iberian endemics known to date. On the basis of the aforementioned characters *N. obcaecatus* seems to be more closely-related to *N. idriacus* Roewer, 1931 known from the eastern area of the Alps and to *N. morisii* Brignoli, 1975 known from Italy. Besides, the conformation of the copulatory organs of these three species are very similar to *Typhlonesticus absoloni* (Kratohvil, 1933) known from Montenegro. Thus, all three species are likely to belong with *Typhlonesticus* as well. Yet, in order to prove or reject this assumption, a molecular phylogeny of all the Mediterranean Nesticidae is to be performed. Our future research is focused on resolving the phylogeny of the Mediterranean Nesticidae (work in progress) and will include most of the Mediterranean species to test the monophyletic status of current genera.

*N. obcaecatus* shows highly troglomorphic characters, such as complete depigmentation, reduction of the eye size and their number (only six eyes), and is known from a single cave. These data alongside its phylogenetic uniqueness (basal position and a deep genetic distance from other Iberian congeners) suggest that this species may be considered a relict representative of an old colonization to Iberia, and should be a candidate for protection.

Climatic relict hypothesis assume that adaptation and speciation to caves are mainly driven by climatic factors. The Pleistocene glacial cycles has been identified as de main driver of the evolution of cave-dwelling fauna in the Palearctic (Barr 1968; Vandel 1958, 1964). The uncorrected genetic distances between *N. baeticus*, *N. lusitanicus* and *N. luquei* range between 14.3 and 15.5%. Assuming an average substitution rate for arthropod mitochondrial genes between 2% (DeSalle et al. 1987) to 2.3% (Brower 1994) we can conclude that the origin of these species preceded the Pleistocene glacial

cycles and, hence that other climatic or environmental factors may have been responsible for the evolution of these taxa.

## Acknowledgements

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## Appendix 1

Species included in the cladistic analysis and GenBank accession numbers for the *cox1* and *rrnL*. All accession numbers starting with EU are new sequences obtained in the present study.

species	Locality	cox1	rrnL
<i>Nesticus obcaecatus</i>	Cueva del Molino de Aso, Valle de Añisclo, Huesca, Spain	EU746428	EU746437
<i>Nesticus luquei</i>	Cueva de la Piconá, San Pedro de Carmona, Cabuerniga, Cantabria, Spain	EU746430	EU746439
<i>Nesticus lusitanicus</i>	Algar de Marradinhos II Concelho de Alcanena, Portugal	EU746429	EU746438
<i>Nesticus baeticus</i>	Sima Irene, Hornos, Jaén, Spain	EU746431	EU746440
<i>Nesticus baeticus</i>	Sima del Campamento, Hornos, Jaén, Spain	EU746432	EU746441
<i>Nesticus baeticus</i>	Sima de los Alhaurinos, Hornos, Jaén, Spain	EU746433	EU746442
<i>Nesticus ionescui</i>	Pestera Tismana, Tismana, Romania	EU746434	EU746443
<i>Nesticus cellulanus</i>	Manantiales Monte Castro, Sueras, Castellón, Spain.	EU746435	EU746444
<i>Nesticus eremita</i>	Cave Pishurka (=Paganetijeva Pécina), Korcula Is., Croatia.	EU746436	EU746445
" <i>Nesticus</i> "X130	China	AY231024	AY230941

## Appendix 2.

Uncorrected genetic distances of *cox 1* gene between terminal taxa analyzed in the present study. Numbers on terminals refers to different localities (see material examined)

	obcaeca	lusitan	luquei	baet5014	baet3860	baet3812	ionescui	cellula	eremita
lusitanicus	0.173								
luquei	0.180	0.143							
baeticus5014	0.197	0.144	0.153						
baeticus3860	0.199	0.143	0.155	0.002					
baeticus3812	0.199	0.143	0.155	0.002	0.000				
ionescui	0.159	0.164	0.170	0.160	0.161	0.161			
cellulanus	0.153	0.168	0.176	0.168	0.169	0.169	0.110		
eremita	0.159	0.155	0.161	0.153	0.151	0.151	0.115	0.117	
NesticusX130	0.169	0.193	0.195	0.204	0.206	0.206	0.174	0.184	0.170



# Mites of the genus *Neharpyrhynchus* Fain (Acariformes, Harpirhynchidae) from Neotropical birds

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## Abstract

Three new species of parasitic mites of the genus *Neharpyrhynchus* Fain (Acariformes, Harpirhynchidae) are described from Neotropical birds: *N. chlorospingus* **sp. n.** from *Chlorospingus pileatus* (Passeriformes, Emberizidae) from Costa Rica, *N. mironovi* **sp. n.** from *Dacnys cayana* (Passeriformes, Thraupidae) and *N. tangara* **sp. n.** from *Tangara cayana* (Thraupidae) both from Brazil. *Neharpyrhynchus trochilinus* (Fain) is recorded from 3 new host species of the family Trochilidae (Apodiformes), *Panterpe insignis* and *Eugenes fulgens* from Costa Rica, and *Amazilia lactea* from Brazil. Emended diagnosis of the genus and a key to species are provided; all records of *Neharpyrhynchus* species are summarized.

## Keywords

Acariformes, Harpirhynchidae, *Neharpyrhynchus*, systematics, birds, parasites

## Introduction

Mites of the genus *Neharpyrhynchus* Fain (Acariformes, Harpirhynchidae) are permanent and highly specialized parasites of birds, as is the case for all other representatives of the subfamily Harpirhynchinae (Bochkov 2008). The subgenus *Neharpyrhynchus*

Fain was established by Fain (1972) in the genus *Harpirhynchus* Mégnin. Later, Fain (1995) proposed full generic status for *Neharpyrhynchus* and simultaneously revised this genus, which included five species at that time. The life-cycle of these mites was described by Moss et al. (1968) as exemplified by *N. novoplumaris* Moss et al. The last revision of the genus *Neharpyrhynchus* was recently provided by Martinu et al. (2008). To date this genus includes 11 species belonging to five species groups established in that revision: *baile* (3 species), *hippolae* (3 species), *pilirostris* (1 species), *plumaris* (3 species), and *squamiferus* (1 species). In our opinion, however, there are no characters discriminating the *pilirostris* and *hippolae* species groups. Moreover, such differential characters were not provided even by Martinu et al. (2008) and in their key, *N. pilirostris* is placed in the same couplet with *N. pari*, a species from the group *hippolae*. We, therefore, include all species of the *hippolae* group in the *pilirostris* group.

Most species of the genus are known from European passerines and only two species are known from Neotropical birds, *N. baile* Bochkov et al. from *Turdus leucomelas* (Passeriformes, Turdidae) (Bochkov et al. 2007) and *N. trochilinus* (Fain) from hummingbirds (Fain 1972, 1995). In this paper, we describe three new species from Neotropical birds and provide new records for *N. trochilinus*. Additionally, an emended diagnosis of the genus and a key to its species are given. The diagnostic characters of species groups we recognize in the genus *Neharpyrhynchus* and all records of these mites are given in Tables 1 and 2, respectively.

## Material and methods

Birds were examined by naked eye for the presence of harpirhynchids and released back to the wild. S.V. Mironov and I. Literak examined birds in the field. Mites were cleared in lactophenol and mounted in Hoyer's medium. Specimens were studied using a Leica microscope under Nomarsky interference-contrast-phase (DIC) optics. Drawings were made

**Table 1.** Subdivision of the genus *Neharpyrhynchus* Fain on species groups. Characters: **1** Setae *vF*: smooth (0), serrate (1) **2** Setae *3a*: present (0), absent (1) **3** Number of articulated segments of leg I: 4 (0), 2 (1) **4** Number of articulated segments of legs II: 4 (0), 2 (1) **5** Number of articulated segments of legs IV: 2 (0), 1 (1) **6** Ornamentation of anterior region of propodsoma: absent or almost absent (0), present (1).

Groups	Characters						Species
	1	2	3	4	5	6	
<i>baile</i>	1	0	0	0	1	0	<i>N. baile</i> Bochkov et al., <i>N. bochkovi</i> Martinu et al., <i>N. trochilinus</i> (Fain)
<i>plumaris</i>	0	0	1	1	1	1	<i>N. chlorospingus</i> sp. n., <i>N. novoplumaris</i> (Moss et al.), <i>N. plumaris</i> (Fritsch), <i>N. spinus</i> Martinu et al.
<i>pilirostris</i>	0	1	1	1	1	1	<i>N. hippolae</i> Bochkov, <i>N. mironovi</i> sp. n., <i>N. pari</i> Martinu et al., <i>N. pilirostris</i> (Berlese & Trouessart), <i>N. schoenobaenus</i> Martinu et al., <i>N. tangara</i> sp. n.
<i>squamiferus</i>	0	0	0	1	0	1	<i>N. squamiferus</i> (Fain)

**Table 2.** Distribution of *Neharpyrhynchus* spp. on hosts.

“ - The same data as previous; \* - type host; † - probably accidental record or wrong determination; @ - originally determined as *N. plumaris* Fritsch, 1954; § - originally determined as *N. novoplumaris* Moss et al., 1968.

Mite species	Host species	Host family and order	Locality	Reference
<i>N. baile</i> Bochkov et al. 2007	* <i>Turdus leucomelas</i> Vieillot	Turdidae (Passeriformes)	Brazil (Mato Grosso do Sul)	Bochkov et al. (2007)
<i>N. bochkovi</i> Martinu et al. 2008	* <i>Turdus merula</i> Linnaeus	Turdidae (Passeriformes)	Czech Republic	Martinu et al. (2008)
<i>N. chlorospingus</i> sp. n.	* <i>Chlorospingus pileatus</i> Salvin	Emberizidae (Passeriformes)	Costa Rica	Present paper
<i>N. hippolae</i> Bochkov, 2000	* <i>Hippolais icterina</i> (Vieillot)	Sylviidae (Passeriformes)	Russia (Novgorod Prov.)	Bochkov (2000)
<i>N. mironovi</i> sp. n.	* <i>Dacnis cayana</i> (Linnaeus)	Thraupidae (Passeriformes)	Brazil (Minas Gerais)	Present paper
<i>N. novoplumaris</i> (Moss et al., 1968)	* <i>Certhia familiaris</i> Linnaeus	Certhiidae (Passeriformes)	USA (California)	Moss et al. (1968)
“	<i>Cardinalis cardinalis</i> (Linnaeus)	Cardinalidae (Passeriformes)	USA (Maryland, Nebraska)	Moss et al. (1968)
“	‡ <i>Campylorhynchus brunneicapillus</i> (Lafresnaye)	Troglodytidae (Passeriformes)	USA (?)	Moss (1979)
“	‡ <i>Spizella passerina</i> (Bechstein)	Emberizidae (Passeriformes)	USA (?)	Moss (1979)
“	‡ <i>Amphispiza bilineata</i> (Cassin)	Emberizidae (Passeriformes)	USA (?)	Moss (1979)
“	‡ <i>Melospiza fusca</i> (Swainson)	Emberizidae (Passeriformes)	USA (?)	Moss (1979)
<i>N. pari</i> Martinu et al. 2008	* <i>Parus major</i> (Linnaeus)	Paridae (Passeriformes)	Czech Republic	Martinu et al. (2008)
“	<i>Periparus ater</i> (Linnaeus)	Paridae (Passeriformes)	Czech Republic	Martinu et al. (2008)
“	@“	“	unknown	Moss (1979)
“	<i>Cyanistes caeruleus</i> (Linnaeus)	Paridae (Passeriformes)	Czech Republic	Martinu et al. (2008)
“	<i>Poecile montanus</i> (Baldenstein)	Paridae (Passeriformes)	Czech Republic	Martinu et al. (2008)
“	<i>Poecile palustris</i> (Linnaeus)	Paridae (Passeriformes)	Czech Republic	Martinu et al. (2008)
“	@“	Paridae (Passeriformes)	unknown	Moss (1979)
“	§ <i>Baeolophus bicolor</i> (Linnaeus)	Paridae (Passeriformes)	USA (?)	Moss (1979)
<i>N. pilitorris</i> (Berlese & Trouessart, 1889)	* <i>Passer domesticus</i> (Linnaeus)	Passeridae (Passeriformes)	France	Berlese and Trouessart (1889)
“	“	“	Germany	Fritsch (1954); Lawrence (1959)

Mite species	Host species	Host family and order	Locality	Reference
"	"	"	Czech Republic	Martinu et al. (2008)
"	"	"	South Africa	Lawrence (1959)
"	"	"	USA (Kansas)	Fain (1995)
"	<sup>8</sup> <i>Aegithalos caudatus</i> (Linnaeus)	Aegithalidae (Passeriformes)	unknown	Moss (1979)
<i>N. plummaris</i> (Fritsch, 1954)	<sup>*</sup> <i>Fringilla coelebs</i> (Linnaeus)	Fringillidae (Passeriformes)	Germany	Fritsch (1954)
"	"	"	Czech Republic	Martinu et al. (2008)
"	"	"	Russia (Novgorod Prov.)	Bochkov (2000)
"	<sup>8</sup> <i>Muscicapa striata</i> (Pallas)	Muscicapidae (Passeriformes)	Germany	Fritsch (1954)
"	<sup>8</sup> <i>Aythya ferina</i> (Linnaeus)	Anatidae (Anseriformes)	Germany	Fritsch (1954)
<i>N. schoenobaenus</i> Martinu et al. 2008	<sup>*</sup> <i>Acrocephalus schoenobaenus</i> (Linnaeus)	Sylviidae (Passeriformes)	Czech Republic	Martinu et al. (2008)
<i>N. spinus</i> Martinu et al. 2008	<sup>*</sup> <i>Spinus spinus</i> (Linnaeus)	Fringillidae (Passeriformes)	Czech Republic	Martinu et al. (2008)
"	<sup>6</sup> <i>Carduelis cannabina</i> (Linnaeus)	Fringillidae (Passeriformes)	Germany	Fritsch (1954)
<i>N. tangara</i> sp. n.	<sup>*</sup> <i>Tangara cayana</i>	Thraupidae (Passeriformes)	Brazil (Minas Gerais)	Present paper
<i>N. trochilinus</i> (Fain, 1972)	Hummingbird	Trochilidae (Apodiformes)	South America (?)	Fain (1972, 1995)
"	<i>Chrysolampis mosquitos</i> (Linnaeus)	Trochilidae (Apodiformes)	South America (?)	Fain (1995)
"	<i>Panterpe insignis</i> Cabanis & Heine	Trochilidae (Apodiformes)	Costa Rica	Present paper
"	<i>Eugenes fulgens</i> (Swainson)	Trochilidae (Apodiformes)	Costa Rica	Present paper
"	<i>Amazilia lactea</i> (Lesson)	Trochilidae (Apodiformes)	Brazil (Minas Gerais)	Present paper
<i>N. squamiferus</i> (Fain, 1972)	<sup>*</sup> <i>Temenuchus pagodarum</i> (Gmelin)	Sturnidae (Passeriformes)	India (?)	Fain (1972, 1995)

with a camera lucida, and measurements were taken using a calibrated ocular micrometer. Drawings were made by A. V. Bochkov. In the species description, names of the leg and idiosomal setae follow Grandjean (1939, 1944) as adapted by Kethley (1990). Names of the palpal setae follow Grandjean (1946) as adapted by Bochkov (2008). All measurements are given in micrometers ( $\mu\text{m}$ ) and were made according to the standard method (Bochkov et al. 2007): body length = maximum length of the body up to the anterior extremity of the palpal tibia; body width = maximum width taken at whatever level it occurs; gnathosomal length = length taken ventrally from the gnathosomal base to the anterior extremity of the palpal tibia; gnathosomal width = maximum width taken at whatever level it occurs; length of dorsal shield = maximum length, measured in the median line of the shield; and width of dorsal shield = maximum width taken at whatever level it occurs.

The scientific names of birds follow the checklist of Clements et al. (2010).

### Abbreviations:

<b>CM #</b>	Ivan Literak field number;
<b>INBio</b>	National Biodiversity Institute (Instituto Nacional de Biodiversidad), Heredia, Costa Rica;
<b>IPCR</b>	Institute of Parasitology, Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic
<b>IRSNB</b>	Royal Belgian Institute of Natural Sciences (Institut Royal des Sciences Naturelles de Belgique), Brussels, Belgium;
<b>MZUSP</b>	Zoological Museum of the University of São Paulo (Museu de Zoologia da Universidade de São Paulo), Brazil;
<b>SVM #</b>	S. Mironov field number;
<b>UMMZ</b>	University of Michigan Museum of Zoology, Ann Arbor, USA;
<b>ZISP</b>	Zoological Institute of the Russian Academy of Sciences, Saint Petersburg, Russia;
<b>ZISP AVB #</b>	number in collection of ZISP.

## Systematics

### Family Harpirhynchidae Dubinin

### Genus *Neharpyrhynchus* Fain

**Type species:** *Harpyrhynchus plumaris* Fritsch, 1954: 193, figs 11, 12, by original designation.

**Diagnosis.** Females. Subcapitulum bearing setae *n*, *m*, and *elcp*; palp bearing setae *vF*, *dF*, *dG*, *l<sup>n</sup>G*, *dT*, *l<sup>n</sup>T*, *l<sup>n</sup>Ta*. Setae *vF* smooth or serrate, setae (=palpalae) *dF*, *dG*, and *l<sup>n</sup>G* grouped together, strongly thickened and roughly barbed. Membranous part of palpal tarsi bearing 2 microspurs. Idiosoma saccate. Anterior part of propodonotum sclerotized (see remark below); this sclerotized area smooth or sculptured. Dorsal shield

distinctly developed, without ornamentation or finely ornamented. Idiosomal setae: *vi*, *ve*, and *si* set close to each other in anterior part of propodosoma, barbed filiform; *se* and *c2* situated distinctly far from *si*; *h1* – whip-like; *1a*, *1b* – fine, smooth filiform; setae *3a* present or absent; setae *scx* and *ag* absent. Legs I–II moderately reduced, with distinct basal lobes; their pretarsi with pair of angled claws and ciliated empodium each. Leg I with 2–4 articulated segments. Tarsus I with 8 setae (*tc'*, *tc''*, *p'*, *p''*, *a'*, *a''*, *u'*, *u''*) and 1 straight solenidion  $\omega$ II; tibia I with 5 setae (*d*, *l'*, *l''*, *v'*, *v''*), two other proximal segments (if present) devoid of setae. Leg II with 2–4 articulated segments. Tarsus II with 7 setae (*tc'*, *tc''*, *p'*, *a'*, *a''*, *u'*, *u''*) and 1 straight solenidion  $\omega$ III; tibia II with 5 setae (*d*, *l'*, *l''*, *v'*, *v''*), two other proximal segments (if present) devoid of setae. Posterior legs III and IV bearing 4–6 setae each; legs III with 1 segment, legs IV with 1 or more rarely with 2 segments.

Males. Gnathosoma as in female. Idiosoma rhomboid in outline. Anterior sclerotized area of propodosoma absent. Dorsal shield well developed, occupying most part of dorsal idiosomal surface. Genital opening situated in middle part of dorsal shield. Genital setae 3 pairs. Penis originating behind genital opening. Situations of dorsal idiosomal setae typical for subfamily. Setae *3a* present. Legs I and II well developed, without basal lobes, with 5 articulate segments each. Setation of tibia and tarsi as in females, three other proximal segments with setae. Legs III with two segments, both bearing setae; legs IV with one segment.

**Species included:** *N. baile* Bochkov et al., *N. bochkovi* Martinu et al., *N. chlorospingus* sp. n., *N. hippolae* Bochkov, *N. mironovi* sp. n., *N. novoplumaris* (Moss et al.), *N. pari* Martinu et al., *N. pilirostris* (Berlese & Trouessart), *N. plumaris* (Fritsch), *N. schoenobaenus* Martinu et al., *N. spinus* Martinu et al., *N. squamiferus* (Fain), *N. tangara* sp. n., and *N. trochilinus* (Fain).

**Hosts:** Passeriformes: Aegithalidae, Cardinalidae, Certhiidae, Emberizidae, Fringillidae, Muscicapidae, Paridae, Passeridae, Sturnidae, Sylviidae, Thraupidae, Troglodytidae, Turdidae; Apodiformes: Trochilidae.

