Zootaxa 3683 (2): 159–177
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

http://dx.doi.org/10.11646/zootaxa.3683.2.5
http://zoobank.org/urn:lsid:zoobank.org:pub:0D08A084-F77F-4D38-9245-CE0B084D475A

Nyx pholeocola, a new genus and cavernicolous species of tribe Aedini (Diptera: Culicidae) from southern Thailand based on morphological and molecular data

RALPH E. HARBACH[1], IAN J. KITCHING[1], C. LORNA CULVERWELL[1],
THERESA M. HOWARD[1] & YVONNE-MARIE LINTON[2,3]

[1]*Department of Life Sciences, Natural History Museum (NHM), Cromwell Road, London SW7 5BD, UK.*
E-mail: r.harbach@nhm.ac.uk; i.kitching@nhm.ac.uk; l.culverwell@nhm.ac.uk; t.howard@nhm.ac.uk
[2]*Walter Reed Army Institute of Research, Entomology Branch, 503 Robert Grant Avenue, Silver Spring, MD 20910, USA.*
E-mail: lintony@si.edu
[3]*Walter Reed Biosystematics Unit, Museum Support Center, 4210 Silver Hill Rd, Suitland MD 20746, USA (formerly of NHM)*

Abstract

Nyx Harbach & Linton, **gen. nov.**, is introduced as a new mosquito genus of tribe Aedini for a previously unknown cave-dwelling species, *Nyx pholeocola* Linton & Harbach, **sp. nov.**, from southern Thailand. A diagnosis of the genus is provided that features unique anatomical characters of the adult, pupal and larval stages of the type species. The affinities of *Nyx* are discussed in terms of its position in the phylogeny of Aedini. *Nyx* is more closely related to *Borichinda* and *Isoaedes* than to other genera of tribe Aedini. Salient differences that distinguish these three genera are contrasted. The male and female genitalia, pupa and fourth-instar larva of the new species are illustrated. DNA sequence for the second nuclear internal spacer region (ITS2) and the 658-bp barcode fragment of the mitochondrial cytochrome c oxidase I (COI) gene reveal very low similarity with published sequences, supporting the unique status of the new species.

Key words: *Nyx* **gen. n.**, *Nyx pholeocola* **sp. n.**, COI barcode, ITS2, mosquito, taxonomy, systematics

Introduction

Biting adults and immature stages of the new species described in this paper were discovered in the cave at Wat Tham Phanturat, Khlong Sok, near Khao Sok National Park in Surat Thani Province of southern Thailand. Adults reared from these larvae were initially found to be similar to species of genera *Borichinda* and *Isoaedes* using the keys of Rattanarithikul *et al*. (2010) for the aedine fauna of Thailand. Upon closer examination of the adults and their associated larval and pupal exuviae, it became obvious that the species was very different from the species of those genera. Furthermore, comparisons of COI and ITS2 sequences with the extensive collection of COI barcodes in the Barcode of Life Database (BOLD; www.boldsystems.org) and GenBank, respectively, showed that the species does not fall within any currently recognized genus-level taxon of Aedini, and therefore a new genus-species combination is proposed and described herein.

Material and methods

This study is based primarily on individually reared adults with associated larval and pupal exuviae (collection SS74). The specimens were reared from larvae and pupae collected from a rimstone pool (20 x 50 cm) deep inside a cave in southern (peninsular) Thailand (see *Type Series*). In addition, a few females were collected landing on the collectors within the cave (SS75). Pinned link-reared adults of both sexes were examined under simulated natural light; dissected male and female genitalia and larval and pupal exuviae were studied with differential interference contrast optics. Anatomical terminology and abbreviations used in the descriptions and illustrations follow Harbach & Knight (1980, 1982).

Accepted by G. Courtney: 11 Jun. 2013; published: 3 Jul. 2013

DNA extraction was carried out using the commercially available Qiagen DNeasy Kit (Qiagen Ltd, Sussex, England) following the manufacturer's recommended protocol. A 658-bp fragment of the mitochondrial cytochrome oxidase subunit I locus (COI) (corresponding to the barcode fragment of the COI) was amplified using the universal barcoding primers LCO1490 and HCO2198 (Folmer *et al*., 1994) according to the protocol of the Mosquito Barcoding Initiative, as described by Ruiz *et al*. (2010). The rDNA ITS2 PCR was carried out using the primers of Collins & Paskewitz (1996) and the protocols of Linton *et al*. (2001). Sequencing reactions were undertaken in both directions using the Big Dye Terminator Kit® on an ABI3770 automated sequencer (PE Applied BioSystems, Warrington, England) and the original PCR primers. Sequence chromatograms were edited in Sequencher™ v. 4.8 (Genes Codes Corporation, Ann Arbor, MI, USA). Basic Local Alignment Search Tool (BLAST) searches and the Identification System (IDS) within the BOLD database were used to determine homology of the sequences with published or available sequences. DNA aliquots are retained at -70°C in the Frozen Tissue Biorepository of the Smithsonian Institution Museum Support Center, Suitland, MD for future reference. Sequence chromatograms and associated data of the new species sequenced in this study are freely available under the project code NYX within the Mosquitoes of the World Campaign in the BOLD database (www.boldsystems.org). GenBank Accession numbers are as follow: ITS2 (KC860485–KC860486) and COI (KC860482–KC860484).

The phylogenetic relationships of the new taxon with other aedine taxa were examined by including character data for the new species in the morphological data set of Reinert *et al*. (2009). The taxa in this data set comprise 270 species, an outgroup of four non-aedine species and an ingroup of 266 species of Aedini. The new species was coded for the 336 morphological characters derived from eggs (the egg of the new species is unknown), fourth-instar larvae, pupae, and adult males and females listed by Reinert *et al*. (2009) (Appendix), using the protocols described therein. Missing data were coded as "?", characters that could not be scored due to the absence of homologous structures were coded as "–" and multistate characters were treated as unordered. For comparison with the results of Reinert *et al*. (2009), the data set was analyzed using maximum parsimony and implied weighting (with values of the concavity constant, *K*, ranging from 5 to 17), implemented in TNT version 1.1 (Willi Hennig Society Edition) (Goloboff *et al*., 2008). Tree searches were conducted using all four "New Technology" search options: sectorial search, ratchet, tree drifting and tree fusing. For the ratchet, upweighting and downweighting probabilities were set to 5% and the number of replicates to 200. The number of cycles of tree drifting was set to 50. All other search parameters were left at their default settings. Analyses were terminated once the most parsimonious (= fittest) cladogram(s) had been found 10 times. Under "Settings", the General RAM was set to 500 Mb and the maximum number of trees to be held to 10000. Cladograms were rooted between *Culiseta* Felt and the remaining taxa.

Taxonomy

Nyx Harbach & Linton, gen. nov.

Type species: *Nyx pholeocola* Linton & Harbach, **sp. nov.**

Females. Medium-sized mosquitoes. *Head*: Eyes narrowly separated above antennae; vertex with large falcate scales and few broad scales laterally, ocular and interocular areas with smaller falcate scales, erect scales on occiput and back of vertex, interocular setae present; antenna longer than proboscis; maxillary palpus about 0.2 length of proboscis, with 3 normally developed palpomeres and a minute vestigial fourth palpomere. *Thorax*: Scutum with pattern of narrow pale and dark scales; acrostichal and dorsocentral setae present; scutellum with very narrow scales; paratergite narrow, without scales or setae; antepronotum with scales, postpronotum with few narrow scales; postspiracular setae and scales present. *Wing*: Remigium without setae; alula with broad scales on margin. *Legs*: Tarsi dark-scaled; both ungues of fore- and midlegs toothed, hindungues slightly smaller, apparently without teeth. *Abdomen*: Tergum I nearly completely covered with scales and setae, laterotergite with scales. *Genitalia*: Segment VIII retracted into segment VII; cercus relatively long, more than twice length of postgenital lobe; insula without setae; 3 spermathecal capsules.

Males. Essentially as the females. *Head*: Antenna about 0.75 length of proboscis, flagellar whorls long, dense,

directed dorsally and ventrally, flagellomeres 12 and 13 elongate; maxillary palpus short, 0.4 length of proboscis, comprised of 4 palpomeres, fourth vestigial. *Legs*: Ungues of fore- and midlegs enlarged, anterior and posterior unguis of each pair with a tooth. *Genitalia*: Tergum IX lobes poorly developed, separated, with rows of setae; sternum IX with 1–3 medial setae; gonocoxite elongate, with lateral scales; gonostylus long, slender at base, distally flattened, expanded and highly setose; claspette small, tapered to acute apex with a few setae; aedeagus comprised of 2 tergally bent lateral plates, each plate with apical teeth; proctiger strongly developed, paraproct with sharp caudal spine, cercal setae absent.

Pupae. *Cephalothorax*: Seta 7-CT longer than seta 6-CT. *Abdomen*: Seta 3-III long, single; seta 6-III single, seta 6-VII small, posterior to seta 9; seta 9-IV–VI posterior to seta 8, seta 9-VII single, stout; paddle oval, external and internal margins smooth.

Larvae, fourth-instars. *Head*: Seta 4-C short, with 3 or 4 branches; setae 5,6,8,13-C single; seta 14-C longer than seta 15-C, single or double; cervical sclerite present. *Thorax*: Seta 1-P well developed, longer than seta 2-P; setae 1,3-P usually double (1,2); seta 5-P double; seta 5-T small, single. *Abdomen*: Seta 11-I large, 2,3-branched; seta 2-I,II large, 2,3-branched, inserted anterolateral to seta 1, seta 2-III–VI relatively small, single or double, inserted anterior to seta 1; seta 5-II–VII small, usually single; seta 7-II single, about half length of seta 6-II; seta 9-III–VI inserted near and anterior to seta 7; seta 10-II–V inserted lateral to setae 11,12; seta 13-III–V single. *Segment VIII*: Comb scales spine-like, in single row. *Siphon*: Acus present, detached; seta 1-S 2,3-branched, inserted distal to pecten; distal 1 or 2 pecten spines usually slightly more widely spaced. *Segment X*: Saddle incomplete, relatively large, extending below lateral midline, acus not developed, posterior margin with spine-like spicules; seta 1-X single, shorter than saddle; seta 2-X with multiple branches; seta 3-X single; ventral brush (seta 4-X) with 4 or 4.5 pairs of setae on grid (with only transverse bars) and 1 precratal seta.

