

The aquatic annelid fauna of the San Marcos River headsprings, Hays County, Texas

McLean L. D. Worsham¹, Randy Gibson², David G. Huffman¹

1 Freeman Aquatic Biology Station, Department of Biology, Texas State University, San Marcos, TX 78666, U.S.A. **2** U. S. Fish & Wildlife Service, San Marcos Aquatic Resources Center, San Marcos, TX 78666, U.S.A.

Corresponding author: McLean L. D. Worsham (biolyth@txstate.edu)

Academic editor: S. James | Received 20 March 2016 | Accepted 20 May 2016 | Published 19 September 2016

<http://zoobank.org/D8A868ED-45AD-47DF-ACAE-A238A1204B11>

Citation: Worsham MLD, Gibson R, Huffman DG (2016) The aquatic annelid fauna of the San Marcos River headsprings, Hays County, Texas. ZooKeys 618: 1–14. doi: 10.3897/zookeys.618.8560

Abstract

The San Marcos River in Central Texas has been well studied and has been demonstrated to be remarkably specious. Prior to the present study, research on free-living invertebrates in the San Marcos River only dealt with hard bodied taxa with the exception of the report of one gastrotrich, and one subterranean platyhelminth that only incidentally occurs in the head spring outflows. The remainder of the soft-bodied metazoan fauna that inhabit the San Marcos River had never been studied. Our study surveyed the annelid fauna and some other soft-bodied invertebrates of the San Marcos River headsprings. At least four species of Hirudinida, two species of Aphanoneura, one species of Branchiobdellida, and 11 (possibly 13) species of oligochaetous clitellates were collected. Other vermiform taxa collected included at least three species of Turbellaria and one species of Nemertea. We provide the results of the first survey of the aquatic annelid fauna of the San Marcos Springs, along with a dichotomous key to these annelids that includes photos of some representative specimens, and line drawings to elucidate potentially confusing diagnostic structures.

Keywords

Annelida, Clitellata, Hirudinida, Aphanoneura, Branchiobdellida, oligochaetous clitellates, freshwater Nemertea, spring fauna

Introduction

The San Marcos River in Hays County, Texas (29°53.505'N; 97°55.973'W) is a spring fed river supplied with physicochemically stable water from the Edwards Aquifer (Crow 2012; Musgrove and Crow 2012). The spring outflows were impounded by a low head dam in 1849 to form a small reservoir known of as Spring Lake. Spring Lake and the upper 2 or 3 km of the spring run supports a rich biotic community (Edwards and Arnold 1961, Bowles et al. 2007, Gibson et al. 2008). At present there are four species that are federally protected, threatened, or endangered, with some other endemics probably worthy of such designation; three of which are vertebrates. The Comal Springs riffle beetle (*Heterelmis comalensis* Bosse, Tuff, and Brown) is the only protected invertebrate species that occurs in the San Marcos River (SMR). Circumstances associated with the biogeographic history of the headsprings no doubt contributed to the evolution of unique and endemic species. Indeed, many of the endemic invertebrates of Spring Lake and the San Marcos Springs are generally considered marine relicts (Holsinger and Longley 1980, Hershler and Longley 1986, Gibson et al. 2008). Therefore, endemism is high for some of the invertebrate taxa; especially taxa that are poor dispersers and have long inhabited the SMR.

The first studies of invertebrates from the SMR and nearby springs issuing from the Edwards Aquifer led to the description of several new stygobionts (Benedict 1896, Ulrich 1902, Holsinger 1966, Bowman and Longley 1976, Holsinger and Longley 1980, Hershler and Longley 1986) with no attention paid to epigeic invertebrate species. The first study on epigeic invertebrates only reported on trichopterans. Not surprisingly, this study led to the description of a new species, *Protophila arca* (Edwards and Arnold 1961), which was determined to be a San Marcos endemic (Edwards and Arnold 1961). Thirty additional species of trichopterans were later reported from the San Marcos (Bowles et al. 2007). More recent surveys reported additional records of species from the SMR and associated springs (Gibson et al. 2008, Diaz and Alexander 2010, Hutchins et al. 2013).

A study of the diet of the fountain darter, *Etheostoma fonticola* Jordan and Gilbert from the SMR was the first study to report on epigeic invertebrates other than trichopterans, but this diet study only reported on hard-bodied invertebrates (e.g. mollusks and arthropods); additionally, recovered specimens were only identified to order (Schenck and Whiteside 1977). Despite its low taxonomic resolution, findings from this study suggested a remarkable amount of diversity, with twelve separate orders reported from the gut contents of this one species of fish. This diversity was verified by a subsequent diet study of the San Marcos salamander, *Eurycea nana* Bishop whereby numerous taxa (also largely hard-bodied forms), were reported from the SMR for the first time (Diaz 2010).

At the time of this writing, the only reports of free-living soft-bodied invertebrates from the SMR were the mention of a stygobiotic platyhelminth and a stygobiotic hirudinean (Hershler and Longley 1986, Bowles and Arsuffi 1993) and the documentation of the first gastrotrich of the genus *Redudasys* (Gastrotricha: Mac-

rodasyida) in the Northern Hemisphere (K  nneby and Wicksten 2014). Presented herein is the first report of identified annelids from the San Marcos River, with notes on other free-living vermiform fauna; including a new distribution record for a nemertean. This report adds several species to the ever-growing list of invertebrate taxa reported from the San Marcos Springs (SMS) and SMR. Several of these appear to be undescribed taxa that likely have a restricted distribution to the physicochemically stable spring run.

Materials and methods

Invertebrates were collected from January 2013 to August 2014. Several sampling methods were utilized, including a Ponar grab sampler, installation of nets over spring outflows, baited traps, dip netting of vegetation and substrate, and SCUBA diving with suction devices. All collected organisms were transported live to the Freeman Aquatic Biology Station at Texas State University-San Marcos. Specimens were examined under a dissecting and/or compound light microscope and were identified to lowest possible taxon using the most recent literature (Brinkhurst 1964, Brinkhurst and Jamieson 1971, Harman 1973, Spencer 1978, Hiltunen and Klemm 1980, Kathman and Brinkhurst 1998, Pinder 2010, Wetzel et al. 2015).