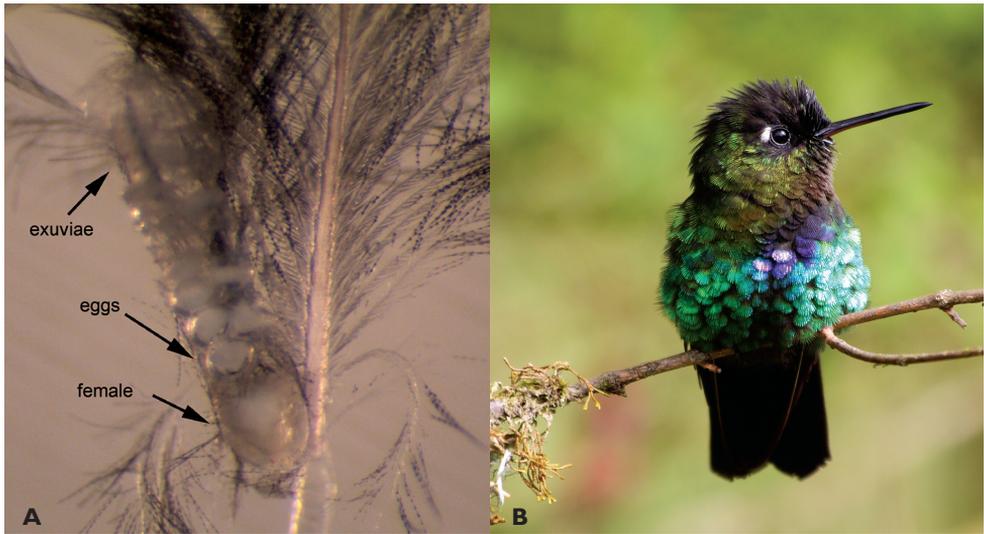
**Remarks.** The sclerotized area on the anterior part of the propodonotum was incorrectly named as the propodosomal (=propodonotal) shield by Martinu et al. (2008). In Harpirhynchidae, actually, the true propodonotal shield is fused with the hysteronotal shield or its remnants to form a common large shield, which can be referred to as the dorsal shield (Bochkov 2008). The sclerotized area in the anterior part of the propodosoma situated anterior to the dorsal shield is formed *de novo* and probably helps to fix the subcapitulum when the female attaches to a feather (Fig. 1).

***Neharpyrhynchus chlorospingus* Bochkov & Literak, sp. n.**

urn:lsid:zoobank.org:act:60C5B820-F280-4D12-9C80-BE5B9D9B2BCD

Figs 2, 3, 6A

**Type material.** Female holotype (ZISP H-T-8, AVB 10-1210-001) and 4 female paratypes (ZISP AVB 10-1210-001, 1–4) from *Chlorospingus pileatus* Salvin (Pas-

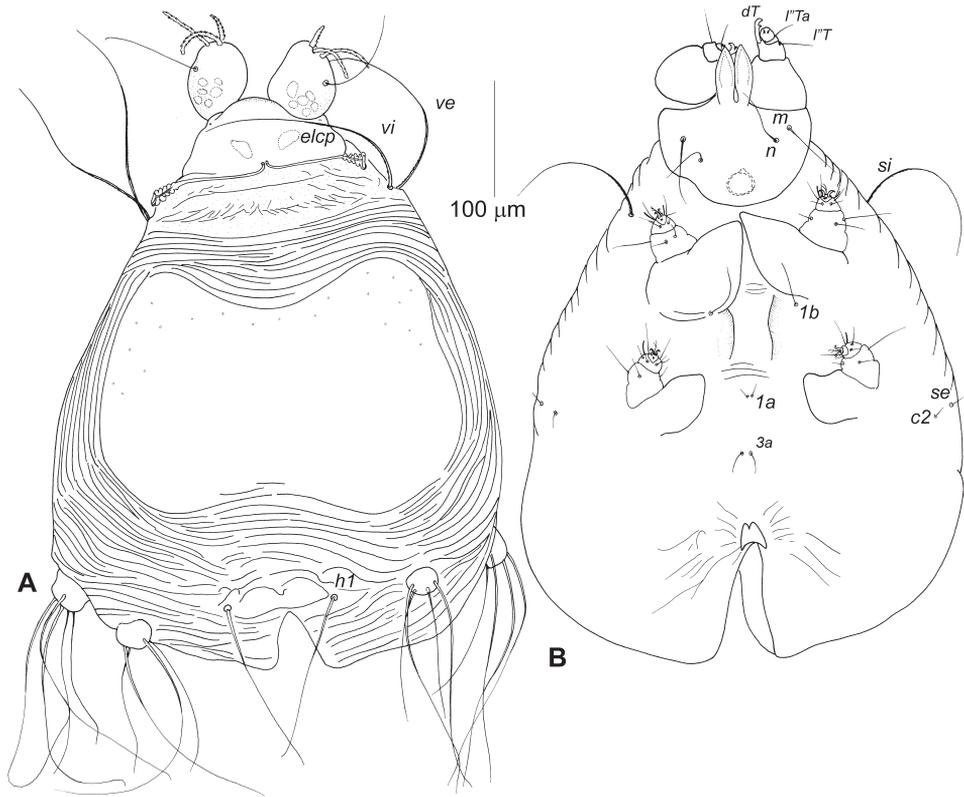


**Figure 1.** **A** *Neharpyrhynchus chlorospingus* sp. n., gravid female attached to host feather (photographed by A. V. Bochkov) **B** *Panterpe insignis* (Trochilidae) – host of *Neharpyrhynchus trochilinus* (Fain) (photographed by Z. Literakova).

seriformes, Emberizidae) [feathers around ear apertures], COSTA RICA: Cerro de la Mueste, 9°34'N, 83°45'W, 13 August 2010, coll. I. Literak et al. (CM 112).

**Type deposition.** Holotype and 3 paratypes deposited in the ZISP, one paratype in the INBio.

**Description.** Female (holotype). Idiosoma, including gnathosoma, 525 long (500–550 in 3 paratypes), 360 wide (350–360) (Fig. 2). Gnathosoma 130 long (130–145), 130 wide (130–140). Palps 50–60 long, moderately inflated dorsally. All palpalae distinctly pectinate (Fig. 3A). Lengths of palpalae: *dF* 35 (35–35), *dG* 20 (20–25), and *l''G* 50 (40–50); *dG* slightly thicker and about 2 times shorter than *dF* and *l''G*. Setae *vF* 100–110 long, smooth. Subcapitulum ventrally with setae *n* and *m*, about 40 and 50 long, respectively. Peritremal branch about 85 long. Idiosoma 425 long (420–440). Anterior region of propodonotum covered by short irregularly situated folds, without scales or tubercles (Fig. 6A). Dorsal shield entire, 165 long in midline (160–170), 300 at maximum width (300–330) (Fig. 2A). Anterior and posterior margins of dorsal shield widely concave. Ventral surface of idiosoma with indistinct transverse striations, without scales or verrucosities (Fig. 2B). Setal lengths: *vi*, *ve*, and *si* – all distinctly barbed, subequal in length, 150–160; *se*, *c2*, and *1a* – all smooth, 10–12; *b1* whip-like, 250 (230–260); *1b* smooth, about 40; *3a* present, about 20. Base of legs I with distinctly developed and slightly attenuated fleshy lobe; base of legs II with moderately developed rounded lobe. Leg I with 2 articulated segments (Fig. 3B). Leg II with 2 articulated segments (Fig. 3C). Legs III, IV with one segment, each bearing 4 (more rarely 5) long setae. One ventral seta of leg III and 2 ventral seta of leg IV 100–120 long, about half the length of other setae situated dorsally or dorsoterminally, 200–250 long.

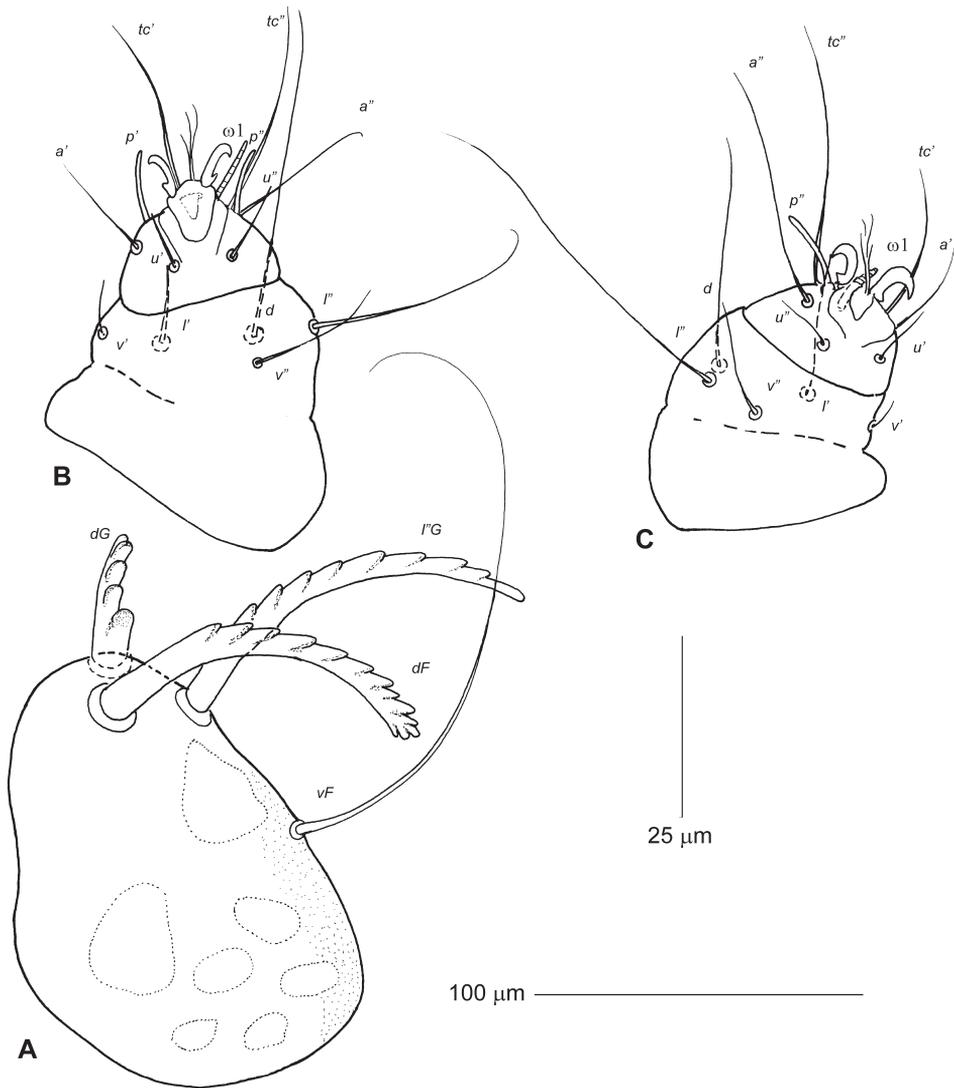


**Figure 2.** *Neharpyrhyrchus chlorospingus* sp. n., female holotype, **A** dorsal view **B** ventral view.

Male. Unknown.

**Etymology.** The species name is derived from the generic name of the host and is a noun in apposition.

**Differential diagnosis.** This species belongs to the “*plumaris*” species group including three species, *N. plumaris* (Fritsch), *N. novoplumaris* (Moss et al.), and *N. spinus* Martinu et al. (Martinu et al. 2008). In females of this group, legs I and II consist of the two articulated segments, palpal setae *vF* are smooth, the anterior region of the propodonomum is covered by short irregular striations, and setae *3a* are present. Within this group, it is close to *N. novoplumaris* described from *Certhia familiaris* Linnaeus (Passeriformes, Certhiidae) [type host] and *Cardinalis cardinalis* (Linnaeus) (Passeriformes, Cardinalidae) from USA (Moss et al. 1968). In females of both of these species setae *dG* are about half the length of *l''G*. In the other two species of the genus, setae *dG* and *l''G* are subequal. Females of *N. chlorospingus* differ from *N. novoplumaris* by the following characters. In *N. chlorospingus*, palpal setae *dF* are slightly shorter than *l''G*, setae *se* and *c2* are about four times shorter than *1b*, the posterior margin of the dorsal shield is widely concave. In *N. novoplumaris*, palpal setae *dF* are slightly longer than *l''G*, setae *se* and *c2* are subequal or only slightly shorter than *1b*, the posterior margin of the dorsal shield is widely convex.



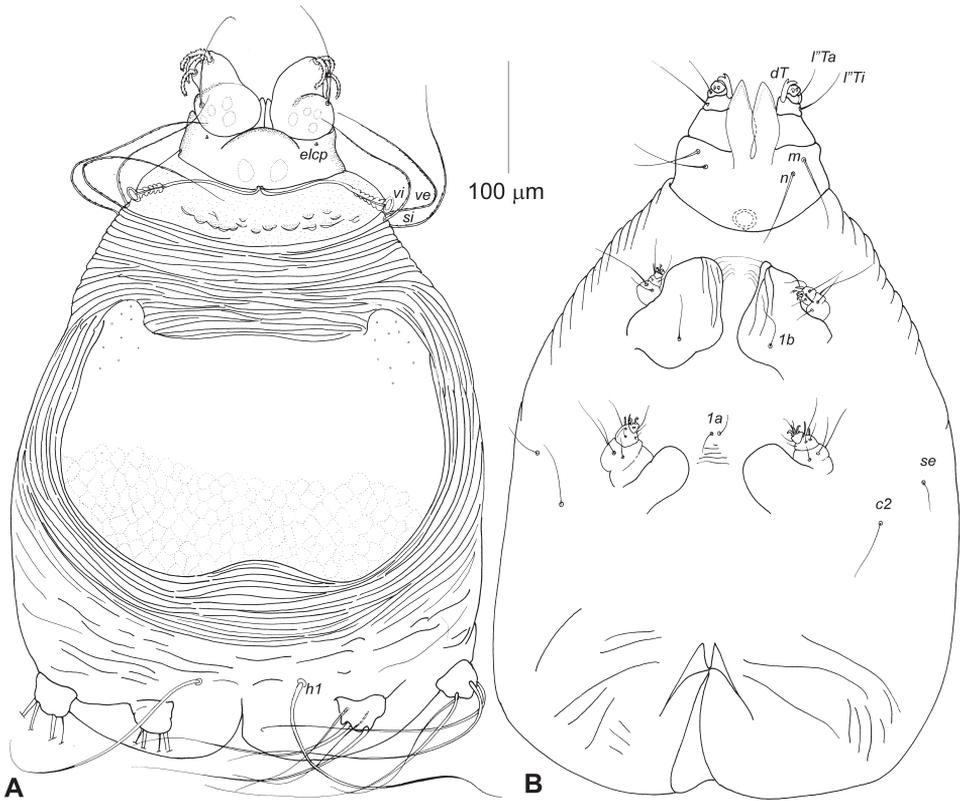
**Figure 3.** *Neharpyrhynchus chlorospingus* sp. n., details of female holotype, **A** palp in dorsal view **B** leg I in dorsal view **C** leg II in dorsal view.

***Neharpyrhynchus mironovi* Bochkov & Literak, sp. n.**

urn:lsid:zoobank.org:act:07B0EB50-C713-417B-AAA4-0B240A93AA7D

Figs 4, 5A–C, 6B

**Type material.** Female holotype (MZUSP), 20 female paratypes (ZISP AVB 10-1210-002, #1–20) on slides and numerous paratypes preserved in alcohol from *Dacnis cayana* (Linnaeus) (Passeriformes, Thraupidae) [feathers around ear apertures, back of the

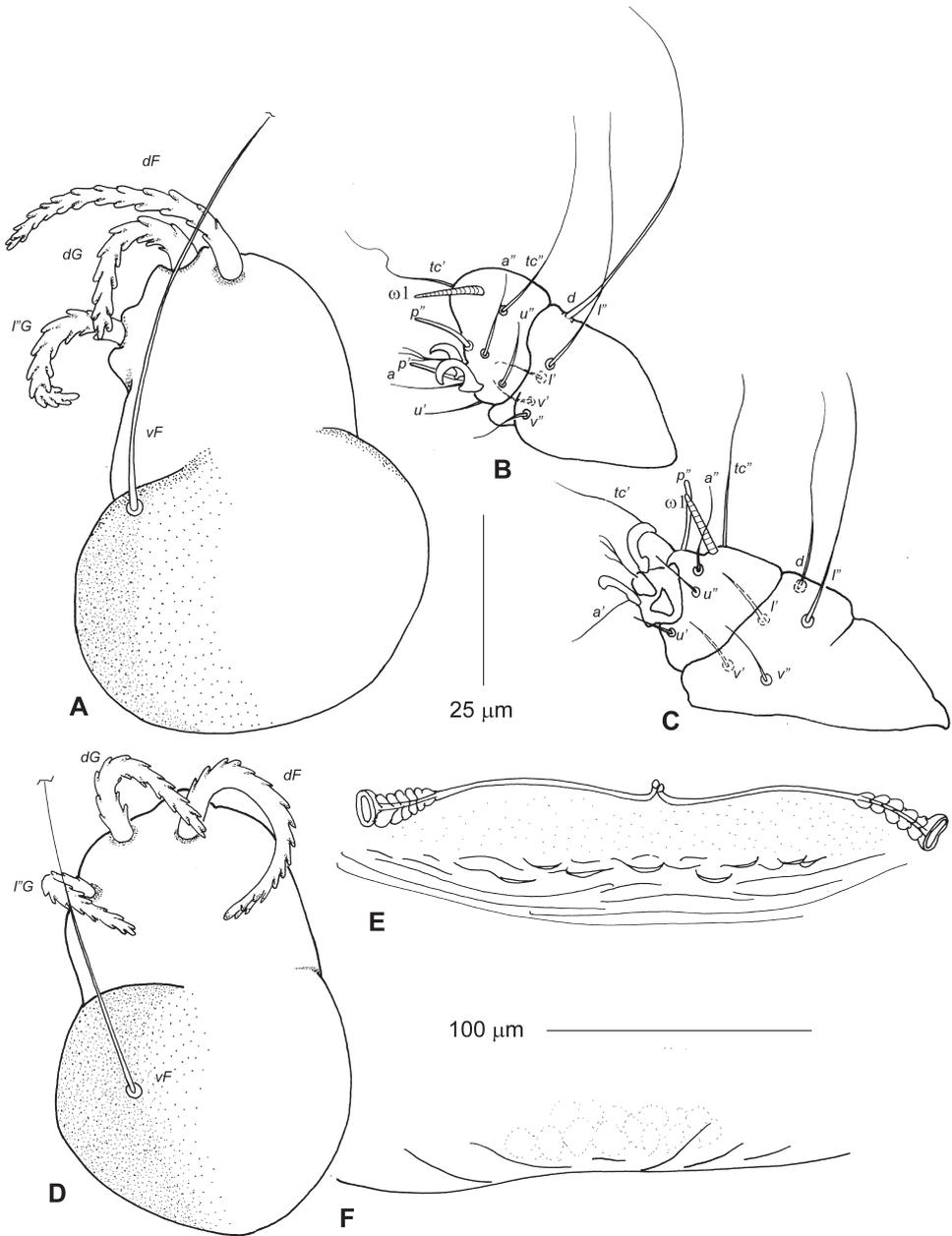


**Figure 4.** *Nebarpyrhynchus mironovi* sp. n., female holotype, **A** dorsal view **B** ventral view.

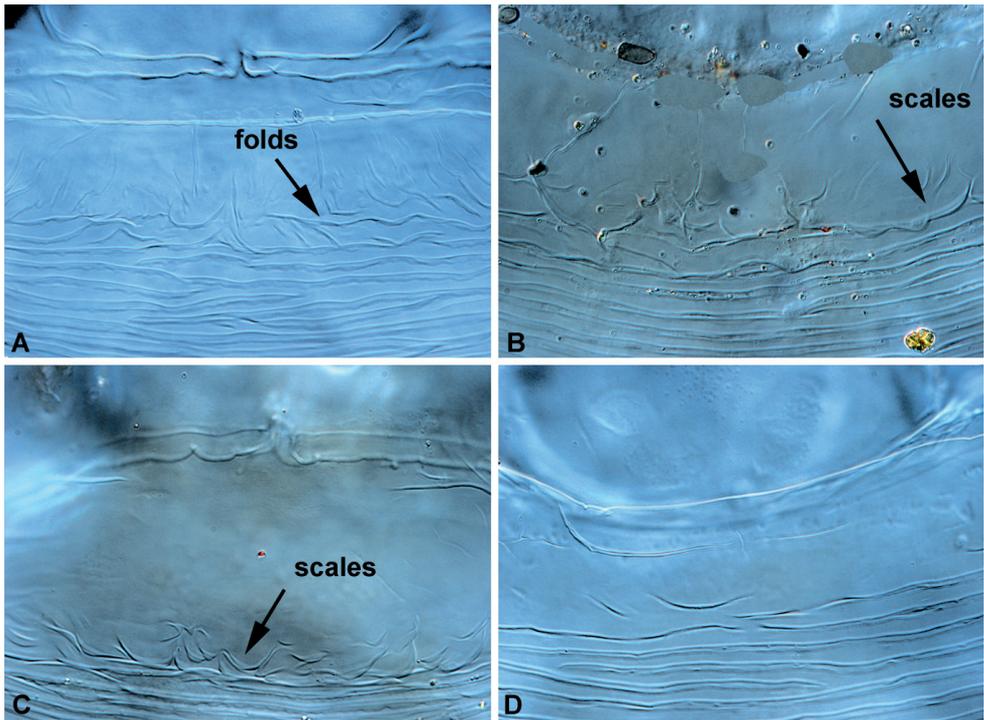
head and neck], BRAZIL: Minas Gerais, Belo Horizonte, Nova Lima, Área de Proteção Permanente (Permanent area for protection) do Condomínio Miguelão, 20°07'S, 43°58'W, 8 September 2010, coll. S.V. Mironov et al. (SVM-10-0908-1).