Eggs. Unknown.

Etymology. Nyx is the Greek goddess of the night – a shadowy figure who stood at or near the beginning of creation and gave birth to a number of personified gods. Her sparse appearances in mythology reveal her as a goddess of exceptional power and beauty. Nyx was rarely and only glimpsed in the shadows of the world, which prompted the generic name proposed here. The two-letter abbreviation *Nx*. is recommended for this genus.

Systematics. The generic status and phylogenetic relationships of *Nyx* were assessed objectively by including character data for *Nyx* in the data set of Reinert *et al*. (2009). The characters used in the analyses and their states observed in *Nyx* are listed in the Appendix. Analyses using implied weights and values of *K* ranging from 5 to 17 each yielded a single most parsimonious (fittest) cladogram (MPC). With the most extreme weighting functions, *K* = 5 and 6, *Nyx* was placed as the sister group to *Isoaedes*, though the placement of this pair with other genera was quite different in the two MPCs. For *K* = 7–13, the pattern of relationships among the genera of the *Stegomyia*-group (the clade comprising genera *Skusea* to *Stegomyia*) was consistently recovered and identical to that shown in figure 1 of Reinert *et al*. (2009), with the addition of *Nyx*, which was placed as a separate branch of the main stem between *Isoaedes* and *Borichinda* (Fig. 1). The branching pattern at the base of the *Stegomyia*-group was thus: (*Skusea* (*Indusius*, *Cancraedes*)) (*Fredwardsius* (*Isoaedes* (*Nyx* (*Borichinda* ((*Diceromyia*, *Ayurakitia*) (*Dendroskusea*, remainder of the *Stegomyia*-group))))))). With *K* = 14 and 15, *Isoaedes*, *Nyx* and *Borichinda* formed a clade with the first two as sister taxa. The weakest weighting with *K* = 16 and 17 showed a similar but inverted pattern to that for *K* = 7–13: (*Dendroskusea* (*Diceromyia*, *Ayurakitia*)) (*Borichinda* (*Nyx* (*Isoaedes* (*Fredwardsius* ((*Skusea* (*Indusius*, *Cancraedes*)), remainder of the *Stegomyia*-group))))). Reinert *et al*. (2009) found a similar "plateau of stability" between *K* = 7 and *K* = 10, and restricted their discussion to a consideration of the MPC found with *K* = 9. As we found a similar pattern, although now extended to *K* = 13, and for ease of comparison, we also restrict subsequent discussion to the MPC found using *K* = 9.

The branch supporting *Isoaedes* as sister to the rest of the *Stegomyia*-group is supported by 15 unambiguously optimized but homoplastic characters (**33: 0**, **54: 0**, **98: 1**, **101: 1**, 158: 2, 188: 1, 198: 0, **226: 0**, 240: 0, **256: 0**, **261: 0**, **262: 1**, **266: 1**, 273: 0, 326:0), which include nine (boldface) of 10 that supported the same branch in the study of Reinert *et al*. (2009). The branch uniting *Nyx* with the remainder of the *Stegomyia*-group to the exclusion of *Isoaedes* is supported by seven unambiguously optimized but homoplastic characters (7:0, **24:1**, **34:1**, **63:1**, **92:1**, **290:1**, **317:1**), and the branch supporting the clade comprising *Borichinda* and the rest of the *Stegomyia*-group to the exclusion of *Isoaedes* and *Nyx* is supported by 10 unambiguously optimized but homoplastic characters (40: 1, **52: 2**, **55: 2**, 58: 1, **64: 1**, **67: 1**, 110: 1, **232: 1**, **255: 0**, 328: 1). The emboldened characters in these two lists are those that supported the clade comprising *Borichinda* and the rest of the *Stegomyia*-group to the exclusion of

Isoaedes in the study of Reinert *et al*. (2009). Thus, six of the original 13 characters that support this clade in Reinert *et al*. (2009) are now placed on the branch below *Nyx* and five on the branch above *Nyx*. The inclusion of *Nyx* rendered the optimization of the remaining character, 53:1, ambiguous. In other words, the addition of *Nyx* has simply resulted in the slotting in of this genus on the original branch between *Isoaedes* and *Borichinda* and the almost equal division of the characters that supported this branch on either side of the node leading to *Nyx*. *Nyx* itself is on a branch supported by 12 unambiguously optimized but homoplastic characters: 36: 1, 37: 0, 65: 1, 143: 2, 157: 2, 159: 0, 161: 0, 182: 3, 221: 1, 282: 1, 304: 2 and 308: 1. These differences clearly support the recognition of *Nyx* as a new polythetically diagnosed genus of tribe Aedini.

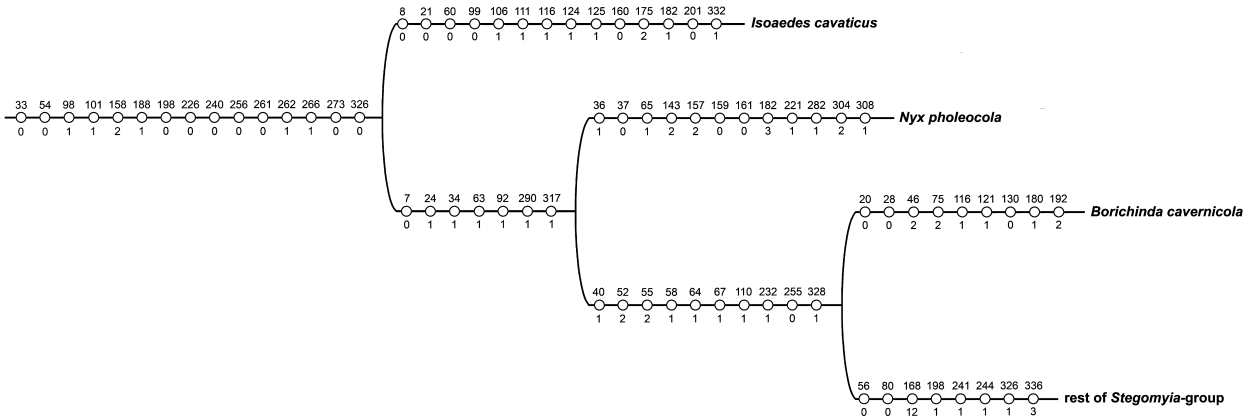

FIGURE 1. Placement of *Nyx* in the phylogeny of tribe Aedini when character data for *Nx. pholeocola* (Appendix) are added to the data set of Reinert *et al*. (2009) and analyzed using implied weighting and a concavity constant (*K*) of 9. Supporting characters are indicated on the branches. All are homoplastic, so *Nyx*, like most generic-level taxa of Aedini, is a polythetic genus that is diagnosed by a unique combination of characters.

Nyx pholeocola Linton & Harbach, sp. n.

Female (Figs 2, 3). As described for genus, bearing the following features and specific characters listed in the Appendix. Dark scaling light to dark brown, pale scaling white. *Head*: Dorsum with large semi-erect pale falcate scales and few broad spatulate scales laterally on one or both sides, relatively few pale erect scales that become darker (brown) laterally. Antenna length about 2.0 mm; pedicel with small pale spatulate scales and flagellomere 1 with few small dark spatulate scales on mesal surfaces. Proboscis (except labella) and maxillary palpus (Fig. 2D) dark-scaled; proboscis length about 1.8 mm, slightly longer than forefemur; maxillary palpus bare beneath, with relatively few setae on dorsal and lateral surfaces, length 0.3 mm. *Thorax*: Integument light brown, darker dorsally. Scutum (Fig. 2B) with pattern of coarser pale scales on background of finer dark scales, pale scaling as follows: acrostichal line bifurcating into lateral prescutellar lines, lateral patch on scutal fossa continuous with anterior dorsocentral area and posterior fossal line that joins posterior dorsocentral line and narrow line on margin of supraalar area; scutellum with pale falcate scales on mid and lateral lobes; brown setae on antepronotum and posteriorly on postpronotum, golden to golden-brown setae on upper proepisternum, postspiracular area, upper and lower mesokatepisternal areas, prealar area, upper mesepisternal area and 1 or 2 (usually 2) on lower anterior area of mesepimeron; pale falcate scales on postpronotum and few scattered pale falcate scales on postpronotum, pale spatulate scales on upper proepisternum, upper and lower posterior mesokatepisternum and upper anterior mesepimeron anteroventral to upper mesepimeral setae. *Wing*: Length about 3.0 mm, entirely dark-scaled. *Halter*: Integument pale, capitellum darker dorsally with faint pale scales. *Legs*: Anterolateral surface of forecoxa with pale spatulate scales and prominent golden-brown setae, midcoxa with pale spatulate scales on anterior side of mid-lateral row of prominent setae, hindcoxa without scales, with posterolateral row of prominent setae; ventral surface of trochanters with pale scales and setae distally; femora with distinct apical pale rings (knee spots) and subdued posteroventral pale stripes extending to or near apex; forefemur length about 1.7 mm. *Abdomen*: Terga II–VIII with relatively narrow straight basal pale bands, often narrower medially on terga VII and VIII and occasionally

obsolescent medially on tergum VIII; sterna dark-scaled from segment II posteriorly. *Genitalia* (Fig. 3): Segment VIII fully retracted into segment VII (Fig. 2F); tergum VIII broad anteriorly, index about 0.7, relatively long stout setae on caudal margin, sparse short setae anterior to these, basolateral seta present, broad scales intermixed with setae; sternum VIII broadened laterally at midlength, index about 0.6, median posterior emargination separating broad lateral lobes, setae on caudal 0.67 progressively longer toward margin of lobes, setae 1–3-S in posterolaterally directed lateral line, basolateral seta present; tergum IX index about 0.6, caudal margin with broad median concavity separating setose lobes; upper and lower vaginal lips relatively broad, moderately pigmented; postgenital lobe (PGL) somewhat heart-shaped, middle of posterior margin slightly concave and lateral margins broadly convex, ventral length about 0.06 mm, width at midlength about 0.08 mm; cercus with more or less straight mesal margin and curved outer margin, broad in proximal half and progressively narrower to blunt apex, distal area of dorsal and lateral surfaces with scattered setae, length about 0.16 mm, greatest width about 0.08 mm, index about 2.0, cercus/dorsal PGL index about 3.2; spermathecal capsules with few small pores near orifice.