Results

At least 4 species of epigeic Hirudinida, 2 species of Aphanoneura, 1 species of Branchiobdellida, and 11 (possibly 13) species of oligochaetous clitellates are present in the SMR and identified herein. At least 3 species of free-living Platyhelminthes and 1 species of Nemertea were also collected. The species of Nemertea is the first record of the phylum from the SMR, though this phylum has been documented elsewhere in the Guadalupe drainage basin (Ourso and Hornig 2000). See Table 1 for list of vermiform taxa identified in this study.

Dichotomous key to Annelida of San Marcos Springs

1a	Parasitic or commensal	2
1b	Free-living	5
2a (1a)	Chaetae absent.....	3
2b	Chaetae present; commensal on gastropods (in mantle cavity); body usually quite small, <4 mm	<i>Chaetogaster cf. limnaei</i>
3a (2a)	Parasitic on exterior of vertebrates.....	4
3b	Parasitic on exterior of crayfish of Family Cambaridae (Figure 1)	
Order Branchiobdellida (Family Cambarincolidae)	

Table 1. List of annelid and other vermiform taxa collected from San Marcos River headsprings.

Phylum	Class	Subclass	Order	Family	Genus/species	Describer
Annelida	Clitellata	Hirudinida	Arhynchobdellida	Erpobdellidae ^[†]		
Annelida	Clitellata	Hirudinida	Rhynchobdellida	Piscicolidae		
Annelida	Clitellata	Hirudinida	Rhynchobdellida	Glossiphoniidae	<i>Placobdella parastica</i>	Say, 1924
Annelida	Clitellata	Hirudinida	Rhynchobdellida	Glossiphoniidae	<i>Helobdella</i> cf. <i>papillata</i>	Moore, 1952
Annelida	Clitellata	Oligochaeta	Lumbriculida	Lumbriculidae	Lumbriculidae sp ₁	
Annelida	Clitellata	Oligochaeta	Lumbriculida	Lumbriculidae	Lumbriculidae sp ₂	
Annelida	Clitellata	Oligochaeta	Haplotaxida	Naididae	<i>Sylaria lacustris</i>	Linnaeus, 1767
Annelida	Clitellata	Oligochaeta	Haplotaxida	Naididae	<i>Chaetogaster</i> cf. <i>limnaei</i>	K. von Baer, 1827
Annelida	Clitellata	Oligochaeta	Haplotaxida	Naididae	<i>C.</i> cf. <i>diaphanus</i>	Gruithuisen, 1828
Annelida	Clitellata	Oligochaeta	Haplotaxida	Naididae	<i>C.</i> cf. <i>crystallinus</i>	Vejdovský, 1883
Annelida	Clitellata	Oligochaeta	Haplotaxida	Naididae	<i>Pristina leidyi</i>	F. Smith, 1896
Annelida	Clitellata	Oligochaeta	Haplotaxida	Naididae	<i>Nais pseudobdusa</i>	Piguet, 1906
Annelida	Clitellata	Oligochaeta	Haplotaxida	Naididae	<i>Dero</i> (<i>Dero</i>) cf. <i>obtusa</i>	d'Udekem, 1855
Annelida	Clitellata	Oligochaeta	Haplotaxida	Naididae	<i>D.</i> (<i>Aulophorus</i>) cf. <i>furcatus</i>	Müller, 1773
Annelida	Clitellata	Oligochaeta	Haplotaxida	Haplotaxidae	<i>Haplotaxis</i> cf. <i>gordiioides</i>	Hartmann, 1821
Annelida	Clitellata	Oligochaeta	Haplotaxida	Aeolosomatidae	<i>Aeolosoma</i> cf. <i>variegatum</i>	Vejdovský, 1884
Annelida	Aphanoneura	Aphanoneura		Aeolosomatidae	<i>A.</i> cf. <i>quaternarium</i>	Ehrenberg
Annelida		Aphanoneura	Tricladida	Dugesidae	<i>Schmidtea</i> sp.	
Platyhelminthes	Turbellaria		Tricladida	Dugesidae	<i>Dugesia</i> sp.	
Platyhelminthes	Turbellaria		Macrostomida			
Platyhelminthes	Turbellaria		Seriata	Kenkiidae	<i>Sphalloplana mohri</i> ^[‡]	Hyman, 1939
Nemertea	Enopla		Hoplomenertea	Tetrastemmatidae	<i>Prostoma</i> cf. <i>gracense</i>	Böhmig, 1892

[†] Both a stygobiotic and epigeic species were collected.

[‡] This species was not collected by the authors but was included for completeness.

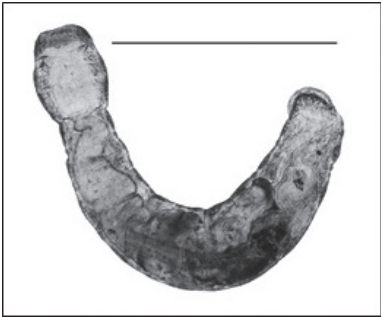


Figure 1. Branchiobdellida from crayfish host (Cambaridae) (scale 1 mm).

- 4a (3a) Parasitic on fishes; anterior sucker about half the diameter of caudal sucker; body small (<2.5 cm)..... **Family Piscicolidae**
- 4b Usually parasitic on turtles; body large, (>2.5 cm) ... ***Placobdella parasitica***
- 5a (1b) Chaetae absent..... **6**
- 5b Chaetae present **7**
- 6a (5a) Multiple pairs of eyes (may be discrete and not visible).....
..... **Family Erpobdellidae**
- 6b Single pair of closely spaced conspicuous eyes..... ***Helobdella* sp.**
- 7a (5b) Dorsal chaetae absent (at least on 10 or more anterior segments)..... **8**
- 7b Dorsal chaetae present (Figure 2)..... **10**
- 8a (7a) Ventral chaetae bifid, and at least three and up to nine per bundle (Figure 3)..... **9**

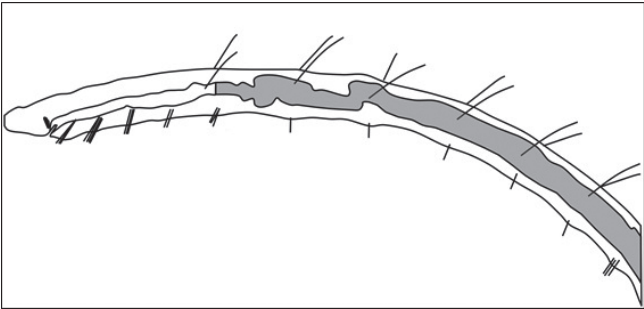


Figure 2. Drawing of generalized aquatic oligochaete showing anterior end and example positions of dorsal and ventral chaetae.