**Type deposition.** Holotype and 10 paratypes deposited in the MZUSP, 6 paratypes in the ZISP, 2 paratypes in the UMMZ, and 2 paratypes in the IPCR. Alcohol preserved paratypes deposited in the MZUSP and ZISP.

**Description.** Female (holotype). Idiosoma, including gnathosoma, 675 long (660–680 in 10 paratypes), 425 wide (420–435) (Fig. 4). Gnathosoma 135 long (130–140), 150 wide (140–155). Palps 65–75 long, distinctly inflated dorsally. All palpalae distinctly pectinate (Fig. 5A). Lengths of palpalae: *dF* 40 (38–40), *dG* 30 (28–33), and *l'G* 30 (30–35); *dF* only slightly longer than *dG* and *l'G*. Setae *vF* about 100 long, smooth. Subcapitulum ventrally with setae *n* and *m*, about 80 long. Peritremal branch about 120 long. Idiosoma 525 long (510–530). Anterior region of propodonotum covered by short rounded scales situated irregularly in its posterior half (Fig. 6B). Dorsal shield entire, 200 long in midline (190–200), 350 at maximum width (350–370) (Fig. 4A). Anterior margin of dorsal shield almost straight, with pair of lateral



**Figure 5.** *Neharpyrhynchus* spp, details of females. *N. mironovi* sp. n., holotype (**A-C**), **A** palp in dorsal view **B** leg I in dorsal view **C** leg II in dorsal view; *N. tangara* sp. n. (**D-F**), **D** palp in dorsal view **E** anterior part of propodonotum **F** posterior margin of dorsal shield. Scale bars: **A-D** = 25 μm; **E** and **F** = 100 μm.



**Figure 6.** *Neharpyrhyynchus* spp, anterior part of propodonotum, **A** *N. chlorospingus* sp. n. **B** *N. mironovi* sp. n. **C** *N. tangara* sp. n. **D** *N. trochilinus* (Fain).

anteriorly directed projections; posterior margin with distinct median concavity. This shield covered by fine rhomboid-like pattern, almost indistinct in anterior half and more clearly discernible in posterior half. Ventral surface of idiosoma with indistinct transverse striations, without scales or verrucosities (Fig. 4B). Setal lengths: *vi*, *ve*, and *si* - all distinctly barbed, subequal in length, 160–175; *se* and *1a* 12–25, *c2* 50–60 - all smooth; *h1* whip-like, 250 (250–280); *1b* smooth, 30–40, *3a* absent. Base of legs I with distinctly developed fleshy lobe partially covering leg segments; base of legs II with moderately developed rounded lobe. Leg I with 2 articulated segments (Fig. 5B). Leg II with 2 articulated segments (Fig. 5C). Legs III, IV with one segment, each bearing 4 long setae. One ventral seta of leg III and 2 ventral seta of leg IV about 150 long, about half the length of other setae situated dorsally or dorsotermally, 250–300 long.

Male. Unknown.

**Etymology.** The species is named in honour of the prominent Russian acarologist Dr. Sergey V. Mironov (ZISP).

**Differential diagnosis.** It is close to species of the group “*pilirostris*”. In all these species, setae *vF* are smooth, only two articulated segments on legs I and II are present, and setae *3a* are absent. Among species of this group, *N. mironovi* is close to *N. pari* by the presence of four setae on leg III and by irregular ornamentation of the anterior part of the propodosoma. The new species differs from *N. pari* by the following characters.

In females of *N. mironovi*, the palps are distinctly inflated dorsally, the ornamentation of the anterior part of the propodonotum is scale-like and present only in the posterior half of this region, setae *c2* are 50–60 long. In *N. pari*, the palps are moderately inflated dorsally, the anterior part of the propodonotum is fully ornamented by verrucosities and setae *c2* are 5–6 long.

***Neharpyrhynchus tangara* Bochkov & Literak, sp. n.**

urn:lsid:zoobank.org:act:5C1328B7-9A79-445A-AFB6-BC5402DFC36C

Fig. 5D–F, 6C

**Type material.** Female holotype (MZUSP), 20 female paratypes (ZISP AVB 10-1210-003, 1–20) on slides and numerous paratypes preserved in alcohol from *Tangara caryana* (Linnaeus) (Passeriformes, Thraupidae) [feathers around ear apertures, back of the head and neck], BRAZIL: Minas Gerais, Belo Horizonte, Nova Lima, Água Limpa, 20°13'S, 43°56'W, 31 August 2010, coll. S.V. Mironov et al. (SVM-10-0831-12).

**Type deposition.** Holotype and 10 paratypes deposited in the MZUSP, six paratypes in the ZISP, 2 paratypes in the UMMZ, and 2 paratypes in the IPCR. Alcohol preserved paratypes deposited in the MZUSP and ZISP.

**Description.** Female (holotype). Idiosoma, including gnathosoma, 600 long (600–620 in 10 paratypes), 380 wide (380–400). Gnathosoma 125 long (125–130), 150 wide (145–150). Palps 60 long, distinctly inflated dorsally. All palpalae distinctly pectinate (Fig. 5D). Lengths of palpalae: *dF* 35 (35–40), *dG* 30 (29–33), and *l<sup>o</sup>G* 29 (30–32); *dF* only slightly longer than *dG* and *l<sup>o</sup>G*. Setae *vF* about 90 long, smooth. Subcapitulum ventrally with setae *n* and *m*, about 70 long. Peritremal branch about 110 long. Idiosoma saccate, 500 long (500–520). Anterior region of propodonotum covered by short rounded scales situated irregularly in its posterior half (4E, 5C). Dorsal shield entire, 190 long in midline (180–200), 330 at maximum width (330–350). Anterior margin of dorsal shield almost straight with pair of lateral anteriad directed projections; its posterior margin almost straight (4F). This shield covered by fine rhomboid-like pattern, almost indistinct in anterior half and slightly better discernible in posterior half. Ventral surface of idiosoma with indistinct transverse striations, without scales or verrucosities. Setal lengths: *vi*, *ve*, and *si* - all distinctly barbed, subequal in length, 150–170; *se*, *c2*, and *1a* 8–12 - all smooth; *h1* whip-like, 230 (210–240); *1b* smooth, 30–35, *3a* absent. Legs as in previous species.

Male. Unknown.

**Etymology.** The species name derives from the generic name of the host and is a noun in apposition.

**Differential diagnosis.** This new species is closest to *N. mironovi* and differs by the following characters. In females of *N. tanagra*, setae *c2* are 8–12 long, the posterior margin of the dorsal shield is almost straight. In *N. mironovi*, setae *c2* are 50–60 long, the posterior margin of the dorsal shield is widely concave. Both species are collected from the hosts belonging to the family Thraupidae.

***Neharpyrhynchus trochilinus* (Fain, 1972)**

Fig. 6D

*Neharpyrhynchus* (*Neharpyrhynchus*) *trochilinus* Fain, 1972: 55.*Neharpyrhynchus trochilinus* Fain 1995: 80, figs 17, 18; Bochkov et al. 2007: 38; Martinu et al. 2008: 207, fig. 1 [types in IRSNB].

**Material examined.** 26 females (ZISP AVB 10-1210-004, 1–26) from *Panterpe insignis* Cabanis & Heine (Apodiformes, Trochilidae) [feathers of neck], COSTA RICA: Cerro de la Mueste, 9°34'N, 83°45'W, 14 August 2010, coll. I. Literak et al. (CM 199); 10 females from same host (ZISP AVB 10-1210-005, 1–10) and locality, 11 August 2010, coll. I. Literak et al. (CM 13); 10 females (ZISP AVB 10-1210-006, 1–10) from same host and locality, 13 August 2010, coll. I. Literak et al. (CM 151).

20 females (ZISP AVB 10-1210-007, 1–20) from *Eugenes fulgens* (Swainson) (Passeriformes, Trochilidae) [feathers of head, chest, and neck], COSTA RICA: Cerro de la Mueste, 9°34'N, 83°45'W, 13 August 2010, coll. I. Literak et al. (CM 152).

27 females (ZISP AVB 10-1210-008, 1–27) from *Amazilia lactea* (Lesson) (Apodiformes, Trochilidae) [feathers of head and neck], BRAZIL: Minas Gerais, Belo Horizonte, Nova Lima, Área de Proteção Permanente (Permanent area for protection) do Condomínio Miguelão, 20°07'S, 43°58'W, 4 September 2010, coll. S.V. Mironov et al. (SVM-10-0904-1).

**Hosts and distribution.** This species was briefly diagnosed from both sexes collected from an unidentified species of hummingbird (Trochilidae) that originated from South America (exact locality unknown) and died in the Zoo of Antwerp (Belgium) during its quarantine. Later on, Fain (1995) provided the full description of this species based on the type specimens and newly obtained specimens from *Chrysolampis mosquitus* (Linnaeus) (Trochilidae) that also originated in South America (without exact locality) and died in the Zoo quarantine. The trochilids, *Panterpe insignis*, *Eugenes fulgens* (Costa Rica), and *Amazilia lactea* (Brazil) are new hosts for this mite species. It is probable, that this species is associated exclusively with hummingbirds and is widely distributed on representatives of this host family.

**Remarks.** The longitudinally subdivided dorsal shield of this species is an artifact sometimes induced by the mite mounting. In this species, actually, the dorsal shield is entire. It differs from the closely related *N. baile* Bochkov et al. by the following characters. In females of *N. trochilinus*, setae *dF*, *dG*, and *l"G* are subequal, legs III and IV with 5–6 setae each, setae *si* and *se* 25–35 long. In *N. baile*, setae *dF* is about 1.5 times longer than *dG* and *l"G*, legs III and IV as a rule with 4 setae each, setae *si* and *se* are 6–12 long.

**Keys to species of the genus *Neharpyrhynchus* Fain (females)**

(based on Martinu et al. 2008)

- 1 Anterior margin of propodonotum without ornamentation or just with few striations. Palpal seta *vF* serrate. Legs II with 4 articulate segments ..... **2**
- Anterior margin of propodonotum ornamented. Palpal seta *vF* smooth. Legs II with 2 articulate segments ..... **4**
- 2 Palpal setae *dF* 1.4–1.5 times longer than *dG* and *l''G*. Setae *si* and *c2* short, 6–12 long. Leg IV normally with 4 setae (rarely with 5 setae) ..... **3**
- Palpal setae *dF*, *dG*, and *l''G* subequal in length. Setae *si* and *c2* relatively long, 25–35 long. Leg IV normally with 5 setae (rarely with 6 setae) .....  
..... ***N. trochilinus* Fain, 1972**
- 3 Lengths of palpal setae *dF*, *dG*, and *l''G* 58–70, 40–49, and 42–53, respectively. Leg III normally with 5 setae (rarely with 4 setae) .....  
..... ***N. bochkovi* Martinu et al., 2008**
- Lengths of palpal setae *dF*, *dG*, and *l''G* 45–54, 30–44, and 30–39, respectively. Leg III normally with 4 setae (rarely with 5 setae) .....  
..... ***N. baile* Bochkov et al., 2007**
- 4 Legs I with 2 segments; legs IV with 1 segment. Scales on ventral surface of idiosoma absent ..... **5**
- Legs I with 4 segments; legs IV with 2 segments. Scales on ventral surface of idiosoma present ..... ***N. squamiferus* (Fain, 1972)**
- 5 Setae *3a* absent ..... **6**
- Setae *3a* present ..... **11**
- 6 Posterior margin of dorsal shield without distinct median suture. Anterior part of propodosoma ornamented by verrucosities, flat cells or widely rounded scales ..... **7**
- Posterior margin of dorsal shield with distinct median suture reaching 1/3 of shield length. Anterior part of propodosoma ornamented by irregular striae (folds) ..... ***N. schoenobaenus* Martinu et al., 2008**
- 7 Palpal femur distinctly inflated dorsally. Anterior part of propodonotum ornamented only in posterior half by widely rounded scales ..... **8**
- Palpal femur moderately inflated dorsally. Anterior part of propodonotum fully ornamented by verrucosities or closed cells ..... **9**
- 8 Setae *c2* 8–12 long. Posterior margin of dorsal shield almost straight .....  
..... ***N. tanagra* sp. n.**
- Setae *c2* 50–60 long. Posterior margin of dorsal shield widely concave .....  
..... ***N. mironovi* sp. n.**

- 9 Anterior part of prodorsum covered by irregularly situated verrucosities, not forming transverse rows. Dorsal shield covered by fine ornamentation. Legs III normally with 4–6 setae..... **10**
- Anterior part of prodorsum covered by verrucosities forming 4–5 transverse rows. Dorsal shield without ornamentation. Legs III with 4 setae .....  
.....***N. pilirostris* (Berlese & Trouessart, 1889)**
- 10 Dorsal shield 140–165 long, covered by fine longitudinal striation. Legs III with 5–6 setae..... ***N. pari* Martinu et al., 2008**
- Dorsal shield 165–195 long, covered by fine irregular transverse scale-like striation. Legs III with 4–5 setae .....***N. hippolae* Bochkov, 2000**
- 11 Palpal setae *dG* about half the length of *l''G*..... **12**
- Palpal setae *dG* and *l''G* subequal ..... **13**
- 12 Palpal setae *dF* slightly shorter than *l''G*. Setae *se* and *c2* about 4 times shorter than setae *1b*. Posterior margin of dorsal shield widely concave.....  
..... ***N. chlorospingus* sp. n.**
- Palpal setae *dF* slightly longer than *l''G*. Setae *se* and *c2* subequal or only slightly shorter than setae *1b*. Posterior margin of dorsal shield widely convex ..... ***N. novoplumaris* Moss et al., 1968**
- 13 Anterior part of propodonorium covered by longitudinal striation only in posterior part. Palpal setae *vF* 75–80 long. Dorsal shield 307–345 wide, covered by fine longitudinal scale-like pattern..... ***N. plumaris* (Fritsch, 1954)**
- Anterior part of propodonorium completely covered by longitudinal striations. Palpal seta *vF* 98–108 long. Dorsal shield 275–280 wide, devoid ornamentation ..... ***N. spinus* Martinu et al., 2008**

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We thank Dr. S.V. Mironov (ZISP, Russia) who provided us with *Neharpyrhynchus* samples collected by him in Brazil. We thank Drs. M. Capek, F.A. Hernandez, F. Kounek, M. Literakova, B. Calvo Rodriguez, O. Sychra, and M. Valim for the cooperation in the field. Mites were collected from birds under permits No. 24789–1 (IBAMA, Brazil) and No. 120-2010-SINAC (MINAET, Costa Rica). This research was supported by grants No. 10-04-00160 and No. IAA601690901 from the Russian Foundation for Basic Research and the Grant Agency of the Academy of Sciences of the Czech Republic, respectively.

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# *Stylobates birtlesi* sp. n., a new species of carcinoecium-forming sea anemone (Cnidaria, Actiniaria, Actiniidae) from eastern Australia

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‡ [urn:lsid:zoobank.org:author:55D84D7A-40A1-454D-8A7F-755C8958E1D9](https://zoobank.org/urn:lsid:zoobank.org:author:55D84D7A-40A1-454D-8A7F-755C8958E1D9)

§ [urn:lsid:zoobank.org:author:78F6BEE6-F495-4151-A1D0-7C301F4F92B3](https://zoobank.org/urn:lsid:zoobank.org:author:78F6BEE6-F495-4151-A1D0-7C301F4F92B3)

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## Abstract

We describe a new species of carcinoecium-forming sea anemone, *Stylobates birtlesi* sp. n., from sites 590–964 m deep in the Coral Sea, off the coast of Queensland, Australia. An anemone of this genus settles on a gastropod shell inhabited by a hermit crab, then covers and extends the shell to produce a chitinous structure termed a carcinoecium. *Stylobates birtlesi* sp. n. is symbiotic with the hermit crab *Sympagurus trispinosus* (Bass, 1911). The nature of marginal sphincter muscle and nematocyst size and distribution distinguish *Stylobates birtlesi* sp. n. from other species in the genus. The four known species of *Stylobates* are allopatric, each inhabiting a separate ocean basin of the Indo-West Pacific. We also extend the known range of *Stylobates loisetteae* in the Indian Ocean off the coast of Western Australia.

## Keywords

Anthozoa, Hexacorallia, deep sea, symbiosis, hermit crab

## Introduction

*Stylobates* Dall, 1903, of family Actiniidae Rafinesque, 1815, is an exclusively deep-water genus of sea anemones in which three species are known: *Stylobates aeneus* Dall, 1903, from Hawai'i and Guam in the Pacific Ocean, *Stylobates cancrisocia* (Carlgren, 1928a), from the Indian Ocean off Africa, and *Stylobates loisetteae* Fautin, 1987, from the Indian Ocean off Western Australia. We describe *Stylobates birtlesi* sp. n. from specimens collected in the Coral Sea off the northeastern coast of Australia. In addition, we report previously unpublished localities for *Stylobates loisetteae*.

A distinctive feature of *Stylobates* is the chitinous carcinoecium it produces; a carcinoecium is a shell-like structure inhabited by a hermit crab. Carcinoecia are produced by bryozoans (e.g. Vermeij 1993) as well as many species of cnidarians (e.g. Williams and McDermott 2004), including hydrozoans (e.g. Millard 1975), zoanthids (e.g. Muirhead et al. 1986, Ates 2003) and sea anemones (e.g. Carlgren 1928a, b, Ross 1971, 1984, Dunn and Liberman 1983, Daly et al. 2004). Of anemones that attach to gastropod shells inhabited by hermit crabs, animals of some species (e.g. those belonging to *Calliactis*) form a thin layer of chitin over the shell. Those of other species (e.g. those belonging to *Stylobates* and *Paracalliactis*) not only cover but extend the shell, producing a carcinoecium. The ability to form a carcinoecium is a convergent attribute of anemones (Gusmão and Daly 2010); anemones possessing this ability belong to four families (Daly et al. 2004).