Male. Generally smaller but otherwise similar to the female excepting obvious sexual differences. *Head*: Antenna with strongly developed whorls of numerous long setae projecting dorsally and ventrally, 2 terminal flagellomeres disproportionately long compared to other flagellomeres; length about 1.7 mm. Proboscis with indication of false joint 0.6 from base. Maxillary palpus (Fig. 2C, E) about 0.4 length of proboscis, slender, dark-scaled, palpomeres 1–3 without scales ventrally, apex of palpomere 3 with few short setae, palpomere 4 bud-like. *Wing*: Generally paler, veins with fewer scales, tertiary fringe scales absent; length about 2.4 mm. *Abdomen*: Tergum and sternum VIII with straight posterior margins; sternum VIII (post-rotation dorsal position) mainly pale-scaled with incomplete posteromedian dark band; tergum VIII mainly dark-scaled. *Genitalia* (Fig. 3): Tergum IX lobes weakly produced, narrowly joined medially by membrane, small setae extend laterally along posterior margin from apex of each lobe; sternum IX about as long as broad, with 1–3 small centrally located setae; gonocoxite elongate, more or less cylindrical, mesal membrane developed to apex, basal dorsomesal lobe slightly produced with dense cluster of relatively long setae, dorsal surface with short setae, lateral and ventral surfaces with long setae and spatulate scales, apicodorsal lobe weakly developed with patch of setae; gonostylus long, about 0.75 length of gonocoxite, narrow proximally, dorsoventrally flattened and laterally expanded in distal 0.67, lateral margins of expanded portion lined with relatively long setae to apex; gonostylar claw relatively long, slightly longer than 0.1 length of gonostylus, dorsoventrally flattened and slightly bent ventrad; claspette relatively slender and slightly compressed, with 1 stout apical seta and a small dorsomesal seta at its base; proctiger about 0.25 length of gonocoxite, paraproct distally tapered and bent tergally, without apical teeth; cercal sclerite poorly defined, more or less membranous, fused with paraproct; aedeagus with lateral plates bent tergally, joined distally, tergolateral margin of each lined with short teeth to apex; paramere nearly as long as aedeagus, articulated with apex of basal piece near midlength.

Pupa (Fig. 4A, B). As described for genus, bearing the following features and specific characters listed in the Appendix; character and positions of setae as illustrated. *Cephalothorax*: Moderately but unevenly pigmented, scutum darker posteriorly. Setae 1–3,6,11-CT single; setae 4,5,7–9,10,12-CT branched. *Trumpet*: Moderately and evenly pigmented; length about 0.5 mm, tracheoid weakly developed on one side of trumpet, length about 0.08 mm, pinna about 0.13 mm, width at midlength about 0. 08 mm, index about 6.3. *Abdomen*: Moderately pigmented, posterior area of tergum I and anterior fold lines of sterna II–VIII darker; length about 3.2 mm. Seta 1-I broom-like, with short thick stem and numerous (approximately 70) thin simple branches; setae 3,6-I long, single; seta 11-I usually present, single; alveolus of seta 13-I usually present; seta 1-II multiple branched, 1-III–VII with fewer branches (usually 2) on succeeding posterior terga; seta 6-II–VI relatively long, usually single, sometimes double on segments II and III; seta 10-II usually absent; seta 9-VIII with 2–4 long aciculate branches, nearly half length of paddle. *Genital lobe*: Moderately pigmented; long in male, length about 0.3 mm, about 0.8 length of paddle; about 0.1 mm in female. *Paddle*: Lightly and evenly pigmented, midrib darker, midrib distinct to distal area before seta 1-Pa; length about 0.8 mm, width about 0.6 mm, index 1.3; seta 1-Pa long, single.

Larva, fourth-instar (Fig. 5). As described for genus; bearing the following features and specific characters listed in the Appendix; character and positions of setae as illustrated (larva reconstructed from larval exuviae, relative positions of setae may differ slightly from those shown). *Head*: Nearly round in dorsal view, length and width about 0.8 mm; moderately pigmented, collar darker. Dorsomentum with 7–9 teeth on either side of slightly larger median tooth, teeth nearest median tooth abutted, lateral 2 or 3 teeth separated. Setae 5,6,8–10-C single. *Antenna*: Moderately pigmented, bent mesad, length about 0.3 mm; seta 1-A inserted at mid-length, setae 2,5-A

very long, 2-A nearly twice length of 5-A (Fig. 5B). *Thorax*: Integument hyaline, smooth. Seta 1-P double or triple; setae 3,8-P single or double; setae 14-P,M and 13-T more strongly developed (longer) than in *Isoaedes*, 14-P double, 14-M double or triple, 13-T with 4 or 5 branches; setae 13-M and 8-T double or triple. *Abdomen*: Integument hyaline, smooth. Seta 3-I–VII normally single, moderately long, all longer than seta 4; seta 6-I–VI with long, stout, aciculate branches, 6-VI noticeably shorter than 6-I–V; seta 2-I,II,VII anterolateral to seta 1, 2-III–VI more or less directly anterior to seta 1; seta 8-II–VI normally single; seta 10-II–VI approaches length of following segment; seta 13-III,IV single or double, longer than following segment. *Segment VIII*: Comb comprised of 6–9 spine-like scales in single row, with minute basolateral denticles. *Siphon*: Moderately pigmented, slightly swollen at level of seta 1-S, surface smooth; length about 0.9–1.0 mm, width at base about 0.3 mm, index 3.0–3.3; acus present, very small, detached from siphon; pecten with 10–12 spines with few denticles basally on ventral margin, distal 1 or 2 spines more widely spaced; seta 1-S branched, inserted more or less at level of distal pecten spine. *Segment X*: Saddle moderately pigmented; dorsal length about 0.3–0.4 mm; siphon/saddle index 2.3–3.3. Seta 1-X single, simple, slightly shorter than saddle; setae of ventral brush with 2 or 3 branches, mostly 3-branched, precratal seta single, simple. Dorsal and ventral anal papillae same length, short and relatively stout, slightly longer than saddle.

Egg. Unknown.

DNA sequence. ITS2 and COI fragments were amplified from two specimens (SS74-19 and SS74-40, see type series below). The sequences of the ITS2 fragments were identical. These fragments (primers removed), which also include a short portion of the flanking 5.8S and 28S genes, were 287 bases long and 50.87% AT rich (65A, 81T, 74C, 67G). The sequence shares greatest similarity (94%) with that of *Borichinda cavernicola* Rattanarithikul & Harbach (GenBank accession EF370410), reflecting the cladistic relationship noted above. The COI barcode fragments (658 bp) exhibited no intraspecific variation and the haplotype was 68.38% AT rich (184A, 266T, 115C, 93G). Despite the presence of COI sequence for *Bc. cavernicola* in GenBank (Harbach *et al*., 2007), the COI gene of *Nx. pholeocola* proved to be most similar (89%) to that of *Stegomyia albopicta* (Skuse) (HQ398902), which emphasizes the need for further phylogenetic investigations before the true affinities of *Nyx* are resolved. Future studies should also include DNA sequences for *Isoaedes cavaticus* (Reinert).

Etymology. The name *pholeocola* is a Latin noun (masculine or feminine) derived from *pholeos*, meaning hole, cave or den, and *-cola*, meaning dweller or inhabitant. It was chosen because the species was discovered in and is only known to inhabit the cave at Wat Tham Phanturat in Khlong Sok, near Khao Sok National Park in southern Thailand (see below). The specific name is feminine in agreement with the gender of *Nyx*.

Bionomics. Larvae and pupae of *Nx. pholeocola* were collected from a rimstone pool (20 x 50 cm) in Wat Tham Phanturat cave, Khlong Sok, near Khao Sok National Park in Surat Thani Province in southern Thailand. The water in the pool was clear and cold, 10 cm deep and devoid of vegetation and plant matter. The cave harbours an enormous number of bats that are likely to be the primary source of food for adult females. Females, however, avidly attacked collectors inside the cave; thus, *Nx. pholeocola* may have the potential to opportunistically transmit zoonotic pathogens between bats and humans.

Distribution. *Nyx pholeocola* is only known from the cave at Wat Tham Phanturat Khlong Sok, near the Khao Sok National Park in the Surat Thani Province of southern Thailand (Fig. 4C).

Type series. Eighty-six specimens: 21 ♀, 2 ♀ genitalia, 11 ♂, 4 ♂ genitalia, 16 fourth-instar larval exuviae [Le] and 35 pupal exuviae [Pe]. *Holotype*, ♂ (SS74-24), with LePe on microscope slide, THAILAND: *Surat Thani Province*, Khlong Sok (near Khao Sok National Park), Wat Tham Phanturat cave (08º 53' 59.6" N, 98º 31' 31.2" E), shallow rimstone pool (20 x 50cm), 23.i.2008 (Y.-M. Linton *et al*.). *Paratypes* (same data as holotype except specimens of collection SS75 captured landing on humans in cave; specimens with dissected genitalia on microscope slides are indicated with an asterisk*), 3♀ (SS75-1, -3, -4); 7 ♀Pe (SS74-2, -4, -5 [dissected head on microscope slide], -6*, -8, -10, -13*); 11 ♀LePe (SS74-23, -25 -35, -36, -37, -38, -39, -40 [♀ used for DNA extraction; COI (KC860482, body; KC860484, leg only), ITS2 (KC860486)] -41, -42, -46 [LePe of -35 to -46 retained in 80% ethanol]); 7♂Pe (SS74-3*, -7*, -17, -19 [♂ used for DNA extraction: COI (KC860483), ITS2 (KC860485)], -20*, -22*, -31); 3 ♂LePe (SS74-9 [dissected head on microscope slide], -26, -43 [LePe retained in 80% ethanol]); 7 Pe (SS74-11, -14, -15, -27, -28, -29, -30). All specimens are deposited in the Natural History Museum, London.