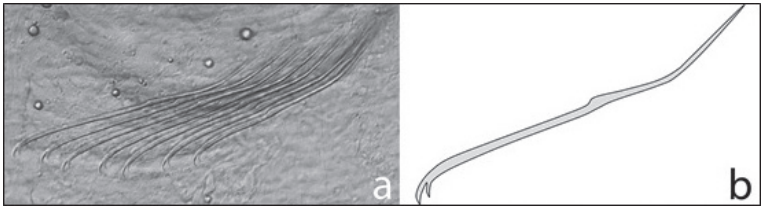


Figure 3. *Chaetogaster*: **A** photograph of typical bifid ventral chaetal bundle **B** drawing showing shape of one chaeta.

- 8b Ventral chaetae 1 per bundle with simple point and tip curved towards posterior of worm (Figure 4); worm elongate, up to 10 cm or more in length, but usually 4–5 cm*Haplotaxis* cf. *gordioides*

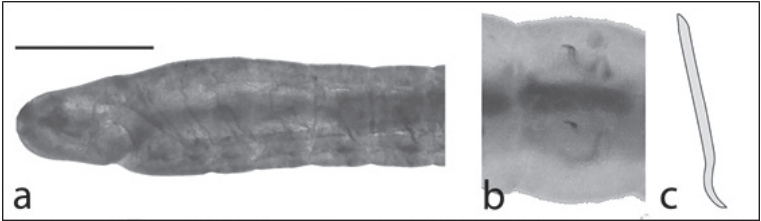


Figure 4. *Haplotaxis* cf. *gordioides*: **A** lateral view of anterior end showing prostomium and ventral mouth (scale 750 μ m) **B** ventral view of one segment showing the two single ventral chaetae **C** drawing of one ventral chaeta.

- 9a (8a) Prostomium more conspicuous than other *Chaetogaster* spp.; only ventral chaetae present; worm usually small, total length <4 mm (Figure 5)
..... *Chaetogaster* cf. *diaphanus*



Figure 5. *Chaetogaster* cf. *diaphanus*: **A** drawing of entire body (scale 250 μ m) **B** photo of anterior end showing prostomium protruding forward from mouth.

- 9b Prostomium inconspicuous with cleft (Figure 6); numerous chaetae per posterior ventral bundles; worm usually relatively large, with total length \geq 4 mm.....*Chaetogaster* cf. *crystallinus*

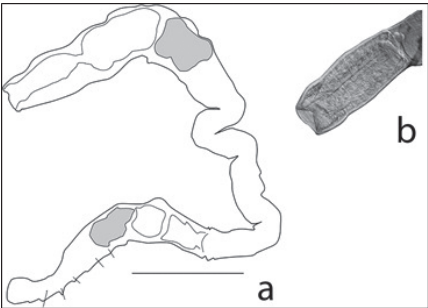


Figure 6. *Chaetogaster* cf. *crystallinus*: **A** outline drawing of entire body showing positions of chaetae that are limited to only ventral bundles of segments (scale 1 mm) **B** photo of anterior end showing cleft in prostomium.

- 10a (7b) Dorsal chaetae usually more than 1 per bundle and found on anterior portions of worm **11**
- 10b Dorsal chaetae short, only 1 per bundle, only found on posterior of worm; ventral chaetae 1 per bundle with simple point curved posteriad; worm elongate, up to 10 cm long, usually 4–5 cm.....***Haplotaxis cf. gordioides***
- 11a (10a) Ventral chaetae two per bundle and with simple point (Figure 7); worm usually quite large, total length >3 cm **12**

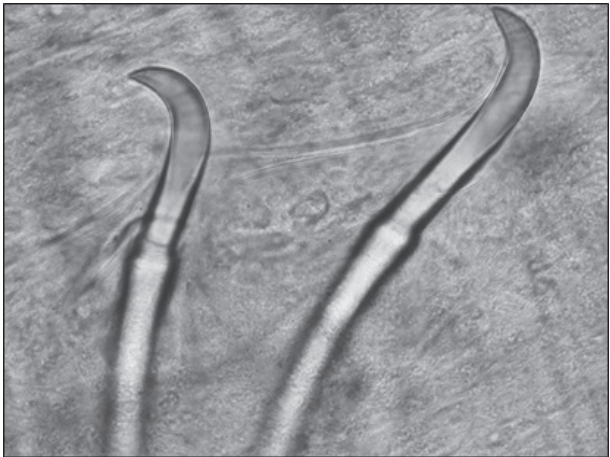


Figure 7. Paired chaetae typical of both dorsal and ventral bundles found on several lumbricolid taxa.

- 11b Ventral chaetae bifid, more than two per bundle with usually 3-9 per bundle in most species (Figure 8)..... **13**

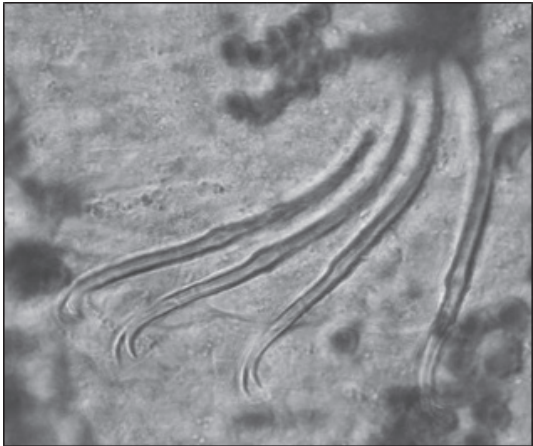


Figure 8. Multiple bifid ventral chaetae.

- 12a (11a) Prostomium modified into elongated proboscis (Figure 9)
..... **Lumbriculidae sp₁** (may be two species)

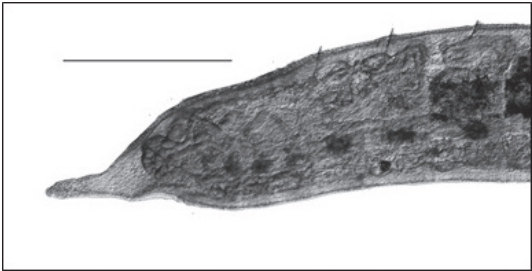


Figure 9. Lumbriculidae sp₁: lateral photo of anterior end showing prostomium with conspicuous proboscis (scale 500 μm).