The genus and species *Stylobates aeneus* were described by Dall (1903, p. 61), who was initially under the impression that the shell of “flexible, horny consistency,” which was inhabited by a hermit crab and covered by a sea anemone, was that of a gastropod. He later corrected his mistake, recognizing that “These specimens were secretions from the bases of the Actinias” (Dall 1919, p. 80). Nonetheless, the holotype of *Stylobates aeneus* Dall, 1903, consisting only of the carcinoecium, is still housed in the mollusc collection of the United States National Museum of Natural History (USNM). Carlgren (1928a) described *Isadamsia cancrisocia* as a new genus and species of carcinoecium-forming anemone, making no reference to Dall (1903). Dunn et al. (1981) synonymized the genera *Isadamsia* and *Stylobates*. According to International Code of Zoological Nomenclature Article 12.2.8 (International Commission on Zoological Nomenclature 1999), having been published before 1931, the name *Stylobates aeneus* is available for the anemone because it is a “description of the work of an organism.”

The allopatric distribution that we found for the four species of *Stylobates* is similar to that of other deep-sea invertebrates in the tropical Indo-West Pacific, such as scleractinian corals and squat lobsters.

## Methods

The holotype and five paratypes of *Stylobates birtlesi* sp. n. were trawled by ORV *Franklin* during the Cidaris I expedition on the northeastern continental slope of Queens-

land in 1986 (Anonymous 1986). The holotype was photographed live (Figure 1a, b) within a few minutes of being brought on deck. Four paratypes were trawled by RV *Soela* off the northeast coast of Queensland and one voucher was trawled by FRV *Iron Summer* off the southeast coast of Queensland. All specimens were preserved in 70% ethanol. Specimens of *Stylobates loisettae* were collected on the RV *Southern Surveyor* expedition to the northwestern coast of Australia in 2007.

Cnida preparations were made from the tentacles, mesenterial filaments, actinopharynx, and column by smashing tissue with water under a coverslip. Preparations were examined using differential interference (Nomarski) optics at 1000×. For each tissue type, the length and width were measured for each type of cnida. Representative cnidae were photographed using an Olympus digital camera. Histological sections were stained with Gomori trichrome (Menzies 1959).

The holotype, four paratype lots, and one voucher of *Stylobates birtlesi* sp. n. are deposited at Museum of Tropical Queensland, Townsville [MTQ], and one paratype lot is deposited at the Division of Invertebrate Zoology collection of the University of Kansas Biodiversity Institute, Lawrence [KUDIZ]. New records for *Stylobates loisettae* are based on specimens in the Western Australian Museum, Perth [WAM]. Separated hermit crab specimens are deposited at Queensland Museum South Bank, Brisbane [QM].

## Results

### Family Actiniidae Rafinesque, 1815

#### Genus *Stylobates* Dall, 1903

*Stylobates* Dall, 1903: p. 62

*Isadamsia* Carlgren, 1928a: p. 167

**General.** Because Dall (1903) had been under the impression that the carcinoecium upon which he based his description was that of a gastropod, the first description of the anemone was by Carlgren (1928a) for *Isadamsia cancrisocia* from the east coast of Africa. Carlgren (1928a, 1949) is the only person to have defined the genus, and his definition was based on the single species he knew. We update the definition of *Stylobates* to incorporate information from all four known species.

Deep-sea Actiniidae with very wide pedal disc that covers a gastropod shell inhabited by a hermit crab. Anemone pedal disc secretes carcinoecium. Column smooth, thin-walled. Marginal sphincter muscle endodermal, circumscribed, palmate or pinnate. Tentacles hexamerously arranged; fewer than mesenteries at base. Longitudinal muscles of tentacles and radial muscles of oral disc ectodermal. Mesenteries of 5–6 orders; those of lowest orders complete and sterile, those of highest orders incomplete and fertile. Retractor muscles weak, diffuse; parietobasilar and basilar muscles distinct.

**Type species (by monotypy):** *Stylobates aeneus* Dall, 1903.

## Species description

### *Stylobates birtlesi* sp. n.

urn:lsid:zoobank.org:act:6B1BF135-854D-4495-BC5D-0D5FAE79EF7D

Figures 1–5

## Material examined.

### Holotype:

MTQ G57579 (one specimen) (Figure 1).

Type locality: 17°45.99'S, 148°39.09'E, 958–964 m; Coral Sea, off Tully, Queensland, Australia (FRV Franklin, Cidaris I expedition, Station 15–4). Bottom temperature 5.5°C, rocks/mud sediment. Collected 9 May 1986, by RA Birtles and P Arnold. Hermit crab present.

### Paratypes:

MTQ G57580 (one specimen).

Locality: 17°52'S, 147°08'E, 680–740 m; Coral Sea, off Tully, Queensland, Australia (FRV Franklin, Cidaris I expedition, Station 48–3). Bottom temperature 8.2°C, mud sediment. Collected 17 May 1986, by RA Birtles and P Arnold. Hermit crab separated from carcinoecium, registered as QM W16502 (crustacean collection).

MTQ G57581 (two specimens).

Locality: 17°51.71'S, 147°09.93'E, 881–920 m; Coral Sea, off Tully, Queensland, Australia (FRV Franklin, Cidaris I expedition, Station 49–3). Bottom temperature 6.1°C, rocks/shell debris/sticky mud sediment. Collected 17 May 1986, by RA Birtles and P Arnold.

MTQ G57582 (one specimen).

Locality: 18°01.69'S, 147°20.53'E, 899–918 m; Coral Sea, off Tully, Queensland, Australia (FRV Franklin, Cidaris I expedition, Station 50–3). Bottom temperature 6.2°C, mud sediment. Collected 17 May 1986, by RA Birtles and P Arnold. Hermit crab separated from carcinoecium, registered as QM W16499 (crustacean collection).

KUDIZ 003352 (one specimen).

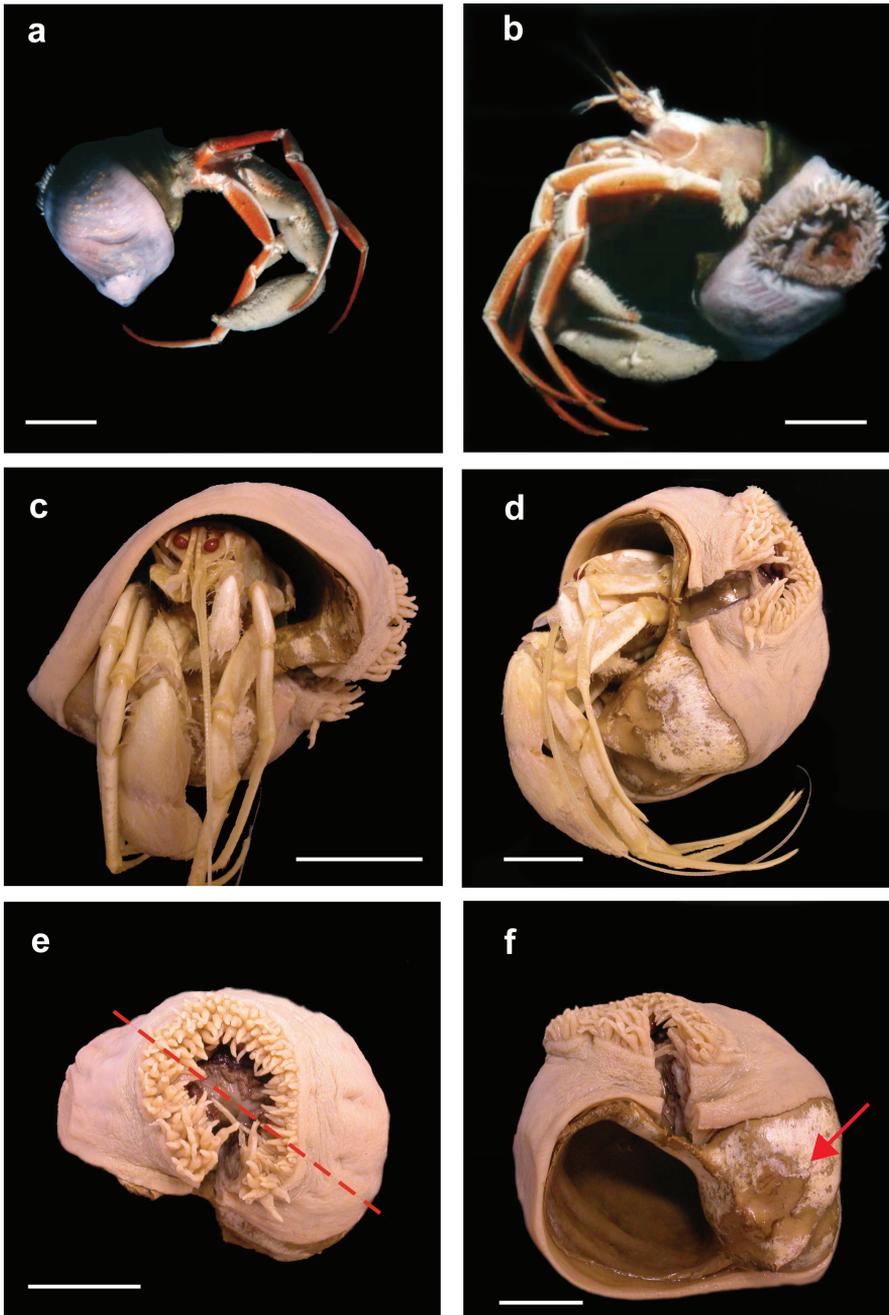
Locality: 18°01.69'S, 147°20.53'E, 899–918 m; Coral Sea, off Tully, Queensland, Australia (FRV Franklin, Cidaris I expedition, Station 50–3). Bottom temperature 6.2°C, mud sediment. Collected 17 May 1986, by RA Birtles and P Arnold. Hermit crab separated from carcinoecium, registered as QM W16499 (crustacean collection).

MTQ G64680 (four specimens).

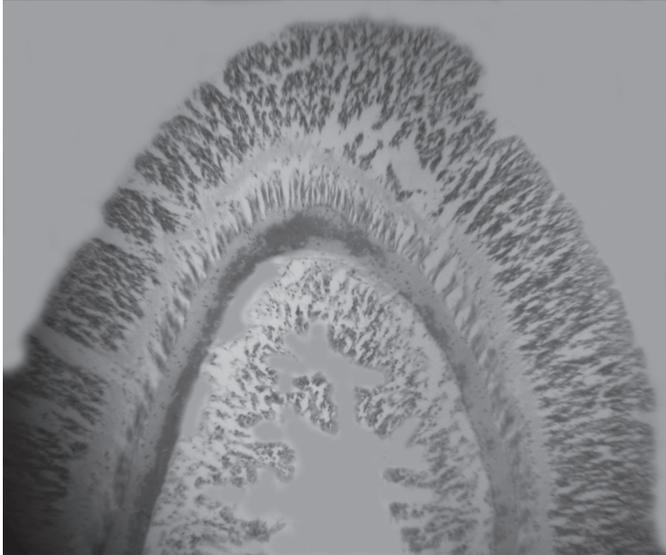
Locality: 16°55'S, 151°34'E, 880 m; Coral Sea, northeast Queensland, Australia (RV Soela, Station CO685A78). Collected 6 December 1985, by P Davie. Hermit crabs separated from carcinoecia, registered as QM W16514 (crustacean collection).

### Voucher:

MTQ G58760 (one specimen).



**Figure 1.** *Stylobates birtlesi* sp. n. holotype MTQ G57579 **a, b** soon after collection (photo: RA Birtles) **c, d** preserved specimen with *Sympagurus trispinosus* showing position of oral disc of anemone **e** preserved specimen: shortest tentacles beside longest ones (on right side of oral disc in this view); tentacles grade in length between longest and shortest around other side of oral disc (dashed line indicates directive axis) **f** preserved specimen without hermit crab showing aperture and part of carcinoecium not covered by anemone (arrow). Scale bars 20 mm.



**Figure 2.** Longitudinal section through tentacle of *Stylobates birtlesi* sp. n. paratype MTQ G57580.

Locality: 27°59.37'S, 154°00.12'E, 590 m; off coast of southeast Queensland, Australia (FRV Iron Summer, Shot 2). Collected 31 March 1983, by R Morton.

**Description.** *Base:* Pedal disc concave, attached to carcinoecium. Base of anemone covers most of carcinoecium, except part directly under hermit crab, presumably where hermit crab's chelipeds frequently contact carcinoecium (arrow, Figure 1f).

*Column:* Not cylindrical: wraps around gastropod shell so column much longer on one side than on diametrically opposite side. Smallest specimen with shortest side 4 mm, longest side 50 mm. Largest specimen with shortest side 15 mm, longest side 90 mm. Smooth, thin. Fosse shallow. Live specimens light pink, body wall translucent (Figure 1a, b); preserved specimens beige. Mesenterial insertions visible through body wall; white in live specimens (Figure 1a) and preserved specimens.

*Oral disc:* Oriented toward substrate in life, over umbilicus area (Figure 1c, d). Disc and mouth circular (Figure 1e); disc exposed and mouth agape in all specimens examined. Ectodermal musculature radial.

*Orientation:* Directive axis in line with spire of shell, parallel to parietal wall of aperture (dotted red line, Figure 1e).

*Tentacles:* Beige, slightly darker than column, no pattern. Relatively narrow, tip terete. 96 to more than 200 in largest specimens; at margin, in 3 or 4 cycles. Not of uniform length: shortest ones (1–4 mm) on directive axis, at end of one siphonoglyph, beside longest ones (3–9 mm); tentacle length grades between them around oral disc (Figure 1e). Ectodermal musculature longitudinal (Figure 2).

*Marginal sphincter muscle:* Well developed, circumscribed, palmate (Figure 3a, b).

*Mesenteries and internal anatomy:* Two siphonoglyphs visible in most specimens; ac-tinopharynx ribbed, darker beige than column. Mesenteries to five orders (Figure 4a);

thin, each with oral but no marginal stoma. Retractor muscles diffuse (Figure 4b). Parieto-basilar muscle with short free penon. Sexes presumably separate: three females, one male examined. First three orders complete and sterile, rest incomplete and fertile (Figure 4a).

***Carcinoecium*:** Shape similar to that of dextral, trochoid gastropod shell. Aperture with simple arced elliptical outer lip, fairly straight parietal wall along what would be termed the columella in a gastropod. Bronze color, becomes chalky out of liquid.

***Cnidae*:** Cnidom: Spirocysts, basitrichs, microbasic *p*-mastigophores. Table 1 lists distribution and size of cnidae; Figure 5 depicts each cnida type. The largest specimen (MTQ G57580) possessed small basitrichs (*e*) and (*f*) in the actinopharynx, and small basitrichs (*b*) in the column that were not found in other specimens.

***Habitat*:** Mud and rocks, 590–694 m.

***Distribution*:** From Coral Sea of northern Queensland to southern Queensland coast (Figure 6).

***Symbiont*:** Hermit crab *Sympagurus trispinosus* (Balss, 1911), identified by Dr. Rafael Lemaitre (Curator of Crustacea, Department of Invertebrate Zoology, USNM).

**Etymology.** This species is named for R Alastair Birtles of James Cook University, Townsville, who, with the late P Arnold (MTQ) and M Pichon (Australian Institute of Marine Science), collected this species and photographed it alive.

### **New records of *Stylobates loisetteae* Fautin, 1987**

WAM Z50046 (one specimen).

Locality: 12.1814°S, 123.4177°E, 400 m; Ashmore, Western Australia, Australia (FRV *Southern Surveyor*, Station SS0507/189, Beam Trawl). Collected 6 July 2007, by MP Salotti.

WAM Z50047 (one specimen).

Locality: 13.2247°S, 123.3957°E, 400 m; Kulumburu, Western Australia, Australia (FRV *Southern Surveyor*, Station SS0507/176, Sherman Sled). Collected 5 July 2007, by MP Salotti.

WAM Z50049 (one specimen).

Locality: 13.2650°S, 123.3741°E, 400 m; Kulumburu, Western Australia, Australia (FRV *Southern Surveyor*, Station SS0507/180, Beam Trawl). Collected 6 July 2007, by MP Salotti.

WAM Z50050 (two specimens).

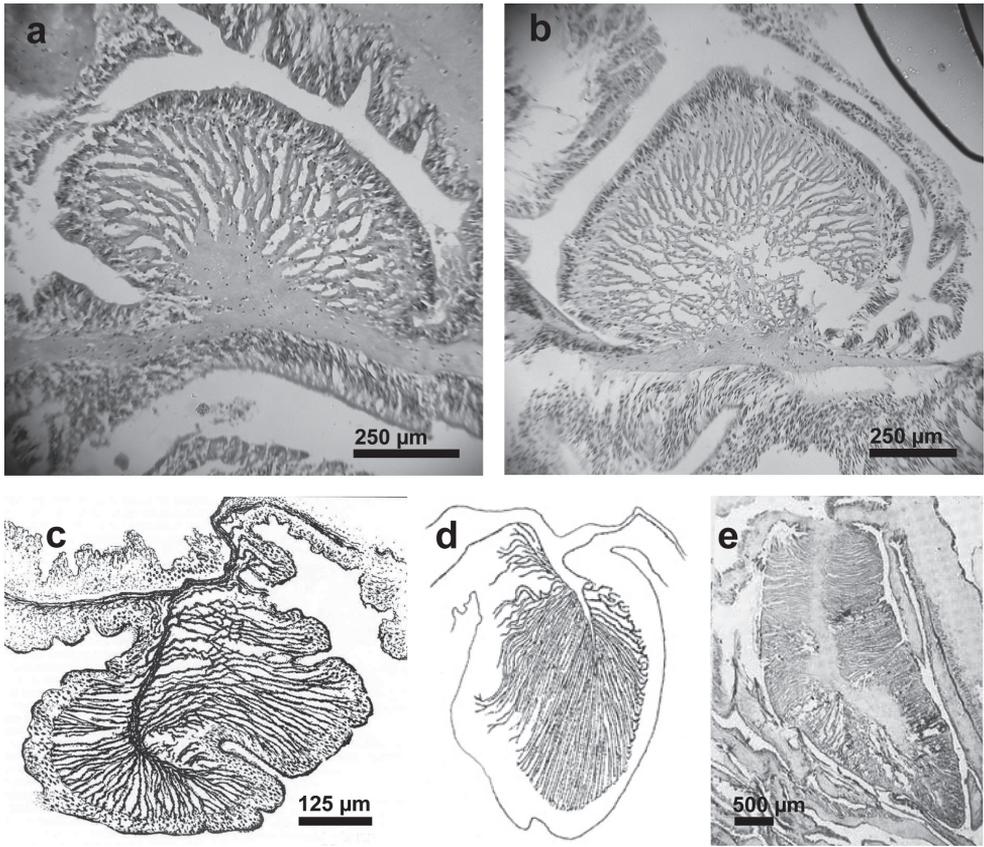
Locality: 15.6102°S, 120.8076°E, 400 m; Lacepede, Western Australia, Australia (FRV *Southern Surveyor*, Station SS0507/130, Beam Trawl). Collected 1 July 2007, by MP Salotti.

WAM Z50058 (two specimens).

Locality: 12.5295°S, 123.4273°E, 400 m; Ashmore, Western Australia, Australia (FRV *Southern Surveyor*, Station SS0507/192, Beam Trawl). Collected 6 July 2007, by MP Salotti.

**Table 1.** Cnidae of all four species of *Syngonostoma*, given as range in length X width of undischarged capsules in  $\mu\text{m}$  (outlier measurements in parentheses). n = number of capsules measured, N = ratio of number of animals in which that type of cnida was found to the number of animals examined (where data are available). Frequency of cnida type indicated by the following: ++ very common, + common, - sporadic. Letters in parentheses correspond to images in Figure 5 for *S. birtlesi* sp. n.