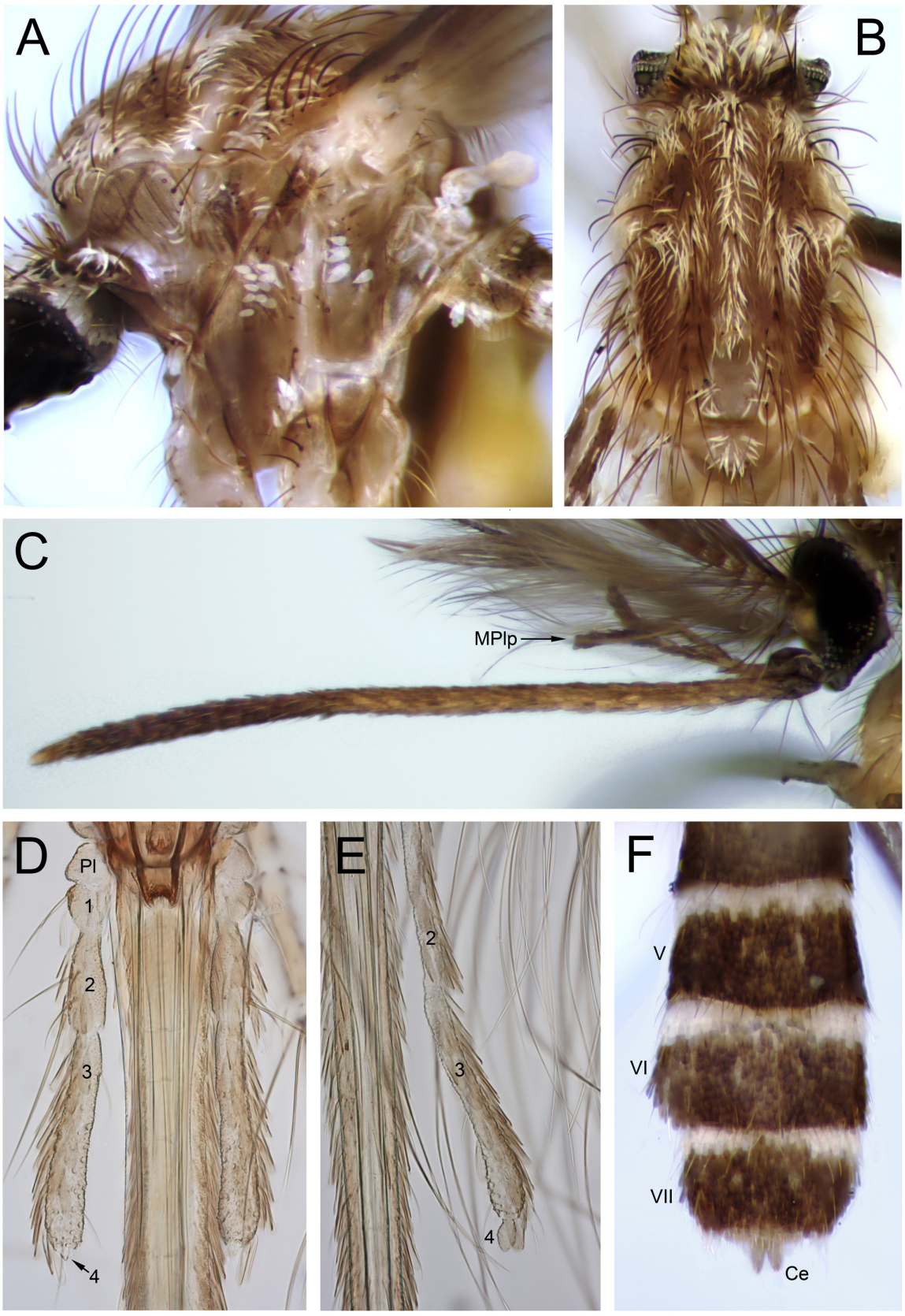

FIGURE 2. Anatomical elements of adult females and males of *Nyx pholeocola*. A, Thorax (left side) of a female. B, Mesonotum (dorsal) of a female. C, Head (left side) of a male showing the short maxillary palpi. D, Maxillary palpi (dorsal) of a female. E, Left maxillary palpus (dorsal) of a male. F, Terminal abdominal segments (dorsal) of a female (segment VIII is fully retracted into segment VII). Ce = cerci; Pl = palpifer; MPlp = maxillary palpus; 1–3 = palpomeres; V–VII = abdominal segments.

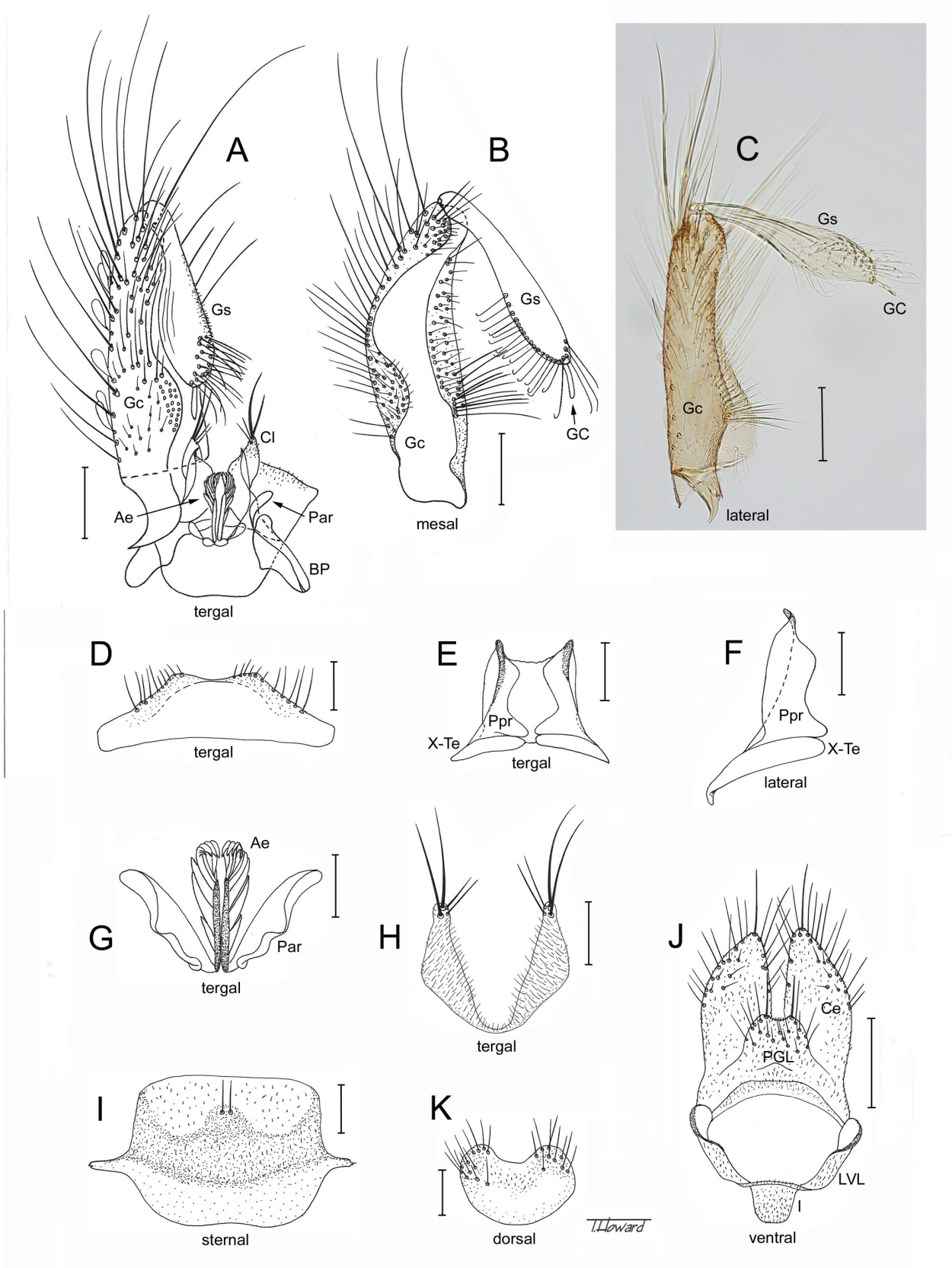

FIGURE 3. Genital structures of *Nyx pholeocola* (aspects as indicated). A–I, Male genitalia: A, with right gonocoxopodite removed; B,C, gonocoxopodite; D, tergum IX; E,F, proctiger; G, aedeagus with parameres; H, claspettes; I, sternum IX. J,K, Female genitalia: J, principal parts; K, tergum IX. Ae = aedeagus; BP = basal piece; Ce = cercus; Cl = claspette; Gc = gonocoxite; GC – gonostylar claw; Gs = gonostylus; I = insula; Par = paramere; PGL = postgenital lobe; Ppr = paraproct; X-Te = tergum X. Scales bars = 0.1 mm (A–C,J) and 0.05 mm (D–I,K).