- 12b Prostomium inconspicuous and without proboscis (Figure 10).....
..... **Lumbriculidae sp₂** (may be two species)

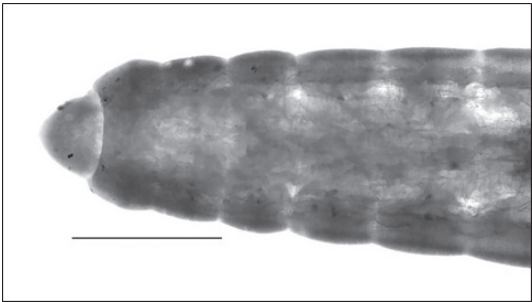


Figure 10. Lumbriculidae sp₂: photo showing inconspicuous prostomium.

- 13a (11b) Gills present on posterior end (digitiform projections; in some cases inconspicuous) **14**
13b Posterior end without gills **15**
14a (13a) Gill fossa with two long parallel accessory palps (Figure 11)
..... ***Dero (Aulophorus) furcatus***

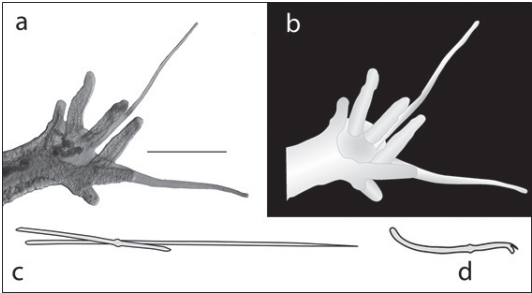


Figure 11. *Dero (Aulophorus) furcatus*: **A** photo of posterior end showing digitiform gills and elongate palps (scale 250 μm) **B** drawing of **A** **C** drawing of typical chaetae bundle **D** drawing of typical ventral chaeta.

- 14b Gill fossa not prolonged, often continuous with gills (Figure 12).....
..... *Dero (Dero) obtusa*

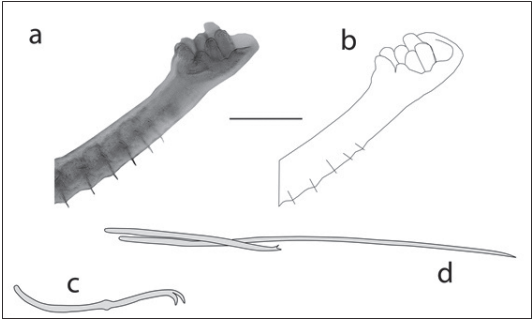


Figure 12. *Dero (Dero) obtusa*; anterior end and typical chaetae: **A** photo of posterior end showing gill fossa (scale 250 μ m) **B** outline drawing of A **C** drawing of typical dorsal chaetae bundle **D** drawing of typical ventral chaeta.

- 15a (13b) Eyes present.....16
15b Eyes absent17
16a (15a) Prostomium with elongate proboscis (Figure 13)..... *Stylaria lacustris*

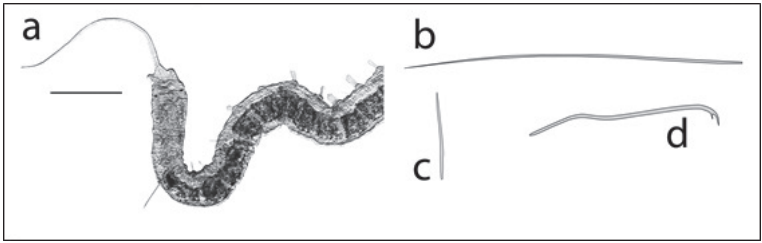


Figure 13. *Stylaria lacustris*, showing elongate prostomial proboscis, eyes, and typical chaetae: **A** photo of anterior end (scale 500 μ m) **B** drawing of dorsal "hair" **C** drawing of dorsal "needle" **D** drawing of ventral chaeta.

- 16b Prostomium protruding conspicuously over mouth, but without proboscis (Figure 14)*Nais pseudobtusa*

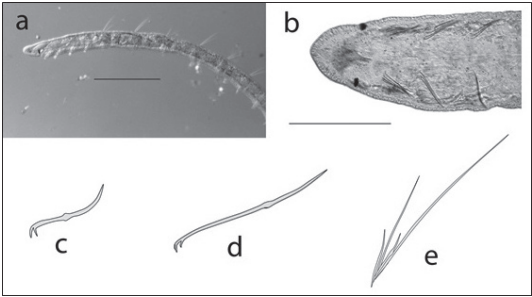


Figure 14. *Nais pseudobtusa*: **A** lateral photo of anterior end showing arrangement of chaetae, eyes, and overhanging prostomium (scale 500 μ m) **B** dorsal photo of anterior end (scale 250 μ m) **C** drawing of typical posterior-ventral chaeta **D** drawing of typical anterior-ventral chaeta **E** drawing of typical bundle of dorsal chaetae.

17a (15b)	Prostomium without proboscis.....	18
17b	Prostomium with elongate proboscis (Figure 15).....	<i>Pristina leidy</i>

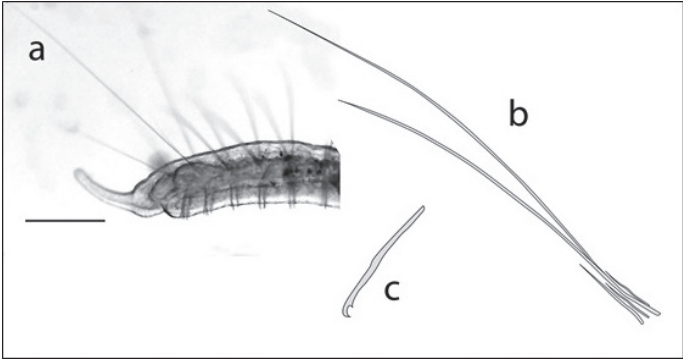


Figure 15. *Pristina* cf. *leidy*: **A** lateral photo of anterior end showing elongate proboscis (scale 200 μm) **B** drawing of typical bundle of dorsal chaetae **C** drawing of typical ventral chaeta.