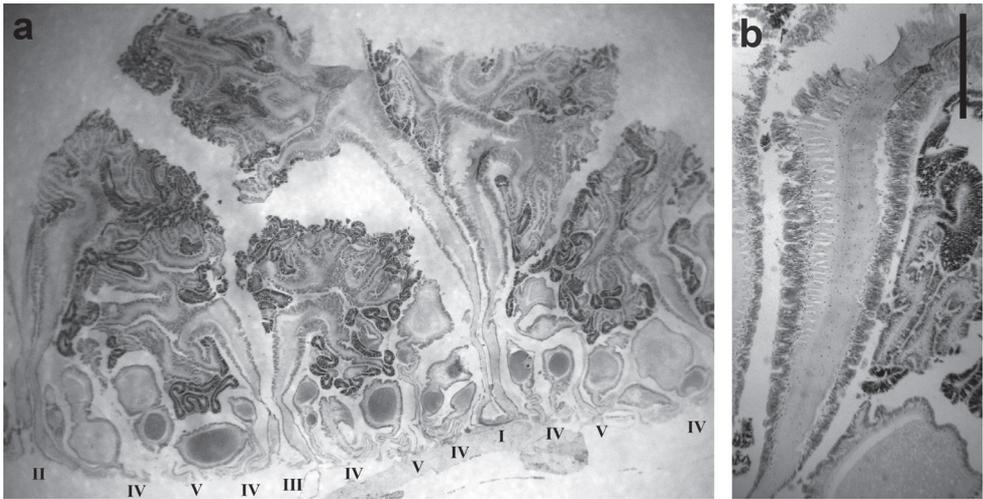
	<i>S. birtlesi</i> sp. n.	<i>S. aeneus</i>	<i>S. cancrisocia</i>	<i>S. loiseteae</i>
Tentacles	Basitrich (a) 26.6-38.8 X 2.6-4.6 n=63 N=6/6 [++]	27.9-36.1 X 3.1-3.9 n=43	27.1-30.3 X 2.5-3.3 n=11	29.8-39.7 X 2.5-3.7 n=70 N=6/6
	Basitrich (b) (11.9) 14.2-19.9 X 2.6-3.4 n=12 N=5/6 [-]			9.9-16.1 X 1.6-2.5 n=11 N=3/6
	Spirocyst (c) 17.9-39.8 (46.3) X 2.2-4.6 n=51 N=7/7 [+	(25.4) 28.7-44.3 X 2.9-4.1 (4.9) n=40	23.8-39.4 X 2.7-3.3 n=10	21.1-55.8 X 2.5-3.7 n=63 N=6/6
Actinopharynx	Basitrich (d) 27.8-37.1 X 2.9-4.3 n=61 N=6/6 [++]	(26.2) 29.5-37.7 X 2.9-4.1 n= 38	27.1-31.2 X 2.5-3.3 n=10	26.0-37.2 X 2.5-3.7 n=59 N=5/5
	Basitrich (e) 19.9-21.2 X 3.3 n=9 N=1/6 [-]	17.2-21.3 X 2.5-3.1 n=9		
	Basitrich (f) 6.4-11.2 X 1.9-2.8 n=20 N=1/6 [-]			
Column	Basitrich (g) 19.9-30.5 X 2.6-4.1 n=65 N=7/7 [++]	29.5-36.1 X 2.9-3.5 n=24	23.0-28.7 X 2.7-3.3 n=12	21.1-33.5 X 2.5-3.7 n=46 N=5/5
	Basitrich (h) 7.3-9.2 X 1.9-3.6 n=10 N=1/6 [-]			
Mesenterial Filaments	Basitrich (i) 27.5-37.1 X 4.4-6.7 n=25 N=6/6 [++]		(25.4) 27.9-32.8 X 4.1-5.5 n=14	28.5-37.2 X 4.7-6.0 n=34 N=5/7
	Basitrich (j) 28.5-33.2 X 2.8-3.8 n=18 N=5/6 [-]	29.5-36.1 X 2.5-3.9 n=21		28.5-37.2 X 2.5-3.5 n=11 N=3/7
	Basitrich (k) 15.9-21.4 X 2.1-3.1 n=51 N=6/6 [++]	15.6-23.0 X 2.1-3.3 n=58	14.8-18.0 X 2.1-2.5 n=8	12.4-16.1 X 1.9-3.1 n=10 N=4/7
	Basitrich (l) 7.9-11.9 X 1.9-2.9 n=21 N=3/6 [+			
Microbasic <i>p</i> -mastigophore (m)	21.2-30.0 X 4.4-7.9 n=45 N=5/6 [++]	21.3-29.5 X 3.9-5.7 n=27	18.9-23.0 X 4.1-5.7 n=12	23.6-32.2 X 3.5-6.2 n=50 N=7/7
Source:	This study	Dunn et al. 1981	Dunn et al. 1981	Fautin 1987



**Figure 3.** Endodermal circumscribed marginal sphincter muscles of *Stylobates* spp. **a, b** Palmate marginal sphincter muscle of *Stylobates birtlesi* sp. n. **a** paratype MTQ G57581 **b** paratype KUDIZ 003352 **c-e** Pinnate marginal sphincter muscles. **c** *Stylobates aeneus* (from Dunn et al. 1981) **d** *Stylobates cancrisocia* (from Carlgren 1928a) **e** *Stylobates loisetteae* (from Fautin 1987).

### Differential diagnosis

Tables 1 and 2 present the major attributes of the four known species of *Stylobates*. *Stylobates birtlesi* sp. n. differs from the others in size and distribution of some of its nematocysts (Table 1), and the nature of the marginal sphincter muscle (Figure 3a, b). Compared to *Stylobates birtlesi* sp. n., *Stylobates aeneus* and *Stylobates cancrisocia* do not possess basitrichs (*b*) in the tentacles nor basitrichs (*l*) in the mesenterial filaments; *Stylobates aeneus* lacks basitrichs (*i*) and *Stylobates cancrisocia* lacks basitrichs (*j*) in the mesenterial filaments. The clearest distinguishing feature of *Stylobates birtlesi* sp. n. is the possession of an endodermal circumscribed marginal sphincter muscle in which the lamellae are arranged in a palmate fashion (Figure 3a, b). This is clearly different to the other three species, which all possess an endodermal circumscribed marginal sphincter muscle in which the lamellae are arranged in a pinnate fashion (Figure 3c–e).

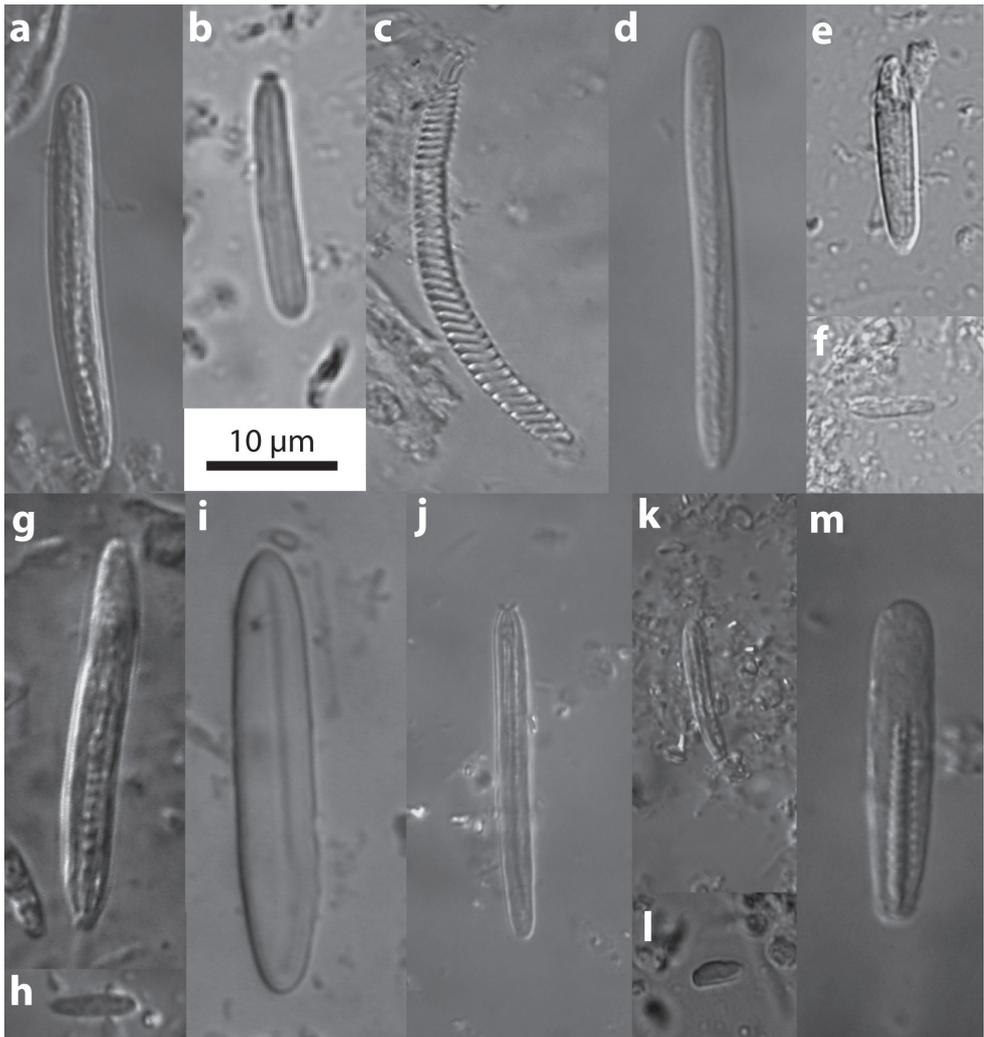


**Figure 4.** Mesenterial musculature and fertility of *Stylobates birtlesi* sp. n. holotype MTQ G57579 **a** mesenterial arrangement, orders indicated with Roman numerals; column wall at base of image **b** diffuse retractor muscle; column wall at base of image. Scale bar 1 mm.

Gross morphology of *Stylobates birtlesi* sp. n. is similar to that of *Stylobates aeneus* and *Stylobates cancrisocia* in position and size of oral disc, and size and arrangement of tentacles. Tentacles of *Stylobates birtlesi* sp. n. (maximum length 9 mm) are shorter than those of *Stylobates loisetteae* (maximum length 20 mm). The tentacles of *Stylobates loisetteae*, in contrast to those of other species, are more or less the same length around the oral disc, and the marginal tentacles are longer than the discal ones. The tentacles of *Stylobates aeneus* and *Stylobates cancrisocia* are arranged like those of *Stylobates birtlesi* sp. n., the longest and shortest ones beside each other (Figure 1e). Diameter of the oral disc of *Stylobates birtlesi* sp. n. (15–40 mm) is similar to that of *Stylobates aeneus* and *Stylobates cancrisocia*, but less than that of *Stylobates loisetteae* (to 55 mm). The position of the oral disc of *Stylobates birtlesi* sp. n. is near the aperture of carcinoecium, like in *Stylobates aeneus* and *Stylobates cancrisocia*, whereas that of *Stylobates loisetteae* is on the side of the ultimate whorl of the carcinoecium, away from the aperture.

## Discussion

The four species of *Stylobates* are distributed allopatrically (Figure 6), in what Cairns (2007) identified as separate biogeographical regions based on distributions of deep-water scleractinian corals. *Stylobates birtlesi* sp. n. occurs in the Coral Sea off the Queensland coast of Australia (southwestern Pacific region); *Stylobates aeneus* is known from Hawai'i and Guam (central Pacific region); *Stylobates loisetteae* occurs in the Indian Ocean off the northwest coast of Australia (southeastern Indian Ocean region); and *Stylobates cancrisocia* is known from the Indian Ocean off east Africa (southwestern



**Figure 5.** Cnidae of *Stylobates birtlesi* sp. n. Refer to Table 1 for list of cnida types and distribution.

Indian Ocean region). Congeneric species of squat lobsters of the genus *Paramunida* have a similar allopatric distribution in the central and the southwestern regions of the Pacific (Baba et al. 2008).

Uchida and Soyama (2001) reported *Isadamsia* sp. J from Japan; that locality is consistent with the distribution of *Stylobates aeneus*. Doumenc (1975) reported *Isadamsia cancrisocia* in the North Atlantic at 3360–3600 m. We are dubious about this identification (and do not include the record in Figure 6) because all records for the occurrence of *Stylobates* are from the Indo-West Pacific and at shallower depths.

Carcinoecium-forming anemones of genera characterized by a mesogleal sphincter muscle are known from the Atlantic: for example, *Paracalliactis consors* (Verrill, 1882) occurs off the northeast coast of the United States at depths of 2085–2665 m, and

**Table 2.** Morphological, biogeographic, and ecological attributes of all four species of *Stylobates*.

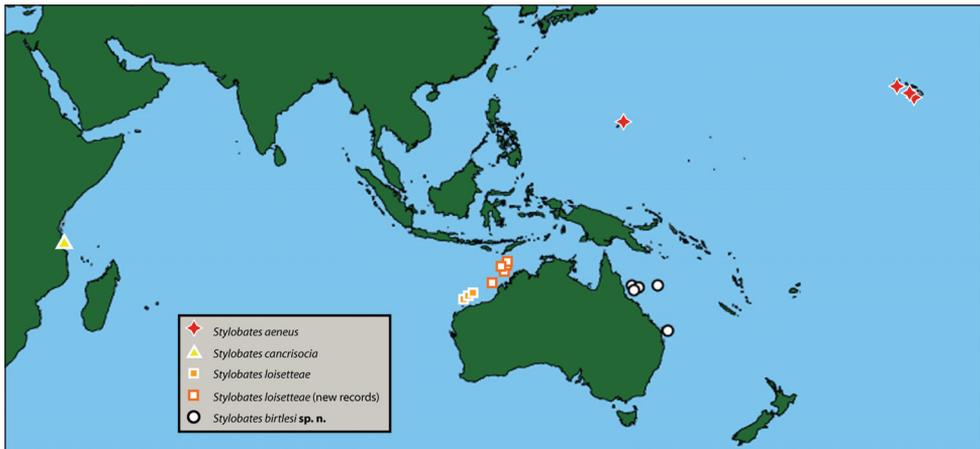
	<i>S. birtlesi</i> sp. n.	<i>S. aeneus</i>	<i>S. cancrisocia</i>	<i>S. loisetteae</i>
<b>Marginal sphincter muscle</b>	endodermal, circumscribed, palmate	endodermal, circumscribed, pinnate	endodermal, circumscribed, pinnate	endodermal, circumscribed, pinnate
<b>Tentacle lengths</b>	differ around oral disc	differ around oral disc	differ around oral disc	marginal greater than oral
<b>Maximum oral disc diameter (mm)</b>	15–40	~20	15–30	to 55
<b>Locality</b>	NE Australia	Guam and Hawai'i	E Africa	NW Australia
<b>Depth (m)</b>	590–964	402–797	818	320–508
<b>Substrate</b>	mud, rock	sand	not recorded	mud
<b>Hermit crab symbiont</b>	<i>Sympagurus trispinosus</i>	<i>Sympagurus dofleini</i>	<i>Sympagurus trispinosus</i>	<i>Sympagurus brevipes</i>

*Adamsia obvolva* Daly et al., 2004, occurs in the Gulf of Mexico at depths of 405–719 m. A specimen of an anemone symbiotic with a hermit crab in the Invertebrate Zoology collection of the California Academy of Sciences (catalog number 35119) from 2630–2660 m off the Pacific coast of Mexico is not *Stylobates*, as it is labelled, based on its mesogleal sphincter muscle.

A specimen in the Invertebrate Zoology collection of WAM (catalog number Z31227) of an anemone that laid down some chitinous material on the gastropod shell to which it is attached is from the same region and depth as *Stylobates loisetteae* off the coast of Western Australia, and has an endodermal sphincter, but does not belong to *Stylobates*, either. This anemone differs from *Stylobates* in that its pedal disc does not cover the whole shell; the chitinous material does not form a carcinoecium; the column is more or less cylindrical and is much thicker than that of *Stylobates*; and the contracted oral disc creates a collar at the margin.

Hermit crabs form symbioses with about 100 species of cnidarians (Williams and McDermott 2004). They occur shallow and deep, in tropical and temperate seas. The hermit crab is thought to be protected by its cnidarian symbiont (e.g. Ross 1971, McLean and Mariscal 1973, Bach and Herrnkind 1980, Brooks 1988, 1989, Brooks and Gwaltney 1993); possible benefits to the cnidarian include transport (Balls 1924, Ross 1974), a firm substrate for attachment (Brooks and Mariscal 1986), and access to food collected by the hermit crab (Ross 1984).

Many carcinoecium-forming species occur in the deep sea, where calcium carbonate (the mineral of mollusc shells) is highly soluble (Correns 1955), resulting in a limited supply of shells (Balls 1924) and rarity of large shells. A hermit crab living in a chitinous carcinoecium need not change shells as it grows, nor will the carcinoecium dissolve (Dunn et al. 1981). In symbioses not involving a carcinoecium, more than one anemone may be attached to a gastropod shell inhabited by a hermit crab, whereas in the *Stylobates/Sympagurus* system, one anemone is associated with one hermit crab. In the most thorough account of this association, Dunn et al. (1981) considered it to be



**Figure 6.** Distribution of species of *Stylobates*.

obligate for the anemone (which receives food, transport, and substrate) and facultative for the hermit crab.

Each species of *Stylobates* is associated with hermit crabs of one species, all belonging to *Sympagurus*. *Stylobates aeneus* occurs with *Sympagurus dofleini* (Bals, 1912), *Stylobates cancrisocia* and *Stylobates birtlesi* sp. n. both occur with *Sympagurus trispinosus* (Bals, 1911) and the hermit crab associated with the newly recorded specimens from the Southern Surveyor cruise of *Stylobates loisetteae* occur with *Sympagurus brevipes* (de Saint Laurent, 1972) (A McCallum, Museum Victoria, pers. comm.); hermit crabs of this species are “frequently associated with actinian-secreted carcinoecium similar to that of *Stylobates*” (McLaughlin et al. 2007, p. 299).

## Conclusion

We describe *Stylobates birtlesi* sp. n., a new species of deep-sea anemone associated with the hermit crab *Sympagurus trispinosus* (Bals, 1911), from specimens collected in the Coral Sea off the Queensland coast of Australia. *Stylobates birtlesi* sp. n. differs from the other three known species of *Stylobates* in some aspects of its nematocysts, and in having a palmate marginal sphincter muscle (in the others it is pinnate). The four species of *Stylobates* are allopatrically distributed in the deep Indo-West Pacific Ocean, a pattern similar to those of deep-sea scleractinian corals and squat lobsters.

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# First record of the adventive oriental aphid *Schizaphis pircicola* (Matsumura, 1917) (Hemiptera, Aphididae) in Europe

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## Abstract

The oriental aphid *Schizaphis pircicola* (Matsumura) is recorded for the first time in Europe, on the ornamental pear tree *Pyrus calleryana* in landscaped areas in Madrid (Spain). Data on the morphology of the forms on primary host (apterous and alate fundatrigeniae and fundatrices), and their biology and distribution are given. The keys for identifying species of *Schizaphis* (*Schizaphis*) in the Iberian Peninsula are updated. Two species of aphids are also recorded for the first time on *Pyrus calleryana*: *S. pircicola* and *Aphis pomi*.

## Keywords

*Schizaphis pircicola*, aphids, adventive species, Spain

## Introduction

The genus *Schizaphis* Börner contains approximately 36 Palearctic species and 6 Nearctic ones. Is a genus resembling *Rhopalosiphum* Koch with little differences between both genus, and for this reason require further taxonomical and molecular study (Blackman and Eastop 1994; Foottit et al. 2008). The species of *Schizaphis* are characterised by more or less cylindrical siphunculi slightly constrained at the apex, ultimate rostral segment short and heart size, absence of dorsal cuticular ornamentation, and alatae with two branches on the medial vein of the wings (Fig. 1D) (Pérez Hidalgo and Mier Durante

2005). Most of the species in the genus are monoecious on species of Poaceae, Juncaceae and Cyperaceae but a few mainly oriental species, are dioecious with species of *Pyrus* as the primary host, where they lay their cold-resistant eggs (Blackman and Eastop 2006).