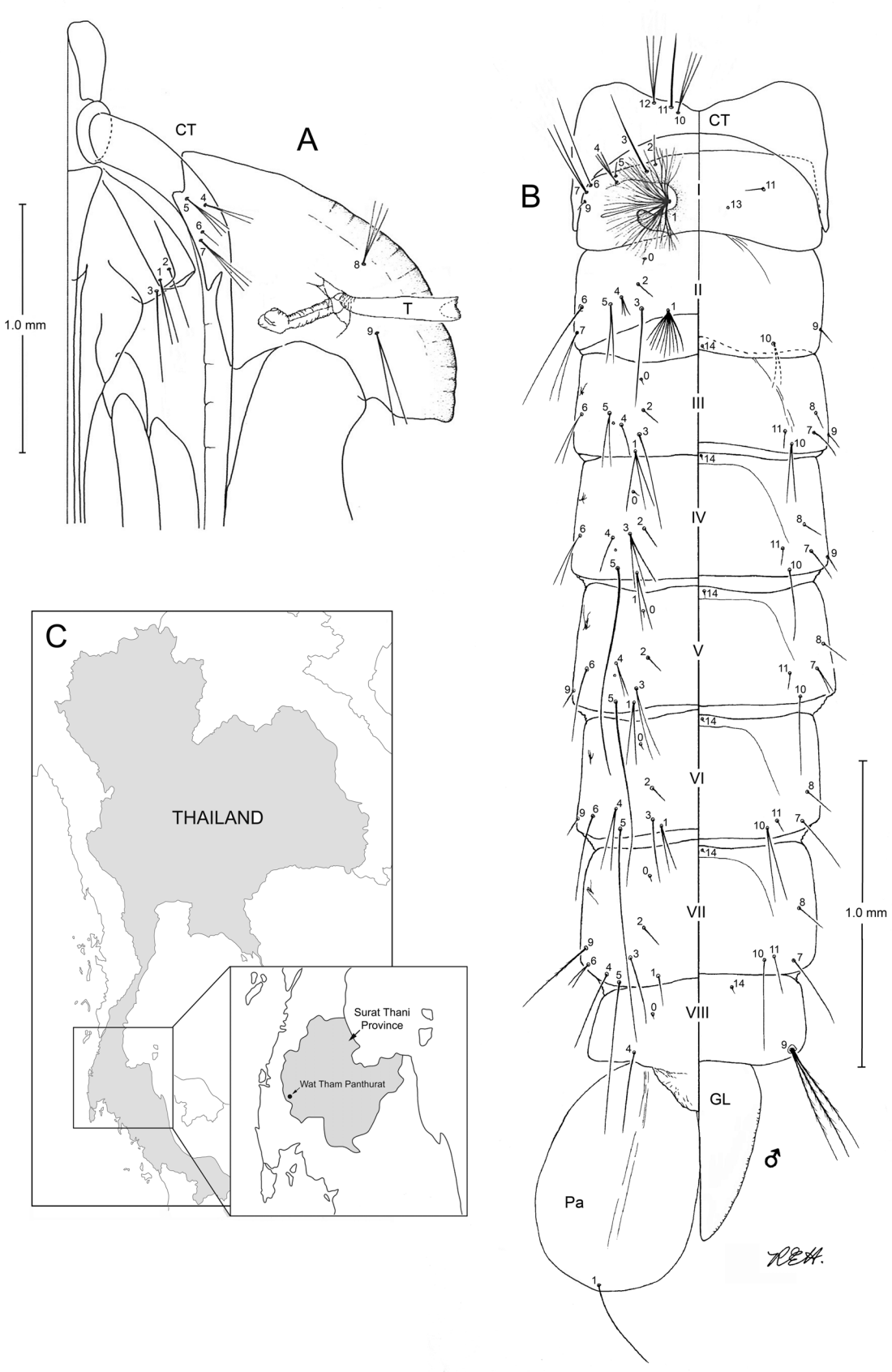

FIGURE 4. A,B, Pupa of *Nyx pholeocola*. A, Left side of cephalothorax, dorsal to right. B, Dorsal (left) and ventral (right) aspects of metathorax and abdomen. C, Outline map of Thailand and Surat Thani Province showing the location of the cave at Wat Tham Panthurat. CT = cephalothorax; GL = genital lobe; Pa = paddle; T = trumpet; I–VIII, X = abdominal segments I–VIII and X; 0–11, 14 = setal numbers for specified areas, e.g. seta 1–II.

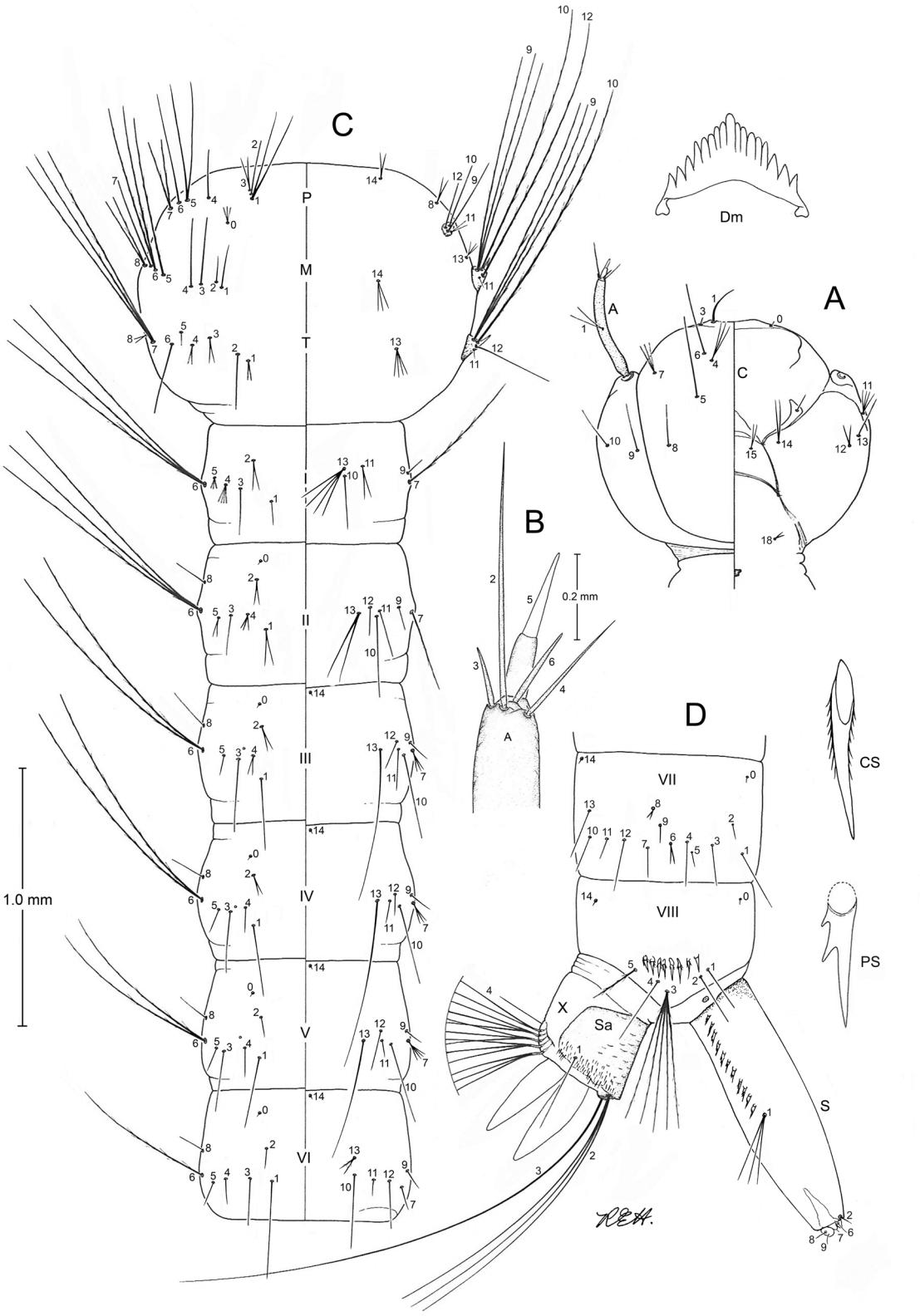

FIGURE 5. Fourth-instar larva of *Nyx pholeocola* (reconstructed from larval exuviae; positions of some setae may differ slightly from those illustrated). A, Head, dorsal (left) and ventral (right) aspects of left side. B, Apex of antenna with setae 2–6-A. C, Thorax and abdominal segments I–VI, dorsal (left) and ventral (right) aspects of left side. D, Abdominal segments VII–X, left side. A = antenna; C = cranium; CS = comb scale; Dm = dorsomentum; M = mesothorax; P = prothorax; PS = pecten spine; S = siphon; Sa = saddle; T = metathorax; I–VIII, X = abdominal segments I–VIII and X; 0–15, 18 = setal numbers for specified areas, e.g. seta 5-C.

Discussion

Between 2005 and 2010, Rattanarithikul and her colleagues published a series of six taxonomic reviews featuring identification keys that recognized 459 mosquito species (classified in 53 genera and 40 subgenera) in Thailand. In the sixth and final publication, Rattanarithikul *et al*. (2010) concluded that "undescribed species almost certainly will be found in the future". The discovery of *Nyx pholeocola* validates this prediction and brings the total of formally named species of Culicidae that are known to occur in Thailand to 460 (now classified in 54 genera).

Nyx is a distinct element of tribe Aedini. The genus and its sole species can be distinguished from the other genera of the tribe in Southeast Asia by incorporating the following information into the keys developed by Rattanarithikul *et al*. (2010).

Nyx pholeocola keys readily to Plate 12 in the key to adult females. This couplet distinguishes *Isoaedes* and *Borichinda*. Disregarding character 1, *Nyx* shares character 2 with *Borichinda* (pattern of scutal pale scaling) and character 3 with *Isoaedes* (scutellum with narrow scales on midlobe). Character 4 pertains to postspiracular and subspiracular scales, which are absent in *Isoaedes* and present in *Borichinda*. *Nyx* differs in having postspiracular scales but no subspiracular scales.

Nyx pholeocola traces to *Borichinda* (*Bc. cavernicola*) at the bottom of Plate 13 in the key to fourth-instar larvae, with slight non-conformity of characters in couplets on Plates 9, 11 and 12. *Nyx* exhibits the alternative character at the top (right) of Plate 9 and the first alternative (top left) of Plate 11, except that seta 4-C is not inserted as far anterior to seta 7-C as indicated; also on Plate 11, seta 4-C does not have many branches – as in the noted exception, *Danielsia albotaeniata* Leicester. *Nyx* exhibits two of the three characters at the bottom (right) of Plate 12, but disagrees with character 3 in having some unevenly spaced pecten spines – as in the noted exception, *Petermattinglyius whartoni* (Mattingly). The characters that identify *Borichinda* on Plate 13 do not distinguish *Bc. cavernicola* from *Nx. pholeocola*. Characters that distinguish larvae of *Nyx* from those of *Borichinda* are listed in Table 1, especially the non-stellate condition of setae 1,3-P, 5-T, 2-I–VI, 11-I, 5-II–VII, 7-II, 13-III–V and the precratal seta, all of which are multiple-branched stellate setae in *Bc. cavernicola*.

TABLE 1. Salient anatomical differences that distinguish the adults, pupae and fourth-instar larvae of *Nyx*, *Borichinda* and *Isoaedes*. Characters that distinguish *Nyx* from *Borichinda* are indicated with an asterisk (*); those that distinguish *Nyx* from *Isoaedes* are indicated with a cross (†).