18a (17a)	Green epidermal glands	<i>Aeolosoma</i> cf. <i>variegatum</i>
18b	Red epidermal glands (Figure 16)	<i>Aeolosoma</i> cf. <i>quarternarium</i>

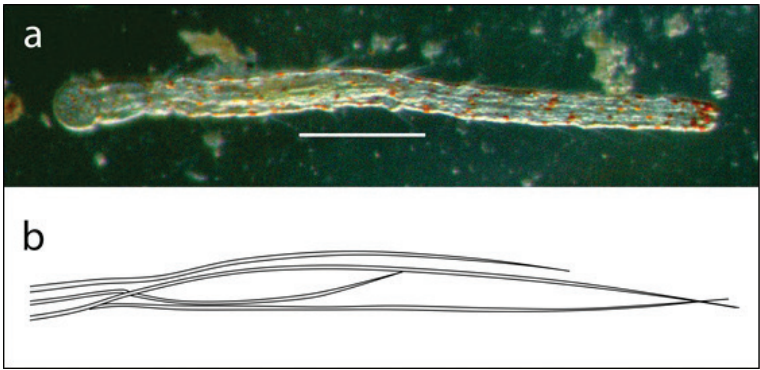


Figure 16. *Aeolosoma* cf. *quarternarium*: **A** photo of entire body showing red epidermal glands and disc-like anterior (scale 200 μm) **B** typical variable bundle of chaetae.

Discussion

The annelids of the SMR headwaters, not surprisingly, proved to be quite diverse. The majority of this diversity was contained within the family Naididae. The naidid annelids that were identified belong to globally common and widely distributed genera and species (Brinkhurst and Jamieson 1971, Martin et al. 2008, Christoffersen 2010, Pinder 2010, Park and Yeon 2013). Though some showed slight morphological differences from published descriptions, these differences did not seem great enough to conclude that they might be new species.

Haplotaxis cf. *gordiioides* (Family Haplotaxidae) was only collected from spring outflows, and the genus is known globally to be exclusively a groundwater taxon with

cryptic microdiversity (Wetzel and Taylor 2001). This collection documents a new stygobiont from the region.

The Family Lumbriculidae may be even more specious in the SMR than indicated herein, as there were four distinguishable forms of lumbriculids collected during this study. However, it could not be determined whether or not the smaller two forms were juvenile forms of the larger two forms. Only the larger two forms are reported herein. Neither of these lumbriculid species could be confidently assigned to any known genus, and it is possible that they represent undescribed endemic species. Along with the collection of two species of *Aphanoneura*, the occurrence of the lumbriculids is highly suggestive that the SMR headsprings is an ancient habitat, as the members of both of these taxa are typically collected from ancient lakes (Martin 1996). One of the lumbriculids (referred to here as Lumbriculidae sp1) was also found to contain larvae of a trichosomoid nematode, as determined by the presence of a stichosome. Therefore, this lumbriculid species is thought to be serving as the intermediate host in the life cycle of a potentially undescribed trichosomoid.

Species of *Helobdella* leeches are typically found free living on the benthic sediments hunting for small arthropods, mollusks, and oligochaetes (Kutschera et al. 2013). Interestingly, a few specimens from this group were found attached to large-mouth bass (*Micropterus salmoides* Lacépède). The method of attachment was quite bizarre. Individual leeches were connected to the ventral anterior surface of the bass with a single point of attachment, and the rest of the worm was enclosed in a mesh-like sack that dangled from the point of attachment. This finding represents an interesting note of life history for this group, as it seems they can also be facultative parasites; however, this is not the first report of *Helobdella* leeches parasitizing vertebrates (Platt et al. 1993, Tiberti and Gentili 2010, Zimić 2015) but is the first report of this genus parasitizing fish that we are aware of.

Two additional oligochaete taxa were collected but have not been included herein because only one specimen of each taxon was collected and specimens were not in suitable condition for identification. A species of leech, which was only rarely collected from turtles, was also not identified. Neither of these oligochaetes or the leech were included in our results. Throughout specimen collections, numerous different forms of soil- and vegetation-dwelling nematodes were also collected. We did not attempt to identify any of these specimens. However, the variety of forms collected suggests that free-living nematodes may be the most specious group of soft-bodied metazoans in the SMR headwaters. The study of the SMR nematode fauna would represent a great contribution to what is known of the invertebrate fauna in this habitat.

Känneby and Wicksten (2014) noted the collection of a new gastrotrich of the enigmatic genus *Redudasys* (Gastrotricha: Macrodasysida) from the SMR headsprings. Theirs is the first report of this genus from the Northern Hemisphere. We also collected gastrotrichs from the SMR, but they were identified to the genus *Chaetonotus* (Gastrotricha: Chaetonotida).

The identifications presented herein represent the first work on identifying annelids of the SMR and all of Central Texas. Therefore, we cannot speculate about how

the diversity of the annelid fauna in the SMR compares to that of other Texas rivers. Greater taxonomic resolution could be achieved through genotyping specimens and we suspect that this would likely reveal appreciable cryptic diversity. Because this is the first annelid study in Central Texas we are hopeful that this will stimulate further research and lead to genotyping and further morphological studies by other authors in the SMR and other bodies of water. Even from the perspective of our incomplete survey, there seems to be compelling evidence that there is much more diversity in the SMR headwaters yet to be described, particularly for the invertebrate fauna.

Acknowledgements

We would like to thank Mark J. Wetzel for confirming and refining our taxonomic identifications; Texas State University for providing supplies, space, and equipment; The Meadows Center for Water and the Environment for providing access to Spring Lake and boat/diving support; and the U. S. Fish & Wildlife Service for providing specimens and resources pertinent to this study through its San Marcos Aquatic Resources Center. We are deeply grateful to Helen Wukasch and Stephen Harding for spending numerous volunteer hours in the lab and field helping collect and prepare collections. We would also like to thank Lindsay Stricklan for graciously providing access to SCUBA equipment making it possible to get this project off the ground and under water. The views presented herein are those of the authors and do not necessarily represent those of the U.S. Fish and Wildlife Service or Texas Parks and Wildlife Department.