Twenty-seven species have been recorded in Europe (Holman 2009; Nieto Nafría et al. 2010): 21 in the nominal subgenus, 5 in the subgenus *Paraschizaphis* and one in the subgenus *Euschizaphis*. Eight of them were recorded in the Iberian Peninsula (Pérez Hidalgo and Mier Durante 2005; Blackman and Eastop 2006): *S. graminum* (Rondani), *S. longicaudata* Hille Ris Lambers, *S. pyri* Shaposhnikov, *S. rotundiventris* (Signoret), *S. (Euschizaphis) palustris* (Theobald), *S. (Paraschizaphis) caricis* (Schouteden), *S. (Paraschizaphis) rosazevedoi* (Ilharco) and *S. (Paraschizaphis) scirpi* (Passerini). Of these, only *S. pyri*, has *Pyrus communis* as its primary host and Cyperaceae as its secondary host; the rest live on Poaceae or Cyperaceae without causing financial loss, except for *S. graminum* which can be a cereal pest (Blackman and Eastop 2000).

A photograph of a colony of aphids on the pear tree of oriental origin *Pyrus calleryana* Decne in “Juan Carlos I park”, Barajas (Madrid, Spain) (Fig. 1) taken on 26th April, 2009 and posted on the “Biodiversidad Virtual” portal (<http://www.biodiversidadvirtual.org/>) enabled the oriental species *Schizaphis pircicola* (Matsumura) to be detected for the first time in Europe. Its presence was confirmed in a study of samples collected the following spring on the same host and in the same place. Its route of entry into Europe is probably linked to when the host plant was imported, as is the case of many other species introduced into Europe (Coeur d’acier et al. 2010).

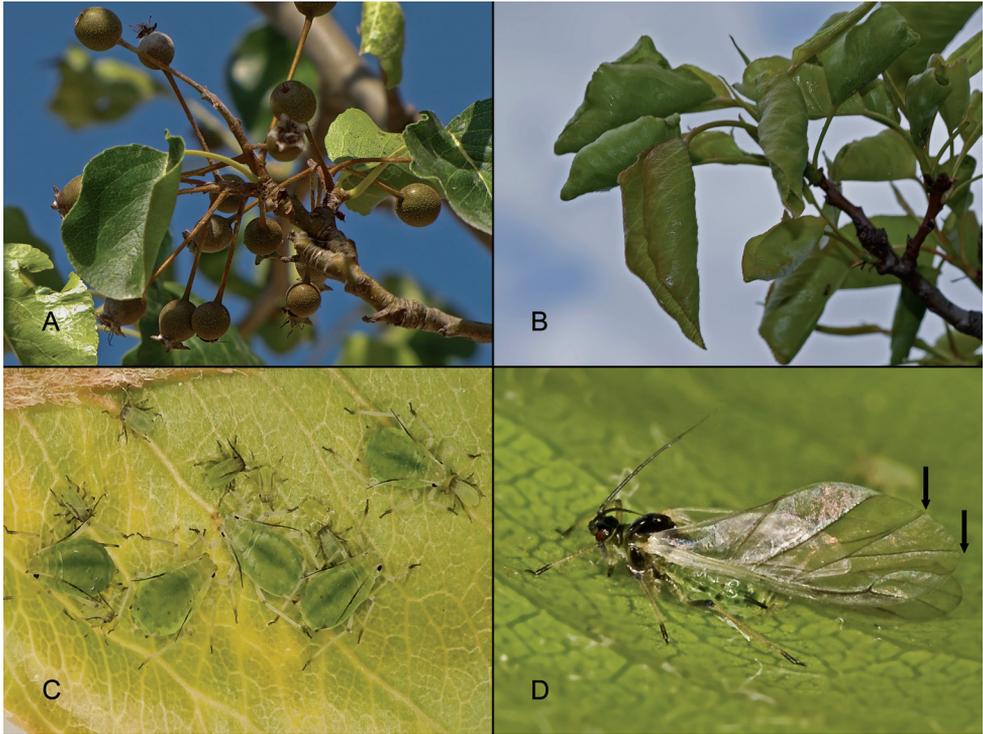
This finding is yet another example of how social networks play an important role in our knowledge of biodiversity and the detection and/or monitoring of invasive or endangered species (Pérez Hidalgo et al. 2009; Silvertown 2010).

## Studied material

Samples containing several apterae (3 fundatrices and 15 fundatrigeniae, measured) and alatae (15 measured) were collected between 11<sup>th</sup> April and 4<sup>th</sup> May, 2010 in “Juan Carlos I park”, Barajas (Madrid, Spain) [40°28'12.77"N, 3°35'6.22"W] (reference M-222). Several populations were also located on the same host on 7<sup>th</sup> May, 2010 in Torrejón de Ardoz (Madrid, Spain) [40°27'23.17"N, 3°28'3.02"W] (M-224) and at the “Vallecas Villa” railway station (Madrid, Spain) [40°22'6.23"N, 3°37'1.15"W] (M-225) on 17<sup>th</sup> May, 2010. These samples are deposited in the aphid collection of the University of León, Spain and the samples of associated ants in the Collection of the Universidad Autónoma de Barcelona, Spain (Dr. Xavier Espadaler).

## Description of the forms of *Schizaphis pircicola* on primary host

The apterous fundatrigeniae (Figs 1C, 2B, 3B, E, H) are between 1.47 and 2.50 mm long and yellowish green to green, with pale antennae and siphunculi bearing dark tips



**Figure 1.** *Pyrus calleryana* with fruit (A), malformation caused by *Schizaphis piricola* (Matsumura) on *P. calleryana* leaves (B), apterous fundatrigeniae (C) and alate fundatrigeniae (D) of *S. piricola*, the arrows indicate the tips of the two branches of the medial vein.

and dark tarsi. Antennae 0.65 to 0.90 times the body; processus terminalis of antennal segment VI 3.13 to 3.92 times its base, and 0.97 to 1.13 times antennal segment III. Antennae without secondary rhinaria. Apical rostral segment 0.90 to 1.09 times second segment of posterior tarsi and usually with 2 accessory setae. Dorsal sclerotization absent. Marginal papillae on abdominal segments I to VII, absent on III to V in some specimens. Dorsoabdominal setae of anterior tergites shorter (17 to 30  $\mu\text{m}$ ) than those of posterior (55 to 90  $\mu\text{m}$ ), ventral setae intermediate in size (45 to 60  $\mu\text{m}$ ). Siphunculi cylindrical, with weakly defined subapical constriction, 0.12 to 0.19 times body and 1.84 to 2.12 times cauda. Cauda 0.17 to 0.19 mm, 1.21 to 1.46 times basal width, bearing 7 to 9 setae. Tibiae of posterior legs 0.44 to 0.50 times body.

The fundatrices (Figs 2A, 3A, D, G) resemble the apterous fundatrigeniae except for the characteristics of the “fundatrix facies” (Lees 1961; Miyazaki 1987): shorter antennae (1.05–1.25 mm and 0.40 to 0.47 times body), processus terminalis of antennal segment VI (1.82 to 2.18 times base and 0.71 to 0.72 times antennal segment III), siphunculi (approximately 0.10 times body and 1.47 to 1.53 times cauda) and legs (posterior tibiae 0.35 to 0.37 times body).

Alatae fundatrigeniae (Figs 1D, 2C, 3C, F, I) 2.10 to 2.52 mm, green, antennae and siphunculi dark, cauda lighter in colour. Well-pigmented marginal sclerites in ter-

guites II to IV, postsiphuncular sclerites fully developed and spinopleural pigmented bands always present on abdominal segments VI to VIII and also occasionally on III to V. Antennae 0.71 to 0.80 times body; processus terminalis of antennal segment VI 3.41 to 5.18 times base; antennal segments III, IV and V bearing 15–32, 7–18, 0–6 secondary rhinaria, respectively. Siphunculi 1.68 to 1.80 times cauda. The remaining characters are similar to those of the apterae.

## Distribution

*Schizaphis piricola* (Matsumura) is an aphid of oriental origin which, according to Holman (2009), had only been recorded in China (north east, south east and Taiwan), Japan and Korea, though Lee et al. (2002) have records for India and Pakistan.

Nevertheless, it is possible that *S. piricola* is now more widely distributed because *Pyrus calleryana* is a very commonly planted ornamental tree species. For example, in the United States there is evidence that this tree species is rapidly becoming invasive in much of its horticultural range (Vincent 2005) and it is possible that the aphid is present at this moment.

## Biology

It is a holocyclic dioecious species with species of pear tree (*Pyrus* sp.) as its primary host and Cyperaceae (*Carex* spp. and *Cyperus rotundus* L.) as secondary host (Miyazaki 1988; Blackman and Eastop 1994; Eastop and Blackman 2005; Blackman and Eastop 2006). It has been recorded on *Pyrus x bretschneideri* Rehder, *Pyrus communis* L., *Pyrus pyrifolia* (Burm. Fil.) Nakai and *Pyrus ursuriensis* Maxim. (Blackman and Eastop 1994; Holman 2009). There are also records on *Prunus persica* (L.) Batsch in Japan (Higuchi and Miyazaki 1969; Moritsu 1983) which we believe should be confirmed. Based on all of these data, this is the first record of an aphid species on *P. calleryana* (Fig. 1).

In spring in Spain, colonies of this species cause the leaves of *P. calleryana* to curl (Fig. 1B) as occurs in *Pyrus pyrifolia* in Japan, Korea and China (Essig and Kuwana 1918), and are attended by the ant *Tapinoma nigerrimum* (Nylander). The fundatrices appear at the beginning of April and the alatae leave the primary host in mid June to colonize their secondary hosts. Efforts to locate the virginogeniae in these hosts in summer, or alatae re-migrating to the primary hosts in autumn, have so far produced no results.

## Damage to the host plant

The direct action of sucking by the aphids (clearly seen in the curling of the leaves), and indirect damage caused by the honeydew they excrete, which covers the leaves, can affect the normal growth of the trees, all the more so if other aphid species (*Aphis pomi*

De Geer 1773 and *Dysaphis* sp.) sometimes forming mixed colonies with *S. piricola*, are present.

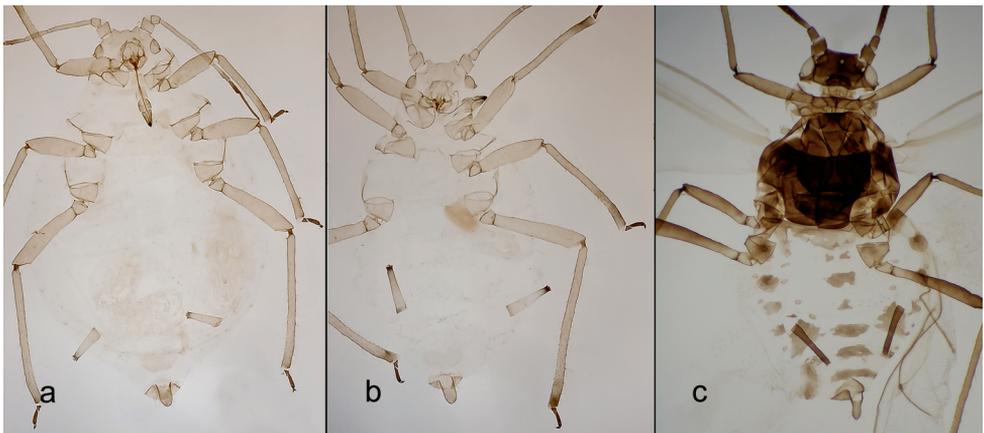
The trees of *Pyrus calleryana* in “Juan Carlos I park” (Barajas, Madrid, Spain), which have been monitored more carefully, were planted two years ago and do not seem to have reached the height and size expected for this species. In any case, a more in-depth study of the population dynamics and auxiliary fauna (coccinellidae, syrphids, etc...) is necessary, taking into account other variables (humidity, temperature, etc...), to be able to reach conclusions on possible damage.

**Identification keys**

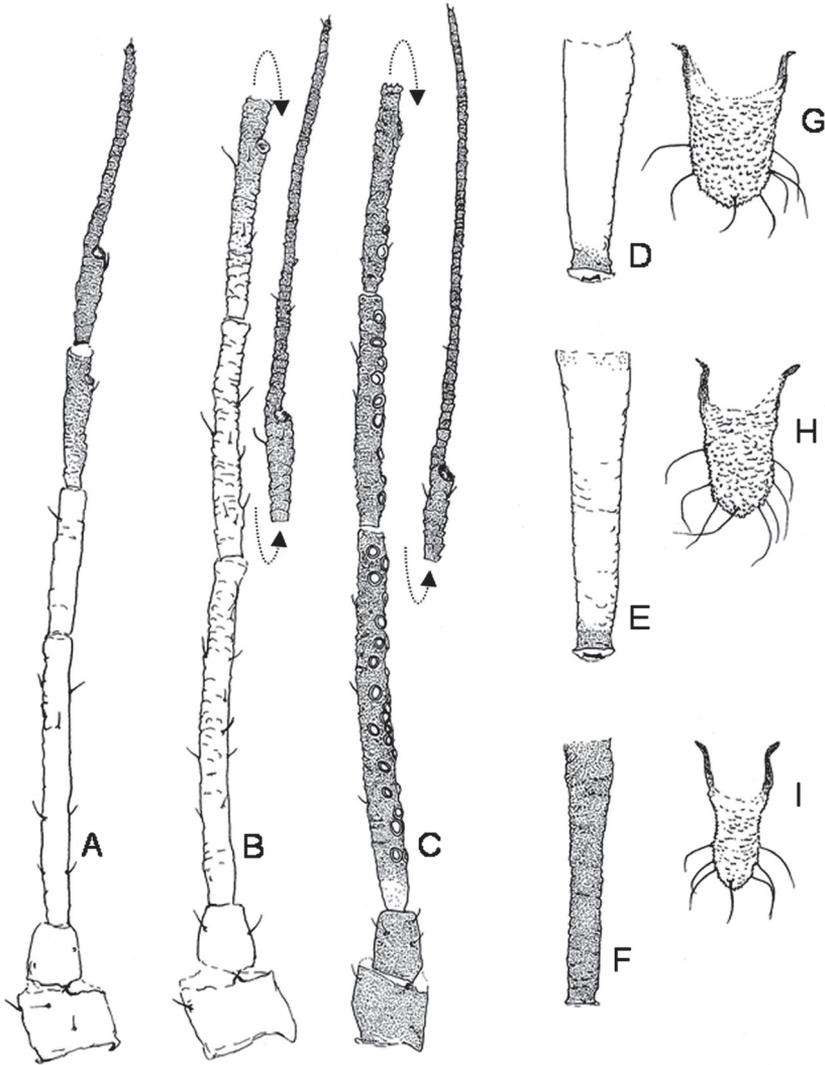
The following keys enable all the species in the subgenus *Schizaphis* recorded in the Iberian Peninsula to be separated.

**Species key (apterae viviparae females)**

- 1 Siphunculi pale with pigmented apex (Figs 2A, B, 3D, E) ..... 2
- Siphunculi entirely dark..... 4
- 2 Siphunculi 0.8 times cauda at most. Usually on *Phalaris arundinacea* ..... *S. (S.) longicaudata*
- Siphunculi at least 0.9 times cauda. On many species of Gramineae and on *Pyrus calleryana* ..... 3
- 3 Siphunculi 1.1 to 1.6 times cauda. On many species of Gramineae ..... *S. (S.) graminum*
- Siphunculi 1.82 to 2.1 times cauda in apterous fundatrigeniae (Figs 2B, 3E) and 1.47–1.53 in fundatrices (Figs 2A, 3D). On *Pyrus calleryana* (primary host) ..... *S. (S.) piricola*



**Figure 2.** Fundatrix (A), apterous fundatrigenia (B) and alate fundatrigenia (C).



**Figure 3.** Antennae (A, B, C), siphunculi (D, E, F) and cauda (G, H, I) of fundatrix (A, D, G), apterous fundatrigenia (B, E, H) and alate fundatrigenia (C, F, I) of *Schizaphis piricola* (Matsumura).

- 4      Processus terminalis of antennal segment VI 3.6 to 4.5 times its base. Siphunculi 2.5 times cauda at most. Marginal papillae on abdominal segments I, VI and VII. On *Pyrus communis* (primary host) or Ciperaceae (secondary host) ...  
 ..... **S. (S.) pyri**
- Processus terminalis of antennal segment VI 4.7 to 6.8 times its base. Siphunculi at least 2.5 times cauda. Marginal papillae on abdominal segments I and VII only. On *Cyperus* and seldom other plants ..... **S. (S.) rotundiventris**

**Species key (alate viviparous females)**

- 1 Siphunculi pale with pigmented apex..... 2
- Siphunculi entirely dark (Figs 2C, 3F) ..... 3
- 2 Siphunculi 0.8 times cauda at most. Normally on *Phalaris arundinacea* .....  
..... **S. (S.) longicaudata**
- Siphunculi at least 0.9 times cauda. On many species of Gramineae and on  
*Pyrus calleryana* ..... 3
- 3 Siphunculi same size as cauda. Bearing 4–10, 0–4 and 0–1 secondary rhy-  
naria on antennal segments III, IV and V, respectively. On many species of  
Gramineae .....**S. (S.) graminum**
- Siphunculi (Figs 2C, 3F) 1.6 to 1.8 times cauda. Bearing 18 to 32, 7–18 and  
2–6 secondary rhynaria on antennal segments III, IV and V, respectively. On  
*Pyrus calleryana* (primary host)..... **S. (S.) piricola**
- 4 Siphunculi 0.1 times body at most and 1.5 to 1.8 times cauda. Processus ter-  
minalis of antennal segment VI 3.6 to 5.0 times its base and 1.1 to 1.6 times  
antennal segment III, and approximately 2.0 times siphunculi. On *Pyrus*  
*communis* (primary host) and Ciperaceae (primary host)..... **S. (S.) pyri**
- Siphunculi at least 0.1 times body and 1.1 to 3.0 times cauda. Processus ter-  
minalis of antennal segment VI 4.7 to 6.0 times its base and 1.3–1.8 times  
antennal segment III. On *Cyperus*..... **S. (S.) rotundiventris**

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# A new species of *Simulium* (*Nevermannia*) (Diptera, Simuliidae) from Thailand, with keys to members of the *Simulium feuerborni* species-group in Thailand

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## Abstract

*Simulium* (*Nevermannia*) *maeaiense* sp. n. is described on the basis of female, male, pupal and larval specimens collected from Chiang Mai Province, Thailand. This species is assigned to the *feuerborni* species-group of the subgenus *Simulium* (*Nevermannia*), and is distinctive among this species-group in having the female cibarium furnished with numerous dark minute conical processes on the lower part, the female genital fork with a strongly sclerotized horizontal bar on each arm, and six long pupal gill filaments arising nearly at the same level from the common basal stalk and lying in a horizontal plane. Identification keys to seven species of the *feuerborni* species-group reported from Thailand are provided for females, males, pupae and mature larvae.

## Keywords

*Simulium*, black fly, Thailand

## Introduction

The *Simulium feuerborni* species-group within the subgenus *Nevermannia* Enderlein, redefined by Takaoka (2003), is a small homogeneous group consisting of 24 named and 1 unnamed species (Adler and Crosskey 2010). The majority of species of this group are distributed in the Oriental Region and only 4 are known in eastern parts (China, Korea and Japan) of the Palearctic Region. This group is relatively rich in species diversity in Thailand, being represented by 5 endemic species: *S. (N.) fangense* Takaoka and Choochote, *S. (N.) fruticosum* Takaoka and Choochote, *S. (N.) chiangklangense* Takaoka and Choochote, *S. (N.) vessabutrae* Takaoka and Srisuka, and *S. (N.) wichaii* Takaoka (Takaoka and Choochote 2005, 2006; Takaoka and Srisuka 2010a,b) and one common species: *S. (N.) feuerborni* Edwards, which was originally described from Java (Edwards 1934) and later recorded from Peninsular Malaysia (Takaoka and Davies 1995), Thailand (Kuvangkadilok et al. 1999) and Sumatra (Takaoka et al. 2000).

In Thailand, we collected one more species of the *feuerborni* species-group, which is easily distinguished from all the known species by the female cibarium, the female genital fork, and the arrangement of the pupal gill filaments. It is described here as a new species based on female, male, pupal and mature larval specimens.