Character	*Nyx*	*Borichinda*	*Isoaedes*
Adults			
Compound eyes†	Narrowly separated	Narrowly separated	Contiguous
Maxillary palpus, length (males)*†	0.3 length of proboscis	Slightly shorter than proboscis	0.84 – 0.92 length of proboscis
Palpomeres (males)*†	Four, 4th vestigial	Five	Five
Scutum, pale scaling†	Present	Present	Absent
Scutellum, scales on midlobe*	Narrow	Broad	Narrow
Postspiracular scales†	Present	Present	Absent
Subspiracular scales†	Absent	Present	Absent
Remigial setae*	Absent	Absent	Present
Foreungues (males)*	Both toothed	One toothed	Both toothed
Male genitalia			
Sternum IX (males)†	Long	Long	Shorter
Sternum IX setae†	Present	Present	Absent
Gonostylus*†	Long, enlarged distally	Long, cylindrical	Long, cylindrical
Claspette*	Apex narrow with few setae	Apex expanded with numerous setae	Apex narrow with few setae

.....continued on the next page

TABLE 1. (Continued)

Character	*Nyx*	*Borichinda*	*Isoaedes*
Pupae			
Seta 7-CT*	Longer than 6-CT	As long as 6-CT	Longer than 6-CT
Seta 3-III†	Single	Single	Multiple branches
Seta 6-III†	Single or double	Single	Branched
Seta 6-VII*	Posteromesal to seta 9	Anterior to seta 9	Posteromesal to seta 9
Seta 9-IV–VI*	Posterior to seta 8	Anterior to seta 8	Posterior to seta 8
Seta 9-VII*†	Single	Branched	Branched
Paddle*†	Apex slightly produced	Apex slightly concave	Apex distinctly produced
Fourth-instar larvae			
Seta 4-C*	Short, 3,4 branches	Short, 7–11 branches	Short, 3–6 branches
Seta 14-C*†	Single or double, longer than 15-C	Branched, shorter than 15-C	Single, shorter than 15-C
Seta 1-P*	Longer than 2-P	Shorter than 2-P	Longer than 2-P
Setae 1,3-P*†	Usually double (2,3)	Stellate	Single
Seta 5-P†	Double	Double	Single
Seta 5-T†	Small single	Large, stellate, multiple branches	Small, single
Seta 2-I,II*†	Large, 2,3 branches	Large, stellate	Small, single
Seta 2-III–VI*†	Small, single or double, anterior to seta 1	Large, stellate, far anterolateral to seta 1	Small, single, near mesal to seta 1
Seta 11-I*†	Large, 2,3 branches	Large, stellate	Small, 1,2 branches
Seta 5-II–VI*	Small, usually single	Large, stellate	Small, single
Seta 7-II*†	Single, short (about half length of 6-II)	Short, stellate	Long, 1,2 branches
Seta 9-III–VI*	Near anterior to seta 7	Far anterior to seta 7	Near anterior to seta 7
Seta 10-II–V*	Lateral to setae 11,12	Mesal to setae 11,12	Lateral to setae 11,12
Seta 13-III–V*	Single	Stellate	Single
Seta 5-VII*	Small, single	Large, stellate	Small, 1,2 branches
Comb scales†	Spine-like, in row	Spine-like, in row	Scale-like, in patch
Pecten spines*	Distal 1 or 2 spines more widely spaced	Evenly spaced	Distal spine more widely spaced
Seta 1-X†	Small, single	Large, branched	Small, single
Precratal setae*†	Present (1, single)	Present (2, stellate)	Absent

Acknowledgements

Fieldwork during which *Nyx pholeocola* was discovered was funded by Royal Caribbean Cruises (The ScholarShip LLC) and Friends of the Natural History Museum through donations to support the efforts of the Mosquito Barcoding Initiative (MBI, http://mosquitobarcode.org/). We are especially grateful to Ravinder S. Bhatia (Vice President, The ScholarShip) for his scientific and logistical support, Mark Isenstadt (Director, Vector Solutions, Bangkok, Thailand) for facilitating field collections, and Rudy Meiswinkel, Magdalena Zarowiecki and Dean Smallwood for their assistance in the field. This manuscript was prepared in part while YML held a National Research Council Research Associateship Award at the Walter Reed Army Institute of Research. The opinions and assertions contained herein are those of the authors and are not to be construed as official or reflecting the views of the US Department of the Army or the US Department of Defense.

References

Collins, F.H. & Paskewitz, S.M. (1996) A review of the use of ribosomal DNA (rDNA) to differentiate among cryptic *Anopheles* species. *Insect Molecular Biology*, 5, 1–9.
http://dx.doi.org/10.1111/j.1365-2583.1996.tb00034.x

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

Goloboff, P.A., Carpenter, J.M., Arías, J.S. & Esquivel, D.R.M. (2008) Weighting against homoplasy improves phylogenetic analysis of morphological data sets. *Cladistics*, 24, 1–16.
http://dx.doi.org/10.1111/j.1096-0031.2008.00209.x

Harbach, R.E. & Knight, K.L. (1980) *Taxonomists' Glossary of Mosquito Anatomy.* Plexus Publishing, Marlton, New Jersey, 415 pp.

Harbach, R.E. & Knight, K.L. (1982) Corrections and additions to *Taxonomists' Glossary of Mosquito Anatomy. Mosquito Systematics* (for 1981), 13, 201–217.

Harbach, R.E., Rattanarithikul, R, Howard, T.M., Linton, Y.-M. & Kitching, I.J. (2007) Systematics of a new genus and cavernicolous species of the mosquito tribe Aedini (Diptera, Culicidae) from Thailand. *Proceedings of the Entomological Society of Washington*, 109, 469–488.

Linton, Y.-M., Harbach, R.E., Chang, M.S., Anthony, T.G. & Matusop, A. (2001) Morphological and molecular identity of *Anopheles* (*Cellia*) *sundaicus* (Diptera: Culicidae), the nominotypical member of a malaria vector species complex in Southeast Asia. *Systematic Entomology*, 26, 357–366.
http://dx.doi.org/10.1046/j.1365-3113.2001.00153.x

Rattanarithikul, R., Harbach, R.E., Harrison, B.A., Panthusiri, P., Coleman, R.E. & Richardson, J.H. (2010) Illustrated keys to the mosquitoes of Thailand VI. Tribe Aedini. *Southeast Asian Journal of Tropical Medicine and Public Health*, 41 (Suppl. 1), 1–225.

Reinert, J.F., Harbach, R.E. & Kitching, I.J. (2009) Phylogeny and classification of tribe Aedini (Diptera: Culicidae). *Zoological Journal of the Linnean Society*, 157, 700−794 + 2 online appendices.
http://dx.doi.org/10.1111/j.1096-3642.2009.00570.x

Ruiz, F., Linton, Y.-M., Ponsonby, D.J., Conn, J.E., Herrera, M., Quinones, M.L., Velez, I.D. & Wilkerson, R.C. (2010) Molecular comparison of topotypic specimens confirms *Anopheles* (*Nyssorhynchus*) *dunhami* Causey (Diptera: Culicidae) in the Colombian Amazon. *Memorias do Instituto Oswaldo Cruz*, 105, 899−903.

APPENDIX

Anatomical characters used in the cladistic analysis. See Reinert *et al*. (2009) for character coding and discussions of the characters. Character states observed in *Nyx pholeocola* are listed with the numerical character code of Reinert *et al*. in parentheses.

Eggs

1. *Oviposition, eggs laid*: unknown (?).
2. *Anterior end, shape*: unknown (?).
3. *Width at midlength*: unknown (?).
4. *Outer chorion, cell pattern*: unknown (?).

Larvae (fourth-instars)

5. *Labiogula, length*: < width (0).
6. *Antenna, ratio of length to median length of dorsal apotome (DAp)*: ≥ 0.42 (1).
7. *Antenna, spicules*: absent (0).
8. *Seta 1-A, ratio of length to antennal width at point of attachment*: ≥ 3.1 (1).
9. *Seta 1-A, development*: 2-branched (0).
10. *Setae 2,3-A, insertion on antenna*: apical or nearly apical (0).
11. *Seta 1-C, development*: single, thinner, distal part attenuate (1).
12. *Seta 4-C, insertion relative to seta 6-C*: posterior (2).
13. *Seta 4-C, ratio of length to median length of DAp*: ≤ 0.19 (0).
14. *Seta 5-C, ratio of length to median length of DAp*: 0.41−0.81 (2).
15. *Seta 5-C, development*: single (0).
16. *Seta 6-C, insertion relative to seta 7-C*: anterior (0).
17. *Seta 6-C, ratio of length to median length of DAp*: 0.21−0.38 (1).