References

- Polaszek A, Alonso-Zarazaga M, Bouchet P, Brothers DJ, Evenhuis NL, Krell FT, Lyal CHC, Minelli A, Pyle RL, Robinson N, Thompson FC, van Tol J (2005) ZooBank: the open-access register for zoological taxonomy: Technical Discussion Paper. *Bulletin of Zoological Nomenclature* 62: 210–220.
- Benedict J E (1896) Preliminary descriptions of a new genus and three new species of crustaceans from an artesian well at San Marcos, Texas. *Proceedings of the United States National Museum* 18: 615–617. doi: 10.5479/si.00963801.18-1087.615
- Bishop SC (1941) Notes on salamanders with descriptions of several new forms. *Occasional Papers of the Museum of Zoology, University of Michigan* 451: 1–21.
- Bowles DE, Arsuffi TL (1993) Karst aquatic ecosystems of the Edwards Plateau region of central Texas, USA: a consideration of their importance, threats to their existence, and efforts for their conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems* 3: 317–329. doi: 10.1002/aqc.3270030406
- Bowles DE, Tiemann SG, Easley GW, Bueno-Soria J, Barba-Álvarez R, Armitage B (2007) Caddisfly (Insecta: Trichoptera) assemblages of large springs and spring-runs in central Texas, USA. *Proceedings of the twelfth international symposium on Trichoptera 2007*: 15–29.

- Bowman TE, Longley G (1976) Redescription and assignment to the new genus *Lirceolus* of the Texas troglobitic water slater, *Asellus smithii* (Ulrich) (Crustacea: Isopoda: Asellidae). *Proceedings of the Biological Society of Washington* 88: 489–496.
- Brinkhurst RO (1964) Studies on the North American aquatic Oligochaeta I: Naididae and Opistocystidae. *Proceedings of the Academy of Natural Sciences of Philadelphia* 116: 195–230.
- Brinkhurst RO, Jamieson BM (1971) *Aquatic Oligochaeta of the world*. University of Toronto Press.
- Christoffersen ML (2010) Continental biodiversity of South American oligochaetes: the importance of inventories. *Acta Zoológica Mexicana (Nueva Serie)* 26: 35–46.
- Crow CL (2012) Geochemical and hydrologic data for San Marcos Springs recharge characterization near San Marcos, Texas, November 2008 – December 2010. *US Geological Survey Data Series* 672: 1–19.
- Diaz PH (2010) Diet and mesohabitat associations of the threatened San Marcos salamander (*Eurycea nana*).
- Diaz PH, Alexander ML (2010) Aquatic macroinvertebrates of a spring-fed ecosystem in Hays County, Texas, USA. *Entomological News* 121: 478–486. doi: 10.3157/021.121.0511
- Edwards SW, Arnold CR (1961) The caddis flies of the San Marcos River. *Texas Journal of Science* 13: 398–415.
- Gibson JR, Harden SJ, Fries JN (2008) Survey and distribution of invertebrates from selected springs of the Edwards Aquifer in Comal and Hays Counties, Texas. *The Southwestern Naturalist* 49: 435–441. doi: 10.1894/0038-4909(2008)53[74:sadoif]2.0.co;2
- Harman WJ (1973) New species of Oligochaeta (Naididae) with additional distributional records from Oklahoma and Texas. *The Southwestern Naturalist* 18: 151–164. doi: 10.2307/3670416
- Hershler R, Longley G (1986) Phreatic hydrobiids (Gastropoda: Prosobranchia) from the Edwards (Balcones Fault Zone) Aquifer region, south-central Texas. *Malacologia* 27: 127–172.
- Hiltunen JK, Klemm DJ (1980) A guide to the Naididae (Annelida, Clitellata, Oligochaeta) of North America. EPA-600/4-80-03.
- Holsinger JR (1966) Subterranean amphipods of the genus *Stygonectes* (Gammaridae) from Texas. *American Midland Naturalist* 76: 100–124. doi: 10.2307/2423237
- Holsinger JR, Longley G (1980) *The subterranean amphipod crustacean fauna of an artesian well in Texas*. Smithsonian Institution Press, Washington DC.
- Hutchins BT, Tovar RU, Schwartz BF (2013) New records for stygobionts from the Edwards Aquifer of Central Texas. *Speleobiology Notes* 5: 14–18.
- Jordan DS, Gilbert CH (1886) List of fishes collected in Arkansas, Indian Territory, and Texas, in September, 1884, with notes and descriptions. *Proceedings of the United States National Museum* 9: 1–25. doi: 10.5479/si.00963801.549
- Kanneby T, Wicksten MK (2014) First record of the enigmatic genus *Redudasys* Kisielowski, 1987 (Gastrotricha: Macrotrichida) from the Northern Hemisphere. *Zoosystema* 36: 723–733. doi: 10.5252/z2014n4a2
- Kathman RD, Brinkhurst RO (1998) *Guide to the freshwater oligochaetes of North America*. Aquatic Resources Center, College Grove, 264 pp.
- Martin P (1996) Oligochaeta and Aphanoneura in ancient lakes: a review. *Hydrobiologia* 334: 63–72. doi: 10.1007/BF00017354

- Martin P, Martinez-Ansemil E, Pinder A, Timm T, Wetzel MJ (2008) Global diversity of oligochaetous clitellates (“Oligochaeta”; Clitellata) in freshwater. *Hydrobiologia* 595: 117–127. doi: 10.1007/s10750-007-9009-1
- Musgrove M, Crow CL (2012) Origin and characteristics of discharge at San Marcos Springs based on hydrologic and geochemical data (2008–10), Bexar, Comal, and Hays Counties, Texas. Scientific Investigations Report 2012–5126: 1–105.
- Ourso RT, Hornig CE (2000) Stream and Aquifer Biology of South-Central Texas-A Literature Review, 1973–97. Open File Report, U.S. Geological Survey, 99–243.
- Park HJ, Yeon JB (2013) Aquatic oligochaete (Annelida: Clitellata) fauna from the Jungnang Stream in Seoul, Korea, with eight new Korean records. *KJEE* 46: 507–512. doi: 10.11614/KSL.2013.46.4.507
- Pinder A (2010) Tools for identifying selected Australian aquatic oligochaetes (Clitellata: Annelida). *Museum Victoria Science Reports* 13: 1–26.
- Platt TR, Sever DM, Gonzalez VL (1993) First Report of the Predaceous Leech *Helobdella stagnalis* (Rhynchobdellida: Glossiphoniidae) as a Parasite of an Amphibian, *Ambystoma tigrinum* (Amphibia: Caudata). *The American Midland Naturalist* 129: 208–210. doi: 10.2307/2426450
- Schenck JR, Whiteside B (1977) Food habits and feeding behavior of the fountain darter, *Etheostoma fonticola* (Osteichthyes: Percidae). *The Southwestern Naturalist* 21: 487–492.
- Spencer DR (1978) *Pristina acuminata* Liang, a naidid oligochaete new to North America. *Transactions of the American Microscopical Society* 97: 236–239. doi: 10.2307/3225598
- Tiberti R, Gentili A (2010) First report of freshwater leech *Helobdella stagnalis* (Rhynchobdellida: Glossiphoniidae) as a parasite of an anuran amphibian. *Acta Herpetologica* 5: 255–258.
- Ulrich CJ (1902) A contribution to the subterranean fauna of Texas. *Transactions of the American Microscopical Society* 23: 83–101. doi: 10.2307/3220939
- Wetzel MJ, Taylor SJ (2001) First records of freshwater oligochaetes (Annelida, Clitellata) from caves in Illinois and Missouri, USA. *Journal of Cave and Karst Studies* 63: 99–104.
- Wetzel MJ, Kathman RD, Fend SV, Coates KA (2015) Classification and checklist of the freshwater oligochaetes occurring in North America north of Mexico. 3 February. World Wide Web URL: <http://www.inhs.uiuc.edu/~mjwetzel/FWOligoNACHkfst.html> [accessed: 18 May 2015]
- Zimić A (2015) Commensalism, Predation or Parasitism: First report of the leech *Helobdella stagnalis* Linnaeus, 1758 on yellow-bellied toad, *Bombina variegata* (Linnaeus, 1758). *Ecologica Montenegrina* 2: 62–63.