Identification keys to seven species of the *feuerborni* species-group reported from Thailand are also provided for females, males, pupae and mature larvae.

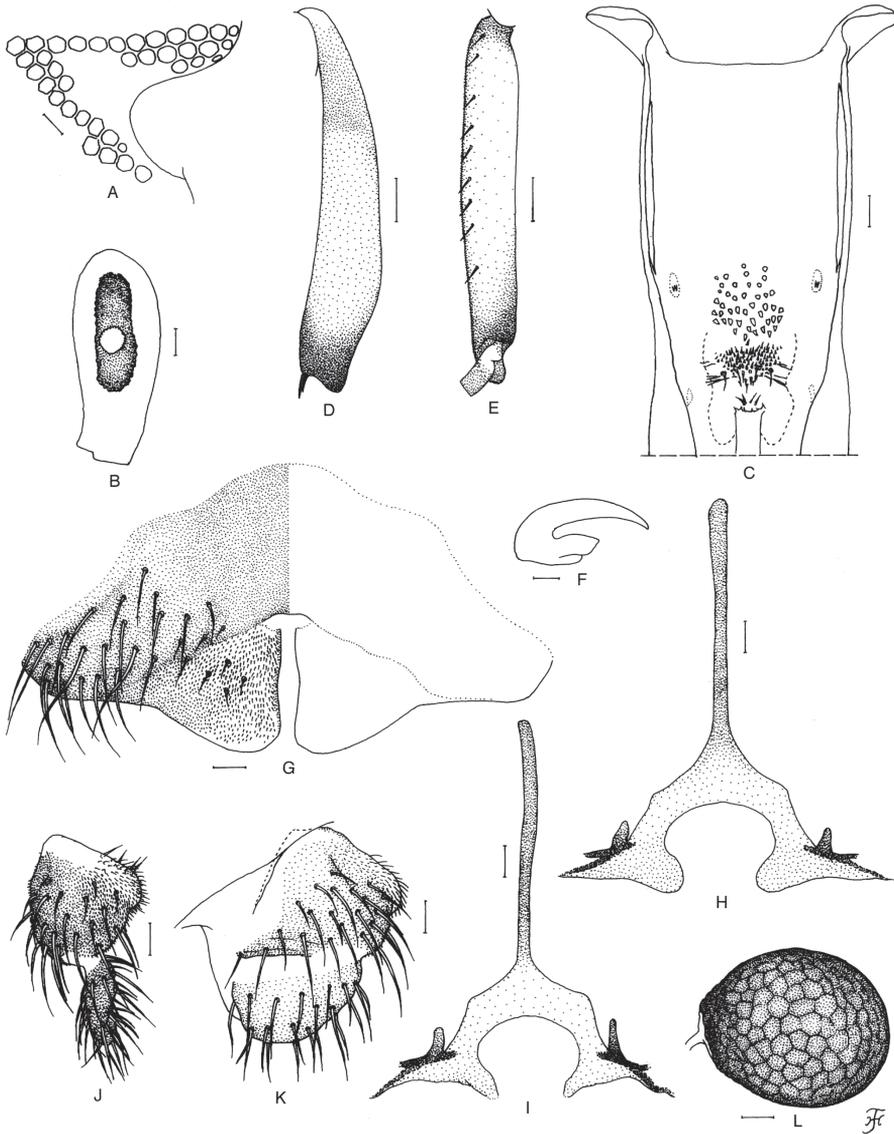
The terms for morphological features used here follow those of Takaoka (2003). Holotype and paratype specimens of the new species are deposited at the Entomology Section, Queen Sirikit Botanic Garden, Chiang Mai, Thailand.

## Systematics

### *Simulium (Nevermannia) maeaiense* sp. n.

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**Description. Female.** Body length 2.4–2.6 mm. **Head.** Slightly narrower than thorax. Frons dark brown, thinly whitish-gray pruinose, densely covered with whitish-yellow recumbent hairs interspersed with several dark brown longer and stouter hairs along each lateral margin. Frontal ratio 1.55–1.79:1.00:2.53–2.76. Frons-head ratio 1.00:5.00–5.54. Fronto-ocular area (Fig. 1A) well developed, triangular, directed laterally and slightly upward. Clypeus dark brown, whitish-gray pruinose, densely covered with whitish-yellow recumbent hairs (except portion near upper margin bare) intermixed with several dark longer and stouter hairs on each side. Labrum 0.88–1.05 times as long as clypeus. Antenna composed of scape, pedicel and 9 flagellomeres, dark brown except scape, pedicel, and base of 1st flagellomere yellow. Maxillary palp consisting of 5 segments, proportional lengths of 3rd, 4th, and 5th segments 1.00:0.81–



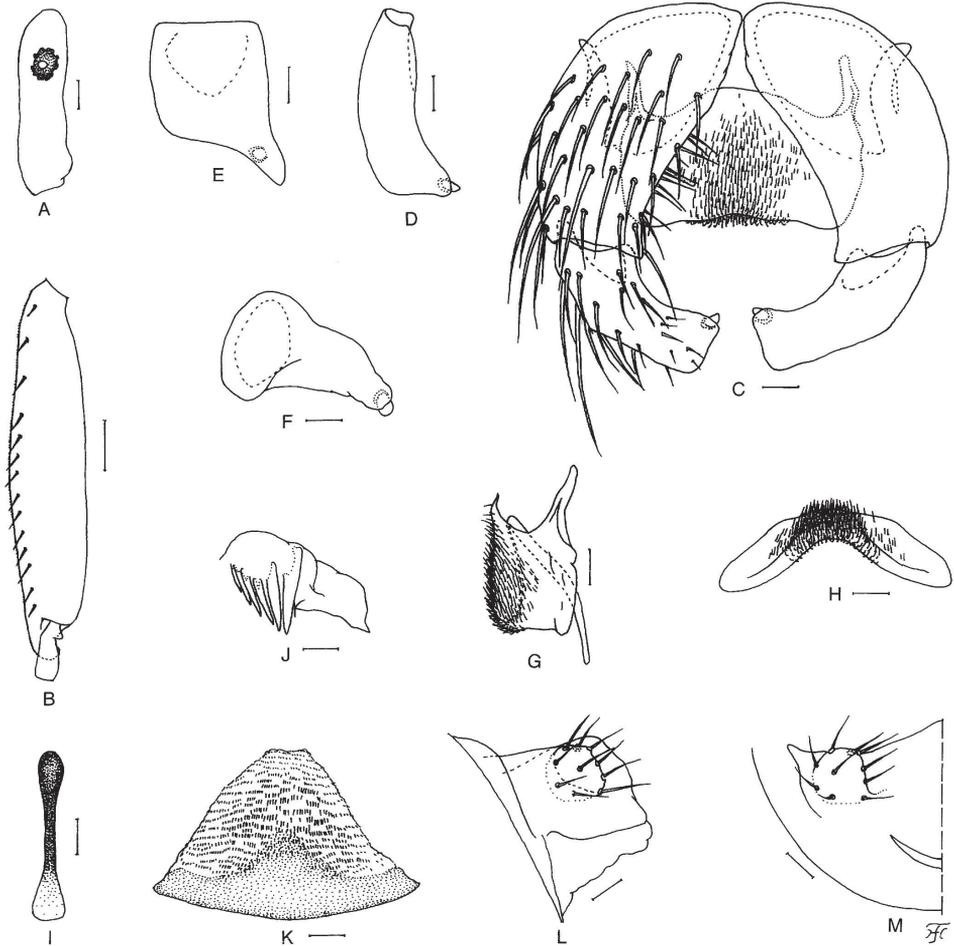
**Figure 1.** Female of *Simulium (Nevermannia) maeaiense* sp. n. **A** fronto-ocular area (right side) **B** 3rd segment of left maxillary palp with sensory vesicle (front view) **C** cibarium **D** left hind tibia (outer view) **E** basitarsus and 2nd tarsomere of left hind leg (outer view) **F** claw **G** 8th sternite and ovipositor valves (ventral view) **H, I** genital forks (ventral view) **J, K** right paraprocts and cerci (**J** ventral view; **K** lateral view) **L** spermatheca (lateral view). Scale bars 0.1 mm for D and E; 0.02 mm for A–C & G–L; 0.01 mm for F.

0.89:1.46–1.56; 3rd segment (Fig. 1B) much enlarged; sensory vesicle (Fig. 1B) elongate, 0.51–0.53 times as long as 3rd segment, with medium-sized opening. Maxillary lacinia with 7 or 8 inner and 10–13 outer teeth. Mandible with 21–23 inner teeth and lacking outer teeth. Cibarium (Fig. 1C) with 40–44 dark minute conical processes

with pointed apices as well as numerous minute spinous processes near lower margin. **Thorax.** Scutum light to medium brown except anterolateral calli ochreous and narrow portion along each lateral margin and part of prescutellar area dark brown, with 3 dark brown narrow longitudinal vittae (1 medial, 2 submedial), thinly whitish-gray pruinose with 3 dark narrow non-pruinose longitudinal vittae (1 medial, 2 submedial) when illuminated at certain angle of light, and densely covered with whitish-yellow recumbent hairs intermixed with several dark brown upright hairs on prescutellar area. Scutellum ochreous, with several dark brown upright hairs as well as whitish-yellow shorter hairs. Postnotum medium to dark brown, whitish-gray pruinose and shiny when illuminated at certain angle of light, and bare. Pleural membrane bare. Katepisternum longer than deep, dark brown, and bare. **Legs.** Foreleg: coxa yellow; trochanter dark yellow; femur dark yellow to light brown with apical cap dark brown; tibia dark brown except median large portion of outer surface light brown; tarsus dark brown; basitarsus slender, slightly dilated, 8.4 times as long as its greatest width. Midleg: coxa medium brown; trochanter yellow; femur yellow with apical cap dark brown; tibia light brown except subbasal portion and apical cap medium to dark brown; tarsus dark brown. Hind leg: coxa light brown; trochanter yellow; femur yellow with apical cap dark brown; tibia (Fig. 1D) light brown except extreme base yellow, subbasal portion medium brown and apical cap dark brown; basitarsus (Fig. 1E) grayish-yellow except base and apical portion medium to dark brown; rest of tarsus dark brown except basal 1/2 of 2nd tarsomere grayish-yellow; basitarsus (Fig. 1E) nearly parallel-sided from base to middle, then slightly narrowed toward apex, 6.16–6.75 times as long as its greatest width, and 0.77–0.83 and 0.63–0.66 times as wide as hind tibia and femur, respectively; calcipala well developed, nearly as long as wide; pedisulcus well developed. Claw (Fig. 1F) with large basal tooth 0.49 times as long as claw. **Wing.** Length 2.6–2.8 mm. Costa with 2 parallel rows of dark brown spinules and dark hairs except on subbasal portion near humeral cross vein with patch of whitish hairs. Subcosta with dark hairs except apical 1/4 bare. Basal portion of radius fully haired;  $R_1$  with dark spinules and hairs;  $R_2$  with dark hairs. Hair tuft on stem vein dark brown. Basal cell absent. **Abdomen.** Basal scale ochreous, with fringe of whitish-yellow long hairs. Dorsal surface of abdomen dark brown to brownish-black except basal 1/2 of segment 2 ochreous, moderately covered with dark brown hairs as well as whitish-yellow hairs; tergites 2 and 6–8 shiny when illuminated at certain angle of light; ventral surface of segment 7 with large sternal plate medially. **Genitalia.** Sternite 8 (Fig. 1G) wide, bare medially but furnished with 18–22 long hairs as well as few short hairs on each side. Ovipositor valves (Fig. 1G) triangular, thin, membranous except inner margin narrowly sclerotized, densely covered with microsetae interspersed with 5–9 short hairs; inner margins slightly concave medially and narrowly or moderately separated from each other. Genital fork (Fig. 1H,I) of inverted Y-form, with well sclerotized stem and relatively wide arms; each arm with lateral plate bearing round or triangular lobe-like projection directed medioposteriorly and short narrow stout projection directed anterodorsally; lateral plate of each arm with strongly sclerotized portion on anterior margin subapically in form of narrow horizontal bar, from which anterodorsally-directed projection

arises. Paraproct in ventral view (Fig. 1J) roughly triangular, slightly longer than its greatest width; anteromedial surface nearly transparent, with 4–6 sensilla; paraproct in lateral view (Fig. 1K) somewhat protruding ventrally beyond ventral margin of cercus, and with 22–27 medium to long hairs on ventral and lateral surfaces. Cercus in lateral view (Fig. 1K) rounded posteriorly, 0.49 times as long as basal width. Spermatheca (Fig. 1L) nearly ovoidal, 1.15 times as long as its greatest width, strongly sclerotized except small area around juncture with duct and duct itself unsclerotized, with distinct reticulate surface pattern and without internal setae; main spermathecal duct narrow, somewhat narrower than both accessory ducts.

**Male.** Body length 2.6–2.9 mm. **Head.** Slightly wider than thorax. Holoptic; upper eye consisting of large facets in 19 vertical columns and 20 horizontal rows. Clypeus brownish-black, not shiny, whitish pruinose, moderately covered with yellow hairs intermixed with dark brown longer hairs except medial portion widely bare. Antenna composed of scape, pedicel and 9 flagellomeres, dark brown except base of 1st flagellomere yellow, scape and pedicel light brown; 1st flagellomere elongate, 2.29–2.33 times as long as 2nd one. Maxillary palp dark brown, composed of 5 segments, proportional lengths of 3rd, 4th, and 5th segments 1.00:1.03–1.12:1.93–1.95; 3rd segment (Fig. 2A) of moderate size; sensory vesicle (Fig. 2A) small, ellipsoidal, 0.17–0.25 times as long as 3rd segment. **Thorax.** Scutum medium to dark brown, with 3 dark longitudinal vittae (1 medial and 2 submedial), whitish pruinose except 3 longitudinal vittae non-pruinose, and slightly shiny when viewed at certain angle of light, densely covered with golden-yellow recumbent hairs interspersed with several dark brown upright hairs on prescutellar area. Scutellum ochreous, with many dark brown upright long hairs as well as golden-yellow shorter hairs. Postnotum dark brown and bare. Pleural membrane bare. Katepisternum longer than deep, medium to dark brown, and bare. **Legs.** Foreleg: coxa yellow; trochanter yellow with somewhat dark portion on outer surface; femur dark yellow to light brown except apical cap dark brown; tibia dark brown though median large area on outer surface light to medium brown; tarsus entirely brownish-black; basitarsus slender, cylindrical, 10.0 times as long as its greatest width. Midleg: coxa medium brown though dark brown on posterolateral surface; trochanter yellow with somewhat dark portion on outer surface; femur dark yellow to light brown with apical cap dark brown; tibia dark brown with median large portion light brown; tarsus dark brown. Hind leg: coxa light to medium brown; trochanter yellow; femur dark yellow to light brown with apical cap dark brown; tibia dark brown with extreme base dark yellow and median large portion light to medium brown; tarsus medium brown except base and apical portion of basitarsus dark brown, and basal 1/2 of 2nd tarsomere light brown; basitarsus (Fig. 2B) enlarged, spindle-shaped, 4.63–4.86 times as long as its greatest width, and 0.88–0.92 and 0.85–0.86 times as wide as greatest widths of hind tibia and femur, respectively; calcipala well developed, nearly as long as basal width; pedisulcus moderately developed. **Wing.** As in female except subbasal patch of pale hairs on costal vein indistinct, and subcosta bare; length 2.4–2.5 mm. **Abdomen.** Basal scale brownish-black, with fringe of pale long hairs. Dorsal surface of abdominal segments entirely dark brown to brownish-black, not

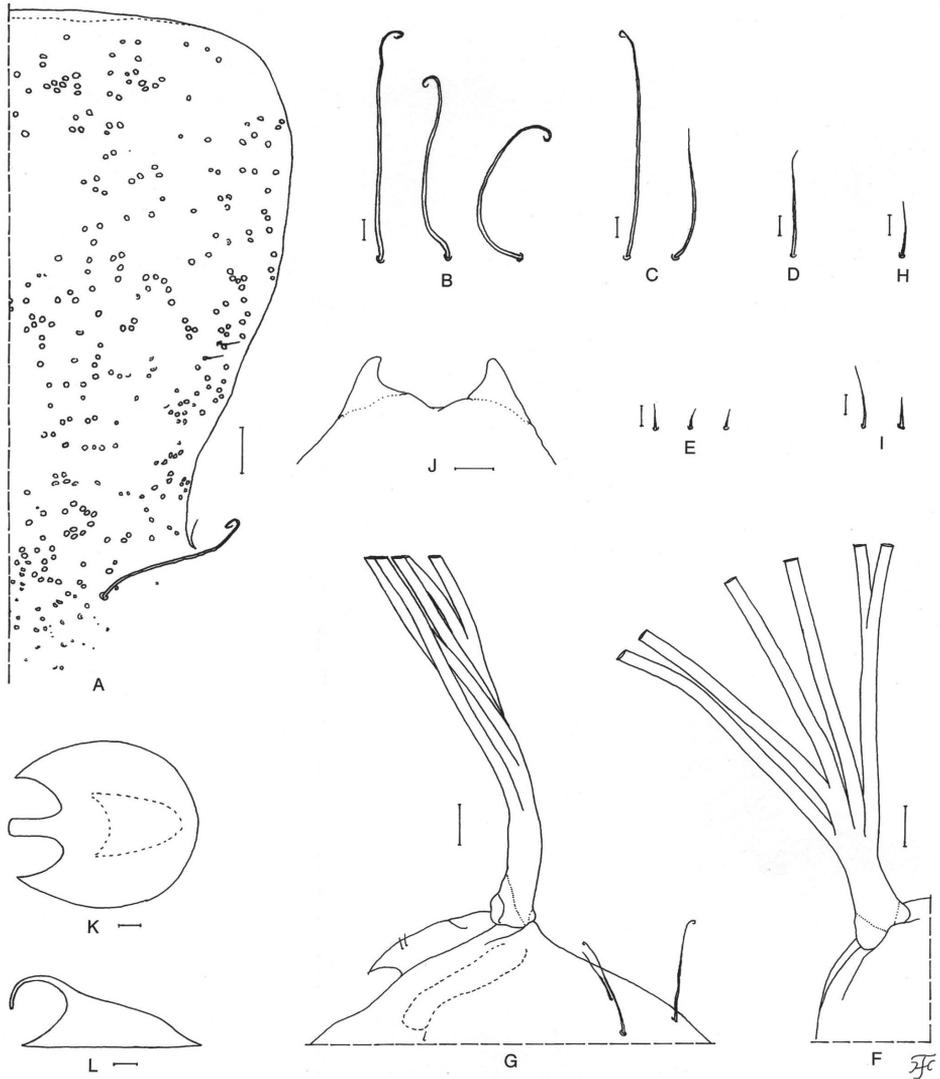


**Figure 2.** Male of *Simulium* (*Nevermannia*) *maeiense* sp. n. **A** 3rd segment of maxillary palp with sensory vesicle (right side, front view) **B** basitarsus and 2nd tarsomere of left hind leg (outer view) **C** coxites, styles and ventral plate (ventral view) **D–F** right styles (**D** medial view; **E** ventrolateral view; **F** end view) **G** ventral plate and median sclerite (lateral view) **H** ventral plate (end view) **I** median sclerite (ventral view) **J** left paramere with 6 hooks (end view) **K** aedeagal membrane and dorsal plate (end view) **L**, **M** right half of 10th abdominal segments with cercus (**L** lateral view; **M** end view). Scale bars. 0.1 mm for B; 0.02 mm for A & C–M.

shiny, and moderately covered with light brown to black short to long hairs and yellow short hairs. **Genitalia.** Coxite in ventral view (Fig. 2C) rectangular, 1.92 times as long as its greatest width. Style in ventral view (Fig. 2C) short, 0.72 times as long as coxite, bent inwardly, with outer margin angled medially and with short stout spine apically; style in medial view (Fig. 2D) gently curved dorsally and nearly parallel-sided; style in ventrolateral view (Fig. 2E) broad, nearly parallel-sided from base to little beyond middle, then abruptly tapered apically; style in end view (Fig. 2F) tapered inward,

with round apex. Ventral plate in ventral view (Fig. 2C) lamellate, subquadrate, 0.52 times as long as its greatest width, well sclerotized except anteromedian portion unsclerotized, with posterior margin slightly concave medially and submedially, and moderately covered with fine short setae on ventral surface except lateral portions widely bare; arm (Fig. 2C) short, slender, directed anteriorly; ventral plate in lateral view (Fig. 2G) with ventral margin nearly straight and arm short, tapered anterodorsally; ventral plate in caudal view (Fig. 2H) with dorsal margin markedly concave, with fine short setae centrally on posterior surface. Median sclerite (Fig. 2G,I) simple, club-shaped, narrow and strongly sclerotized except weakly sclerotized apical portion. Paramere (Fig. 2J) with 5 or 6 hooks decreasing in length toward apex. Aedeagal membrane (Fig. 2K) moderately covered with very minute setae; dorsal plate (Fig. 2K) triangular in shape, thin, weakly sclerotized. Ventral surface of 10th segment (Fig. 2L,M) without any distinct hairs near each posterolateral corner. Cercus (Fig. 2L,M) small, rounded and encircled by 8–12 simple hairs.