18. *Seta 6-C, development*: single (0).
19. *Seta 7-C, insertion relative to seta 5-C*: anterior (0).
20. *Seta 7-C, ratio of length to median length of DAp*: 0.21−0.38 (1).
21. *Seta 7-C, development*: ≥ 3 branches (1).
22. *Seta 12-C, insertion relative to seta 13-C*: mesal (0).
23. *Seta 13-C, development*: single (0).
24. *Seta 14-C, development*: branched (1).
25. *Seta 19-C*: absent (0).
26. *Ventromedian cervical sclerite*: present (1).
27. *Setae 1−3-P, insertion*: not on common support plate (0).
28. *Seta 1-P, length relative to length of seta 2-P*: longer (1).
29. *Seta 2-P, development*: single (0).
30. *Seta 3-P, length relative to length of seta 2-P*: shorter (0).
31. *Seta 4-P, length relative to length of seta 3-P*: longer (1).
32. *Seta 4-P, development*: single (0).
33. *Seta 5-P, length relative to length of seta 6-P*: equal (0).
34. *Seta 5-P, development*: branched (0).
35. *Seta 7-P, development*: single or branched (0,1).
36. *Seta 8-P, ratio of length to length of seta 4-P*: ≥ 1.8 (1).
37. *Seta 8-P, development*: 2-branched (0).
38. *Seta 13-P*: absent (0).
39. *Seta 1-M, ratio of length to length of seta 2-M*: ≤ 2.5 (0).
40. *Seta 1-M, development*: single (rarely 2-branched) (0).
41. *Seta 4-M, development*: single (0).
42. *Seta 7-M, length relative to length of seta 5-M*: shorter (0).
43. *Seta 1-T, development*: slender (0).
44. *Seta 1-T, insertion*: on integument (0).
45. *Seta 2-T, development*: single (0).
46. *Seta 4-T, development*: ≥ 2 branches, not stellate (1).
47. *Seta 6-T, development*: single (0).
48. *Seta 3-I, development*: single (0).
49. *Seta 7-I, ratio of length to length of seta 6-I*: ≥ 0.55 (1).
50. *Seta 7-I, development*: single (0).
51. *Seta 12-I*: absent (0).
52. *Seta 1-II, development*: single or ≥ 2 branches, not stellate (0,1).
53. *Seta 2-II, development*: branched (1).
54. *Seta 3-II, development*: single (0).
55. *Seta 5-II, development*: ≥ 2 branches, not stellate (1).
56. *Seta 6-II, length relative to length of seta 6-III*: equal (1).
57. *Seta 6-II, development*: branched (1).
58. *Seta 7-II, development compared to seta 7-I*: similar (0).
59. *Seta 8-II, development*: single (0).
60. *Seta 6-III, development*: branched (1).
61. *Seta 3-V, ratio of length to length of setae 5-V*: ≤ 1.55 (0).
62. *Seta 1-VII, ratio of length to middorsal length of segment X*: 0.48−0.85 (1).
63. *Seta 2-VII, insertion on tergum VII*: on anterior 0.40 far anterior to seta 1-VII (1).
64. *Seta 2-VII, development*: single (0).
65. *Seta 3-VII, insertion relative to seta 1-VII*: at same level (1).
66. *Seta 3-VII, development*: single (0).
67. *Seta 3-VII, ratio of length to middorsal length of segment X*: ≤ 0.42 (0).
68. *Seta 10-VII, development*: single (0).
69. *Seta 12-VII, insertion relative to seta 13-VII*: posterior (2).
70. *Seta 12-VII, development*: single (0).
71. *Setae 1,2-VIII, insertion*: not on common setal support plate (0).
72. *Seta 1-VIII, ratio of length to length of seta 2-VIII*: 0.50–0.95 (1).
73. *Seta 1-VIII, development*: single (0).
74. *Seta 2-VIII, development*: single (0).
75. *Seta 2-VIII, insertion*: nearer to seta 1-VIII than to seta 3-VIII (0).
76. *Seta 3-VIII, development*: aciculate (1).
77. *Seta 4-VIII, development*: single (0).
78. *Comb, scales*: several in one row (0).

79. *Comb plate*: absent (0).
80. *Siphon, acus*: present (1).
81. *Pecten*: present (1).
82. *Pecten spines, arrangement*: distal one more widely spaced (1).
83. *Seta 1-S, number*: 1 (0).
84. *Seta 1a-S, ratio of length to width of siphon*: 0.43−1.12 (1).
85. *Seta 1a-S, development*: branched (1).
86. *Seta 1a-S, insertion*: distal to pecten (1).
87. *Seta 6-S, length relative to distal width of siphon*: equal (0).
88. *Seta 8-S, length relative to distal width of siphon*: shorter (0).
89. *Seta 9-S, development*: slender, slightly curved (0).
90. *Sclerotization of segment X (saddle)*: incomplete ventrally (0).
91. *Saddle, acus*: absent (0).
92. *Saddle, moderate to well-developed spicules on posterior margin*: present (1).
93. *Seta 1-X, insertion*: on saddle (0).
94. *Seta 2-X, ratio of length to length of seta 3-X*: ≤ 0.70 (rarely 0.80) (1).
95. *Seta 2-X, development*: 3-branched (0).
96. *Seta 2-X, aciculate*: absent (0).
97. *Seta 3-X, development*: single (0).
98. *Ventral brush (seta 4-X), attachment*: on grid with transverse bars (1).
99. *Precratal/preboss setae (i.e. two or more setae anterior to grid or boss)*: present (1).
100. *Seta 4a-X of ventral brush, ratio of length to length of seta 4c-X*: ≥ 0.70 (1).
101. *Seta 4d-X of ventral brush, development*: 3-branched, not plumose (1).

Pupae

102. *Cephalothorax, clear unpigmented spots*: absent (0).
103. *Trumpet, tracheoid area*: present (1).
104. *Trumpet, tracheoid area, development*: weakly developed, at base (0).
105. *Seta 1-CT, development relative to seta 3-CT*: weakly developed, considerably shorter than seta 3-CT (0).
106. *Seta 5-CT, ratio of length to length of seta 4-CT*: ≤ 1.2 (0).
107. *Seta 7-CT, length relative to length of seta 6-CT*: 1.2−5.0 times as long (1).
108. *Seta 11-CT, development*: single (0).
109. *Seta 13-CT*: absent (0).
110. *Seta 3-I, length relative to length of seta 6-I*: shorter (0).
111. *Seta 3-I, development*: single (0).
112. *Seta 6-I, length relative to length of seta 7-I*: longer (1).
113. *Seta 1-II, development*: ≥ 5 branches (1).
114. *Seta 2-II, insertion relative to seta 1-II*: lateral (1).
115. *Seta 2-II, length relative to length of seta 1-II*: shorter (0).
116. *Seta 3-II, insertion relative to seta 2-II*: directly posterior (0).
117. *Seta 3-II, development*: single (0).
118. *Seta 3-II, length relative to length of seta 6-II*: equal or shorter (0).
119. *Seta 3-II, thickness relative to seta 1-II*: thicker (1).
120. *Seta 5-II, insertion relative to seta 4-II*: lateral (0).
121. *Seta 5-II, length relative to length of seta 3-II*: shorter (0).
122. *Seta 6-II, length relative to length of seta 7-II*: longer (1).
123. *Seta 3-III, length relative to length of seta 5-III*: longer (1).
124. *Seta 3-III, development*: single (0).
125. *Seta 6-III, development*: single or branched (0,1).
126. *Seta 2-V, insertion relative to seta 3-V*: anterior (0).
127. *Seta 5-V, length relative to median length of tergum VI*: longer (1).
128. *Seta 2-VI, insertion relative to seta 1-VI*: lateral (1).
129. *Seta 3-VI, insertion relative to seta 1-VI*: lateral (1).
130. *Seta 6-VII, insertion relative to seta 9-VII*: posterior (1).
131. *Seta 9-VII, length relative to length of seta 6-VII*: longer (1).
132. *Seta 9-VIII, insertion relative to posterolateral corner of segment*: on corner (0).
133. *Seta 9-VIII, development*: ≥ 3 branches (1).
134. *Paddle, midrib, development*: strong, extending to near apex of paddle (1).
135. *Paddle, fringe of hair-like spicules*: absent (0).
136. *Paddle, development of apical margin*: rounded (0).

137. *Seta 1-Pa*: present (1).
138. *Seta 1-Pa, ratio of length to length of paddle*: ≤ 0.33 or 0.40−0.60 (0,1).
139. *Seta 1-Pa, development*: single (0).
140. *Seta 2-Pa*: absent (0).

Adults (females except where otherwise noted)

141. *Head, erect scales*: present (1).
142. *Head, position erect scales*: on occiput and vertex (1).
143. *Head, decumbent scales of vertex*: both broad and narrow (2).
144. *Head, ocular line width*: narrow (0).
145. *Head, ocular scales*: all narrow (0).
146. *Eyes, immediately above antennal pedicels*: narrowly to moderately separated (1).
147. *Interocular space, scales*: present (1).
148. *Interocular space, scales*: all narrow (0).
149. *Interocular space, setae*: present (1).
150. *Interocular space, setae*: ≤ 5 (0).
151. *Antennal pedicel, vestiture on mesal surface*: present (1).
152. *Antennal pedicel, mesal surface, vestiture, composition*: few scattered scales (not overlapping and not silvery) and setae (0).
153. *Antennal pedicel, lateral surface, scales*: absent (0).
154. *Apical two flagellomeres, length compared to length of other flagellomeres* (males): disproportionately longer (0).
155. *Antenna, flagellar whorls, development* (males): numerous long setae, normally directed dorsally and ventrally (2).
156. *Maxillary palpus, pale scales*: absent (0).
157. *Maxillary palpomeres, development* (males): 3, palpomere 4 absent or vestigial (2).
158. *Maxillary palpomeres, position of palpomeres 4 and/or 5 relative to palpomere 3* (males): nearly straight (2).
159. *Maxillary palpus, palpomere 3, ratio of length to length of proboscis* (males): ≤ 0.14 (0).
160. *Maxillary palpus, palpomere 5, ratio of length to length of palpomere 4* (males): no homologous structure (–).
161. *Maxillary palpus, ratio of length to length of proboscis* (males): ≤ 0.27 (0).
162. *Maxillary palpus, setae on palpomeres 3 (distally) and 4* (males): few, short to moderately long (0).
163. *Proboscis, length relative to length of forefemur*: longer (1).
164. *Proboscis, pale scales*: absent (0).
165. *Proboscis, pale-scaled band near midlength*: absent (0).
166. *Antepronota*: widely separated (1).
167. *Antepronotal scales*: present (1).
168. *Antepronotal scales*: all narrow (0).
169. *Anterior acrostichal setae*: present (1).
170. *Posterior acrostichal setae*: present (1).
171. *Anterior dorsocentral setae*: present (1).
172. *Posterior dorsocentral setae*: present (1).
173. *Scutal scales*: all narrow (0).
174. *Scutum, erect twisted scales*: absent (0).
175. *Scutal scales, colour*: both pale and dark (1).
176. *Combined anterior acrostichal and anterior dorsocentral areas, large patch of pale scales covering anterior ≥ 0.7*: absent (0).
177. *Anterior acrostichal area, pale-scaled stripe*: present (1).
178. *Posterior acrostichal area, pale-scaled stripe*: present (1).
179. *Anterior dorsocentral area, pale-scaled stripe*: absent (0).
180. *Posterior dorsocentral area, pale-scaled stripe*: absent (0).
181. *Scutal fossal scales*: sparse (0).
182. *Scutal fossal scales, colour*: indefinite arrangement of pale and darker scales (3).
183. *Prescutellar area, median and/or posterior parts, scales*: absent (0).
184. *Prescutellar setae*: present (1).
185. *Prescutellar setae on each side of thorax*: ≥ 6 (1).
186. *Prescutellar area, pale scales on outer margin mesal to setae*: present (1).
187. *Antealar area, scales on anterior part*: present (1).
188. *Antealar area, scales on anterior part, colour*: all pale (1).
189. *Supraalar area, pale scales*: present (1).
190. *Anterior supraalar-posterior antealar area, transverse patch of pale scales*: absent (0).
191. *Scutellum, scales on midlobe*: all narrow (0).
192. *Scutellum, scales on lateral lobes*: all narrow (0).