Ecology and distribution of large branchiopods (Crustacea, Branchiopoda, Anostraca, Notostraca, Laevicaudata, Spinicaudata) of the Eastern Cape Karoo, South Africa

Annah Mabidi^{1,2}, Matthew S. Bird¹, Renzo Perissinotto¹, D. Christopher Rogers³

1 DST/NRF Research Chair: Shallow Water Ecosystems, Nelson Mandela Metropolitan University, P.O. Box 77000, Port Elizabeth 6031, South Africa **2** Africa Earth Observatory Network, Nelson Mandela Metropolitan University, P.O. Box 77000, Port Elizabeth 6031, South Africa **3** Kansas Biological Survey/Biodiversity Institute, Kansas University, Higuchi Hall, 2101 Constant Avenue, Lawrence, KS 66047-3759, USA

Corresponding author: Annah Mabidi (annahanusa@gmail.com)

Academic editor: Saskia Brix | Received 13 May 2016 | Accepted 1 September 2016 | Published 19 September 2016

<http://zoobank.org/59484271-3CEF-4BC7-8E2C-1DE6A83643FD>

Citation: Mabidi A, Bird MS, Perissinotto R, Rogers DC (2016) Ecology and distribution of large branchiopods (Crustacea, Branchiopoda, Anostraca, Notostraca, Laevicaudata, Spinicaudata) of the Eastern Cape Karoo, South Africa. ZooKeys 618: 15–38. doi: 10.3897/zookeys.618.9212

Abstract

A survey of the large branchiopod fauna of the Eastern Cape Karoo region of South Africa was undertaken to provide baseline biodiversity information in light of impending shale gas development activities in the region. Twenty-two waterbodies, including nine dams and thirteen natural depression wetlands, were sampled during November 2014 and April 2015. A total of 13 species belonging to four orders were collected, comprising five anostracans, one notostracan, six spinicaudatans and one laevicaudatan. *Cyzicus australis* was most common, occurring in 46% of the waterbodies. Species co-occurred in 87% of the waterbodies, with a maximum number of six species recorded from the same waterbody. Our new distribution records for *Lynceus truncatus*, *Streptocephalus spinicaudatus* and *S. indistinctus* represent substantial expansions of the previously known ranges for these species. Tarkastad is now the westernmost record for *S. spinicaudatus*, while Jansenville now constitutes the southernmost record for *S. indistinctus*. Large branchiopod distribution data from previous Eastern Cape records were combined with our current data, demonstrating that a total of 23 large branchiopod species have been recorded from the region to date. As the Karoo is one of the few major shale basins in the world where the natural baseline is still largely intact, this survey forms a basis for future reference and surface water quality monitoring during the process of shale gas exploration/extraction.

Keywords

Depression wetlands, environmental monitoring, hydraulic fracturing, invertebrate biogeography, wetland invertebrates

Introduction

Large branchiopod crustaceans belonging to the orders Anostraca (fairy shrimps), Notostraca (tadpole shrimps), Laevicaudata (smooth clam shrimps) and Diplostraca (sub-order Spinicaudata, spiny clam shrimps) are obligatory residents of temporary waterbodies throughout the world (Day et al. 1999, Brendonck et al. 2008, Rogers 2009). Large branchiopods are adapted to these systems and survive drought phases as dormant eggs which can remain in the sediments of a dry wetland for many years (Wiggins et al. 1980, Rogers 2015a). The dormant eggs hatch during favourable environmental conditions and only a fraction of the resting stages hatch per each inundation (Brock et al. 2005, Rogers 2015a, b). This is a bet-hedging strategy aimed at ensuring long-term survival of populations (Brendonck and De Meester 2003, Schwentner and Richter 2015, Rogers 2015a, b). Most large branchiopods are filter feeders, which indiscriminately filter particles from water (Brendonck et al. 2008). However, the notostracans and a few anostracans are omnivorous and predatory as adults (Rogers 2009).

Large branchiopods are primarily restricted to rain-fed (as opposed to groundwater-fed) temporary aquatic habitats, such as ephemeral rock pools, natural depressional wetlands, roadside ditches, farm dams and pools in riverbeds that dry completely in the warm months (Brendonck et al. 2008, Rogers 2009). Ecologically, these temporary aquatic habitats are among the most extreme aquatic environments, with highly variable physico-chemical conditions that vary both during inundations and between inundation events (Meintjes et al. 1994, Williams 2006, Boven et al. 2008, Nhiwatiwa et al. 2011, Rogers 2014b). They are also among the most seriously threatened habitats globally (Semlitsch and Bodie 1998), among other factors due to their relatively small volume and shallow depth, which make them easy targets for infilling, drainage and rapid pollution (Hamer and Brendonck 1997, Williams 2006, Darwall and Brooks 2011, Collen et al. 2014).