**Pupa.** Body length 3.0–3.5 mm. **Head.** Integument (Fig. 3A) dark yellow, moderately or sparsely covered with tubercles; antennal sheaths bare; frons with 2 very short simple trichomes (Fig. 3A) near lateral margin on each side; face with 1 long somewhat stout simple trichome with coiled apex (Fig. 3A) on each side. **Thorax.** Integument dark yellow, moderately (though sparsely on certain portions) covered with round tubercles, with 3 long somewhat stout simple trichomes with coiled apices (Fig. 3B) mediodorsally, 2 simple trichomes (1 long, somewhat stout, with coiled apex, and 1 medium-long, slender, with uncoiled apex) (Fig. 3C) anterolaterally, 1 medium-long somewhat stout simple trichome with uncoiled apex (Fig. 3D) mediolaterally, and 3 very short slender simple trichomes with uncoiled apices (Fig. 3E) ventrolaterally on each side. Gill (Fig. 3F,G) with 6 long thread-like slender filaments, arranged as 2+1+2+1 filaments in horizontal plane from inside to outside; common basal stalk of moderate length, subequal in length to interspiracular trunk, and stalk of inner pair medium-long to long (varying from 0.4 mm to 0.8 mm in individual pupae); stalk of middle pair very short to short; all filaments tapered toward tip, subequal in length (4.4–5.5 mm) and thickness to one another except 2 filaments of inner pair somewhat shorter (3.5–4.3 mm long) and slightly thinner than others; cuticular surface with distinct annular ridges and furrows though becoming less distinct near apex, and densely covered with minute tubercles. **Abdomen.** Dorsally, segments 1 and 2 ochreous, weakly tuberculate; segment 1 with 1 short slender simple seta (Fig. 3H) on each side; segment 2 with 1 short slender simple seta and 5 very short spinous setae (Fig. 3I) on each side; segments 3 and 4 each with 4 hooks and 1 very short spinous seta on each side; segments 5–8 each with spine-combs directed backward in transverse row and comb-like groups of minute spines (though comb-like groups of minute spines indistinct on segment 5) on each side; segment 9 with pair of distinct horn-shaped terminal hooks (Fig. 3J), comb-like groups of minute spines and few to several tubercles. Ventrally, segments 3–8 each with comb-like groups of minute spines; segment 4 with 4 very short simple slender setae, of which 1 somewhat spinous, on each side; segment 5 with pair of bifid hooks submedially and few very short slender setae on each side;

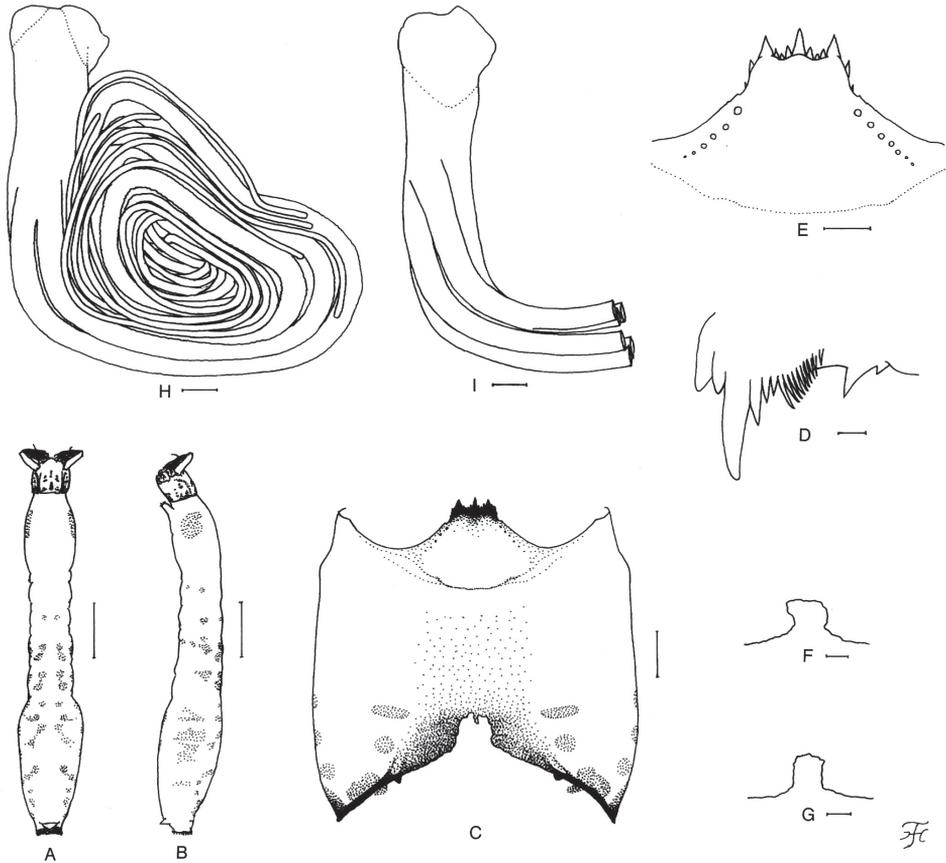


**Figure 3.** Pupa of *Simulium* (*Nevermannia*) *maeaiense* sp. n. **A** frons and part of face showing frontal and facial trichomes and tubercles (left half) **B–E** thoracic trichomes (**B** dorsomedial; **C** anterolateral; **D** mediolateral; **E** ventrolateral) **F, G** basal portions of left gill (**F** dorsal view; **G** lateral view) **H** short seta on dorsal surface of abdominal segment 1 **I** short seta and very short spinous seta on dorsal surface of abdominal segment 2 **J** terminal hooks (end view) **K, L** cocoons (**K** dorsal view; **L** lateral view). Scale bars. 0.5 mm for K, L; 0.1 mm for G, F; 0.05 mm for A; 0.02 mm for B–E & H–J.

segments 6 and 7 each with pair of bifid inner and simple outer hooks (though simple very short seta in place of simple outer hook on segment 7) and few slender very short setae on each side. **Cocoon** (Fig. 3K,L). Wall-pocket shaped, compactly woven without open spaces in web, thin, with anterior margin somewhat thickly woven, and extend-

ing ventrolaterally; anterodorsal projection long, 1.2–1.5 mm, extending forward but sometimes bent downward as shown in Fig. 3L; individual threads invisible; 3.8–4.2 mm long by 2.6–2.9 mm wide.

**Mature larva.** Body length 7.0–7.8 mm. Body (Fig. 4A,B) yellow though somewhat grayish on ventral surface of thorax, with reddish-brown markings on abdomen; i.e., segment 1 on each side with 2 spots (1 laterally, 1 ventrolaterally), segment 2 on each side with 2 spots (1 dorsally, 1 laterally), segment 3 on each side with 6 spots (2 dorsally which are connected or separated, 3 laterally, 1 ventrolaterally), segment 4 on each side with 3 spots (2 dorsally which are connected or separated, 1 laterally), segment 5 on each side with 3 spots (2 dorsally, 1 laterally), segment 6 on each side with 3 spots (2 dorsally which are narrowly connected, 1 laterally), segment 7 on each side with 3 spots (2 dorsally which are usually connected, 1 laterally), segment 8 on each side with 2 spots dorsally, segments 5 and 6 each with faint spot ventromedially; lateral spots on segments 5–7 not well defined, some of other lateral spots very faint or even indistinct in some larvae. Cephalic apotome yellow, with distinct head spots. Lateral surface of head capsule yellow, except eye-spot region white; eyebrow distinct, with 1 dark round spot medially; 2 large spots and 1 small spot near posterior margin and 2 small spots below eye-spot region moderately positive. Ventral surface of head capsule (Fig. 4C) yellow except most of postgenal bridge light to dark brown and basal area on each side of postgenal cleft dark brown; 1 horizontal and 2 round spots on each side of postgenal cleft moderately positive. Cervical sclerite composed of 2 small elliptical pieces, not fused to occiput, widely separated medially from each other. Antenna consisting of 3 segments and apical sensillum, much longer than stem of labral fan; proportional lengths of 1st, 2nd, and 3rd segments 1.00:0.80–0.95:0.72–0.74. Labral fan with 23–25 main rays. Mandible (Fig. 4D) with mandibular serrations consisting of 2 teeth (1 large and 1 small); large tooth making nearly right angle or slightly less with mandible on apical side; comb-teeth composed of 3 teeth shortened from 1st to 3rd; supernumerary serrations absent. Hypostoma (Fig. 4E) with 9 apical teeth in row; median and corner teeth well developed; middle tooth of 3 intermediate teeth on each side smallest; lateral margin nearly smooth or with 1 or 2 very weakly developed teeth apically; 5–7 hypostomal bristles lying slightly divergent posteriorly from lateral margin on each side. Postgenal cleft (Fig. 4C,F,G) small, variable in shape, usually quadrate or rounded, 0.30–0.33 times as long as postgenal bridge. Thoracic cuticle bare; histoblast of pupal gill in outer view (Fig. 4H) with common basal stalk of medium length, from which 4 filaments arising; 2 middle filaments bearing very short stalk, and inner and outer filaments each arising individually; histoblast of pupal gill in inner view (Fig. 4I) showing remaining 2 filaments with relatively long stalk arising just below inner individual filament. Abdominal cuticle bare except both sides of anal sclerite moderately covered with simple colorless setae. Rectal scales present. Rectal organ compound, each lobe with 13–19 long finger-like secondary lobules. Anal sclerite of usual X-form, with anterior arms nearly as long as posterior ones, broadly sclerotized at basal juncture; sensilla absent on and just posterior to basal juncture area; accessory sclerite absent. Last abdominal segment much expanded ventrally forming large ventral papillae. Posterior circling with 75–86 rows of up to 14 hooklets per row.



**Figure 4.** Mature larva of *Simulium* (*Nevermannia*) *maeaiense* sp. n. **A, B** whole body showing color markings on abdomen (**A** dorsal view; **B** lateral view) **C** head capsule showing small postgenal cleft (ventral view) **D** apical portion of mandible **E** hypostoma **F, G** postgenal cleft **H, I** histoblast of pupal gill (**H** left gill, outer view; **I** right gill, inner view; only basal portion shown). Scale bars. 1.0 mm for A & B; 0.1 mm for C; 0.05 mm for E–I; 0.01 mm for D.

**Type specimens.** Holotype female (with associated pupal exuviae and cocoon) (preserved in 80% ethanol) (QSBG 2010-45-24), reared from pupa, collected from a small slow-running stream (width 20 cm, depth 10 cm, water temperature 14.0°C, partially shaded, altitude 1,556 m, 20°04'43.2"N, 99°10'53.6"E), Doi Pha Hom Pok National Park, Mae Ai District, Chiang Mai Province, Thailand, 25.II.2010, by W. Srisuka and S. Suriva. Paratypes: 4 females, 5 males (with associated pupal exuviae and cocoon), all reared from pupae, and 10 mature larva, same data and date as those of holotype.

**Biological notes.** The pupae and larvae of this new species were collected together with *S. (Gomphostilbia) ceylonicum* species-group sp. and *S. (Simulium) doipuiense* Takaoka and Choochote. The habit of biting of the females remains unknown.

**Etymology.** The species name *maeiense* refers to the name of the district, Mae Ai, where this new species was collected.

**Remarks.** *Simulium* (*N.*) *maeiense* sp. n. is readily assigned to the *feuerborni* species-group redefined by Takaoka (2003) by the combination of the following characteristics: male genitalia with a simple lamellate ventral plate (Fig. 2C), a short inwardly-twisted style (Fig. 2E), a simple narrow median sclerite (Fig. 2I) and several parameral hooks (Fig. 2J); the pupal gill with six long thread-like filaments per side (Fig. 3F); and the larval head with a small short postgenal cleft (Fig. 4C).

The female of *S.* (*N.*) *maeiense* sp. n. is distinctive among the *feuerborni* species-group in that the cibarium has numerous minute cone-shaped processes on the lower part (Fig. 1C) and the genital fork bears a strongly sclerotized narrow horizontal bar at the base of the anterodorsally directed projection (Fig. 1H,I) on each arm. This combination of characters has not been reported in any known species of the species-group except two species, *S.* (*N.*) *fruticosum* from Thailand, and *S.* (*N.*) *sasai* (Rubtsov) from Japan, which have, respectively, 11 and 16 minute processes on the lower part of the cibarium, according to the illustrations given by Takaoka and Choochote (2005) and Sato et al. (2005).

The pupa of this new species is also remarkable within this species-group in having the unique arrangement of the gill filaments (Fig. 3F) (i.e., arranged as 2+1+2+1 filaments from inside to outside; two stalks of the inner and middle pairs and two individual filaments arising close together from the common basal stalk nearly at the same level and lying nearly in a horizontal plane).

On the other hand, the male of this new species is very similar to those of other known species in many features including the genitalia and barely distinguished by the combination of the numbers of the vertical columns (19) and horizontal rows (20) of the enlarged upper-eye facets and the number of parameral hooks (5 or 6).

The mature larva of this new species has reddish-brown markings on the abdomen as in most known species of the *feuerborni* species-group, but is easily distinguished from other species by possessing six colored spots on each side of the abdominal segment 3 (Fig. 4B).

In having the cocoon with an anterodorsal projection (Fig. 3K), *S.* (*N.*) *maeiense* sp. n. appears to be somewhat similar to the following six known species: *S.* (*N.*) *fange* from Thailand (Takaoka and Choochote 2006), *S.* (*N.*) *feuerborni* from Java, Sumatra, Peninsular Malaysia and Thailand (Edwards 1934; Kuvangkadilok et al. 1999; Takaoka and Davies 1995, 1996; Takaoka et al. 2000), *S.* (*N.*) *leigongshanense* Chen and Zhang from China (Chen and Zhang 1997), *S.* (*N.*) *mongare* Takaoka and Somboon from Bhutan (Takaoka and Somboon 2008), *S.* (*N.*) *praelargum* Datta from India (Datta 1973) and *S.* (*N.*) *sasai* from Japan (Sato et al. 2005). However, this new species is easily distinguished from these known species in the female by the cibarium and genital fork, in the pupa by the arrangement of the gill filaments, and in the larva by the reddish-brown markings on the abdomen, as already mentioned above.

This new species is distinguished from all six known species of the *feuerborni* species-group in Thailand by the following keys.

## Keys to seven species of the *feuerborni* species-group of the subgenus *Simulium* (*Nevermannia*) in Thailand

### Females\*

- 1 Fore basitarsus 7.3–7.4 times as long as its greatest width; claw with basal tooth 0.42–0.43 times as long as claw..... **2**  
 – Fore basitarsus 7.7–8.4 times as long as its greatest width; claw with basal tooth 0.49–0.50 times as long as claw..... **3**  
 2 Frons-head ratio 1.00:1.48..... *S. chiangklangense*  
 – Frons-head ratio 1.00:1.54..... *S. feuerborni*  
 3 Maxillary lacinia with 10 or 11 inner teeth; mandible with 17 or 18 inner teeth ..... *S. vessabutrae*  
 – Maxillary lacinia with 6–9 inner teeth; mandible with 20–23 inner teeth ... **4**  
 4 Sensory vesicle 0.51–0.53 times as long as 3rd segment of maxillary palp; genital fork with a strongly sclerotized horizontal bar on each arm... *S. maeaiense* sp. n.  
 – Sensory vesicle 0.58–0.66 times as long as 3rd segment of maxillary palp; Genital fork without horizontal bar on each arm ..... **5**  
 5 Labrum 0.92 times as long as clypeus ..... *S. fangense*  
 – Labrum as long as clypeus..... *S. fruticosum*

\* *Simulium wichaii* is not included because its female remains unknown.

### Males\*\*

- 1 Scutum light to dark brown or reddish-brown, with 3 dark longitudinal vittae..... **2**  
 – Scutum brownish-black, without dark longitudinal vittae..... **4**  
 2 Paramere with 3 or 4 hooks ..... *S. feuerborni*  
 – Paramere with 5–7 hooks..... **3**  
 3 Upper eye with large facets in 14 or 15 vertical columns and in 17 or 18 horizontal rows ..... *S. fruticosum*  
 – Upper eye with large facets in 19 vertical columns and in 20 horizontal rows...  
 ..... *S. maeaiense* sp. n.  
 4 Upper eye with large facets in 21 vertical columns and in 21 horizontal rows...  
 ..... *S. fangense*  
 – Upper eye with large facets in 14–17 vertical columns and in 16–18 horizontal rows ..... **5**  
 5 Upper eye with large facets in 14 vertical columns and in 16 or 17 horizontal rows; paramere with 7 or 8 hooks ..... *S. wichaii*  
 – Upper eye with large facets in 17 vertical columns and in 18 horizontal rows; paramere with 9 hooks..... *S. vessabutrae*

\*\* *Simulium chiangklangense* is not included because its male remains unknown.

## Pupae

- 1 Cocoon with anterodorsal projection ..... 2
- Cocoon without anterodorsal projection ..... 4
- 2 Six gill filaments arranged as 2+1+2+1 lying nearly horizontally from inside to outward ..... *S. maeaiense* sp. n.
- Six gill filaments arranged otherwise ..... 3
- 3 Six gill filaments arranged as 4+2 from dorsal to ventral; stalk of ventral pair medium-long to long ..... *S. feuerborni*
- Six gill filaments arranged otherwise; stalk of ventral pair short... *S. fangense*
- 4 Head and thorax covered with dark brown large tubercles ..... *S. wichaii*
- Head and thorax covered with yellowish brown small tubercles ..... 5
- 5 Six gill filaments arranged as 2+(2+2), each pair with medium-long to long stalk; common basal stalk long ..... *S. vessabutrae*
- Six gill filaments arranged otherwise; common basal stalk short to medium-long ..... 6
- 6 Six gill filaments arranged as 4+2 from dorsal to ventral; stalk of ventral pair medium-long ..... *S. fruticosum*
- Six gill filaments arranged as 1+1+2+2 from inside to outside; stalks of pairs short ..... *S. chiangklangense*

## Mature larvae\*\*\*

- 1 Ventral surface of head capsule not darkened on postgenal bridge ..... 2
- Ventral surface of head capsule darkened on postgenal bridge ..... 3
- 2 Labral fan with 16 main rays ..... *S. wichaii*
- Labral fan with 28 main rays ..... *S. vessabutrae*
- 3 Postgenal cleft 0.30–0.43 times as long as postgenal bridge ..... 4
- Postgenal cleft 0.50–0.64 times as long as postgenal bridge ..... 5
- 4 Posterior circling with 75–86 rows of up to 14 hooklets per row ..... *S. maeaiense* sp. n.
- Posterior circling with about 90 rows of up to 16 hooklets per row ..... *S. feuerborni*
- 5 Each lobe of rectal organ with 11 or 12 secondary lobules ..... *S. fruticosum*
- Each lobe of rectal organ with 17–21 secondary lobules ..... *S. fangense*

\*\*\**Simulium chiangklangense* is not included because its larva remains unknown.

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