193. *Paratergal scales*: absent (0).
194. *Parascutellar scales*: absent (0).
195. *Mesopostnotal scales*: absent (0).
196. *Mesopostnotal setae*: absent (0).
197. *Postpronotal scales*: present (1).
198. *Postpronotal scales*: all narrow (0).
199. *Prespiracular setae*: absent (0).
200. *Postspiracular setae*: present (1).
201. *Postspiracular scales*: present (1).
202. *Hypostigmal scales*: absent (0).
203. *Subspiracular scales*: absent (0).
204. *Upper proepisternal setae*: 1−4 and 5−19 (0,1).
205. *Upper proepisternal scales*: present (1).
206. *Lower proepisternal scales*: absent (0).
207. *Upper mesokatepisternal setae*: present (1).
208. *Mesokatepisternal scales*: in two patches (1).
209. *Upper prealar setae*: ≤ 20 (0).
210. *Upper prealar scales*: absent (0).
211. *Lower prealar scales*: absent (0).
212. *Mesepimeral scales*: present (1).
213. *Mesepimeral scales*: in one patch (0).
214. *Lower anterior mesepimeral setae*: present (1).
215. *Mesepimeral fine setae*: absent (0).
216. *Metameron, vestiture*: absent (0).
217. *Metameron, vestiture*: no homologous structure (–).
218. *Upper calypter, setae or hair-like scales* numerous, ≥ 7 (1).
219. *Upper calypter, setae or hair-like scales* (males): numerous, ≥7 (1).
220. *Alula, marginal scales*: present (1).
221. *Alula, marginal scales*: broad (1).
222. *Alula, dorsal broad scales*: absent (0).
223. *Remigium, dorsal setae*: absent (0).
224. *Remigium, insertion of dorsal setae*: no homologous structure (–).
225. *Remigium, ventral setae*: absent (0).
226. *Costal scales*: all dark (0).
227. *Dark pigmentation around radiomedial crossvein and proximal segment of media* $_{3+4}$: absent (0).
228. *Vein* R_2*, length relative to length of* R_{2+3}: longer (1).
229. *Anal vein, point of termination*: noticeably distad of this point (1).
230. *Wing, fringe scales, colour*: uniform (0).
231. *Wing, dorsal tertiary fringe scales on proximal 0.50*: present (1).
232. *Wing, dorsal tertiary fringe scales on proximal 0.50* (males): absent (0).
233. *Wing, dorsal tertiary fringe scales, colour*: uniform (0).
234. *Anteprocoxal scales*: absent (0).
235. *Postprocoxal scales*: absent (0).
236. *Hindcoxa, base relative to dorsal margin of mesomeron*: well below (0).
237. *Fore-, mid- and hindfemora, complete subapical, pale-scaled bands*: absent (0).
238. *Midfemur, median pale-scaled stripe from base to or near apex on anterior surface*: absent (0).
239. *Hindfemur, pale scales dorsally and/or anteriorly at apex*: present (1).
240. *Hindtibia, scales, colour*: dark only (0).
241. *Hindtarsomere 1, basal pale scales*: absent (0).
242. *Hindtarsomere 1, one or more median pale-scaled bands*: absent (0).
243. *Hindtarsomere 1, apical pale scales*: absent (0).
244. *Hindtarsomere 2, basal pale scales*: absent (0).
245. *Hindtarsomere 2, apical pale scales*: absent (0).
246. *Foreungues, development*: both toothed (2).
247. *Foreungues, development* (males): both toothed, larger one with one tooth (4).
248. *Midungues, development* (males): both toothed, larger one with one tooth (4).
249. *Hindungues, development*: both simple (0).
250. *Hindungues, development* (males): both simple (0).
251. *Abdominal tergum I, laterotergite, scales*: present (1).
252. *Abdominal tergum III, median dorsobasal pale-scaled area*: present (1).
253. *Abdominal tergum III, median dorsoapical pale-scaled area*: absent (0).

254. *Abdominal terga, lateral setae* (males): relatively short to moderately long (0).
255. *Abdominal segment VII, cross-section shape*: dorsoventrally flattened (1).

Female genitalia

256. *Intersegmental membrane between segments VII and VIII*: short to intermediate (0).
257. *Tergum VIII, development*: entirely sclerotized, rarely with few small, non-sclerotized areas on distal, lateral and proximal areas, without rod-shaped structures laterally on each side (1).
258. *Tergum VIII, posterior margin*: straight (1).
259. *Tergum VIII, length relative to width*: shorter (0).
260. *Tergum VIII, moderately long to long seta(e) on lateral margins of proximal 0.40*: absent (0).
261. *Tergum VIII, insertion of setae*: on distal 0.60 or less (0).
262. *Tergum VIII, scales*: present, ≥ 14 scales (1).
263. *Sternum VIII, development*: entirely sclerotized (3).
264. *Sternum VIII, posterior margin*: median emargination separating broadly rounded lateral lobes (3).
265. *Sternum VIII, seta 2-S, insertion relative to seta 1-S*: noticeably posterior (0).
266. *Sternum VIII, scales*: present, ≥ 10 scales, often covering much of surface (1).
267. *Tergum IX, width/length ratio*: ≤ 1.8 (1).
268. *Tergum IX, development*: single sclerite, with broad emargination on posterior margin (0).
269. *Tergum IX, setae*: present (1).
270. *Tergum IX, insertion of setae*: on distal area (0).
271. *Postgenital lobe, posterior margin*: emarginate (2).
272. *Postgenital lobe, ratio of ventral width at distal 0.20 to cercus width at midlength*: ≥ 0.66 (1).
273. *Postgenital lobe, ventral index*: 0.47−1.64 (0).
274. *Postgenital lobe, insertion of ventral setae*: on distal area (0).
275. *Upper vaginal sclerite*: present (1).
276. *Lower vaginal sclerite*: absent (0).
277. *Insula, development*: tongue-like (0).
278. *Insular setae*: absent (0).
279. *Insula, insertion of setae*: no homologous structure (–).
280. *Cercus index*: ≤ 2.88 (0).
281. *Cercal scales*: absent (0).
282. *Cercus, distal part*: gently oblique (1).
283. *Cercus/dorsal postgenital lobe index*: ≤ 3.20 (0).
284. *Accessory spermathecae*: present (1).
285. *Accessory spermathecae, development*: two large (1).

Male genitalia

286. *Tergum IX, posterior margin*: two moderately broad to broad lobes (1).
287. *Tergum IX, position of lateral lobes on posterior margin*: widely separated (1).
288. *Tergum IX, setae*: present (1).
289. *Tergum IX, setae*: all slender (0).
290. *Sternum IX, vestiture*: present (1).
291. *Sternum IX, vestiture*: setae (0).
292. *Gonocoxite, dorsomesal apical lobe*: absent (0).
293. *Gonocoxite, dorsomesal basal lobe*: absent (0).
294. *Gonocoxite, scales*: present (1).
295. *Gonocoxite, mesal surface*: entirely membranous (0).
296. *Gonocoxite, seta(e) on basomesal area of dorsal surface*: present (1).
297. *Gonocoxite, setal development on basomesal area of dorsal surface*: all slender (0).
298. *Gonocoxite, lateral setae*: mostly long (1).
299. *Gonocoxite, few to several short blunt-tipped spiniforms in a row on mesal area of ventral surface*: absent (0).
300. *Gonocoxite, row or patch of long moderately broad to broad scales on mesal area of ventral surface*: absent (0).
301. *Gonocoxite, row or patch of long narrow lanceolate setae on mesal area of ventral surface*: absent (0).
302. *Gonostylus, attachment to gonocoxite*: apical (0).
303. *Gonostylus, proximal part*: narrow (0).
304. *Gonostylus, median part*: noticeably broader than proximal part (2).
305. *Gonostylus, distal part*: slightly broader than proximal part (1).
306. *Gonostylus, elongate lobe on lateral surface*: absent (0).
307. *Gonostylus, insertion of elongate lobe on lateral surface*: no homologous structure (–).

308. *Gonostylus, moderately broad to broad lobe on median part of lateral surface*: present (1).
309. *Gonostylus, hornlike projection on distal part of lateral surface*: absent (0).
310. *Gonostylus, seta(e) on distal 0.33*: present (1).
311. *Gonostylus, scales*: absent (0).
312. *Gonostylar claw(s)*: present (1).
313. *Gonostylar claw(s), number*: one (0).
314. *Most proximal gonostylar claw, insertion on gonostylus*: at apex (0).
315. *Most proximal gonostylar claw, ratio of length to length of gonostylus*: ≤ 0.35 (0).
316. *Most proximal gonostylar claw, development*: moderately broad spiniform (1).
317. *Most proximal gonostylar claw, apex*: bluntly pointed (1).
318. *Gonostylus/gonocoxite index*: ≥ 0.73 (3).
319. *Claspette*: present (1).
320. *Claspette, development*: single basal setose plaque, columnar lobe absent (0).
321. *Claspette, subapical thumb-like projection on columnar stem*: no homologous structure (–).
322. *Claspette, ratio of columnar stem length to length of aedeagus*: no homologous structure (–).
323. *Claspette, vestiture*: two simple setae (0).
324. *Claspette filament, distinct transverse striations*: absent (0).
325. *Aedeagus, development*: comprised of two lateral plates (aedeagal sclerites) (1).
326. *Aedeagus, width*: widest in distal 0.33 (0).
327. *Aedeagal teeth*: present (1).
328. *Aedeagal teeth, position*: on distal ≤ 0.55 (0).
329. *Aedeagus, small distal spicules*: absent (0).
330. *Opisthophallus and prosophallus*: absent (0).
331. *Opisthophallus, development*: no homologous structure (–).
332. *Proctiger, sternal arm*: absent (0).
333. *Proctiger, cercal setae*: absent (0).
334. *Proctiger, apical teeth on paraproct*: present (1).
335. *Paraproct, subapical small knoblike or thumb-like process*: absent (0).

Biology

336. *Habitat of immature stages*: fresh-water rock pools (2).