Factors influencing large branchiopod assemblages have been studied extensively (Hamer and Appleton 1991a, b, Thiéry and Puente 2002, Timms and Sanders 2002, Boven et al. 2008, Waterkeyn et al. 2008, Rogers 2009, Padhye and Dahanukar 2015). Annual average rainfall, rainfall season and effective temperature are climatic factors that appear to influence anostracan distribution (Hamer and Brendonck 1997), while local abiotic factors such as waterbody size, number of niches, habitat duration and life history traits influence large branchiopod species richness (Thiéry 1991, Hamer and Appleton 1991b, King et al. 1996, Waterkeyn et al. 2009). Relationships between geochemical substrate properties and the distribution of anostracan species have also been reported (Rogers 2014).

Sixty-six large branchiopod species have been documented within the southern African region to date (Day et al. 1999, Rogers 2013). However, large branchiopod crusta-

ceans are still among the least known of all macroinvertebrates in temporary inland waters of the region and richness is expected to be substantially higher, given that vast areas of the southern African subcontinent remain unstudied (Brendonck et al. 2008). South Africa is no exception, as there is currently limited information on large branchiopod species distribution and their relationships with habitat factors (Hamer and Brendonck 1997, Henri et al. 2014). Information about the distribution and conservation status of large branchiopod species is available for some areas of South Africa. These include the KwaZulu-Natal lowlands (Rayner and Bowland 1985a, Hamer and Appleton 1991a), the mountainous Drakensberg region of KwaZulu-Natal (Hamer and Martens 1998), the Northern Cape (Hamer and Rayner 1996), Western Cape (De Roeck et al. 2007b, 2010), North West and Free State provinces (Henri et al. 2014), as well as the Mpumalanga Highveld region (Riato et al. 2014). Virtually nothing, however, is known about the large branchiopods living in the semi-arid Karoo basin. The known large branchiopod distribution records in this region are few and were mainly obtained from a single sampling expedition in 1996 (Day et al. 1999). This lack of information is of concern, particularly given that the region has recently been earmarked for shale gas exploration through hydraulic fracturing methods (Econometrix 2012, Geel et al. 2015, Tucker and van Tonder 2015, Murray et al. 2015). The hydraulic fracturing process uses large amounts of water and in turn produces large amounts of briny waste water, which when mismanaged may pollute both surface and groundwater systems (McBroom et al. 2012, Mauter et al. 2014). This may compound problems associated with water scarcity in this naturally arid region, which is already experiencing drier conditions as a result of climate change (Hewitson and Crane 1996, Cubasch et al. 2013). Large branchiopods, as obligatory residents of temporary waterbodies, are expected to be among the most threatened by activities associated with shale gas exploration and development through hydraulic fracturing. The inhabitants may be affected by contamination from leakage and spillage of insufficiently treated wastewater and chemicals, sedimentation due to development of additional roads, and landscape fragmentation (Warner et al. 2013, Mauter et al. 2014).

Here, we present large branchiopod diversity and distribution patterns prior to shale gas development. We present data on patterns of species assemblage composition and richness and assess these patterns in the context of environmental parameters for regional branchiopod populations. The survey is the first of its kind for the Karoo region and represents an important step towards understanding large branchiopod communities in this largely unexplored region. This information can be useful in planning and decision-making for development and to monitor changes in the temporary aquatic biota of the region in relation to future impacts.

Materials and methods

Study area

The study area occurs within the Eastern Cape Province of South Africa (Figure 1). Air temperatures in the region are notoriously variable both diurnally and seasonally.

Temperature extremes range from -5°C in winter (mean July daily minimum $< 0^{\circ}\text{C}$) to 43°C in summer (mean January daily maximum $> 30^{\circ}\text{C}$) (Schulze 1997, Mucina et al. 2006). Regional rainfall is highly unpredictable in both space and time. Sporadic rainfall events occur throughout the year, displaying elements of a perennial precipitation regime. However, long term records show that the bulk of the rainfall generally occurs in summer, peaking between December and March (Schulze et al. 1997). Mean annual precipitation ranges from 70 mm in the west to around 400 mm in the east, with a coefficient of variation of annual precipitation of 30–60% (Schulze 1997, Desmet and Cowling 1999). The hot and dry climatic conditions limit the occurrence of perennial aquatic habitats, whilst favouring the presence of temporary waterbodies.

The waterbodies of the region can be divided into three major types. First are depressional systems, which manifest as surface water on a temporary basis predominantly as isolated pools fed by direct precipitation, although some depressions may be connected to a larger drainage network (Ollis et al. 2015). Second are rivers (longitudinal features), which are generally small and temporarily inundated. Very few river systems in the region are genuinely permanent (e.g. Great Fish River). Lastly, dams (reservoirs) provide an exception to the natural surface water regime of the region in that they artificially increase the number of permanent water features in the landscape. These reservoirs are typically utilized for livestock.

Low intensity rangeland agriculture for livestock grazing is the main land-use activity in the region, although sparse amounts of irrigation agriculture and mining do occur (Mucina and Rutherford 2006). The regional geology is characterised by shallow, weakly developed lime-rich soils underlain by Ecca and Beaufort shales of the Karoo Supergroup (mostly glacial, shale and sandstone deposits, van Tonder et al. 2013). Our study area lies in the Nama Karoo biome, between 384 and 1450 m elevation and is dominated by low-shrub vegetation ($< 1\text{m}$ tall) intermixed with grasses, succulents, geophytes and annual forbs (Dean and Milton 1999, Meadows and Watkeys 1999, Mucina et al. 2006).

Sampling sites and methods

Twenty-two lentic habitats (nine dams and thirteen depressional wetlands) were sampled for large branchiopods during November 2014 (austral spring) and April 2015 (austral autumn, see Supplementary Appendix 1 for full locality information for each habitat). The geographic region covered includes the Eastern Cape Karoo area earmarked for shale gas exploration, specifically from the towns of Aberdeen and Jansenville in the west to Tarkastad and Cradock in the east (Figure 1). Site W117 (depression wetland) was only sampled in November 2014, as it was dry during the April 2015 survey. The sites were divided into three size categories according to surface area, as small ($< 499\text{ m}^2$), medium ($500\text{--}1000\text{ m}^2$) and large ($> 1000\text{ m}^2$). Large branchiopods were sampled semi-quantitatively with a D-frame sweep net (1 mm mesh size, 250 mm mouth diameter) by means of a timed collection effort standardised according to

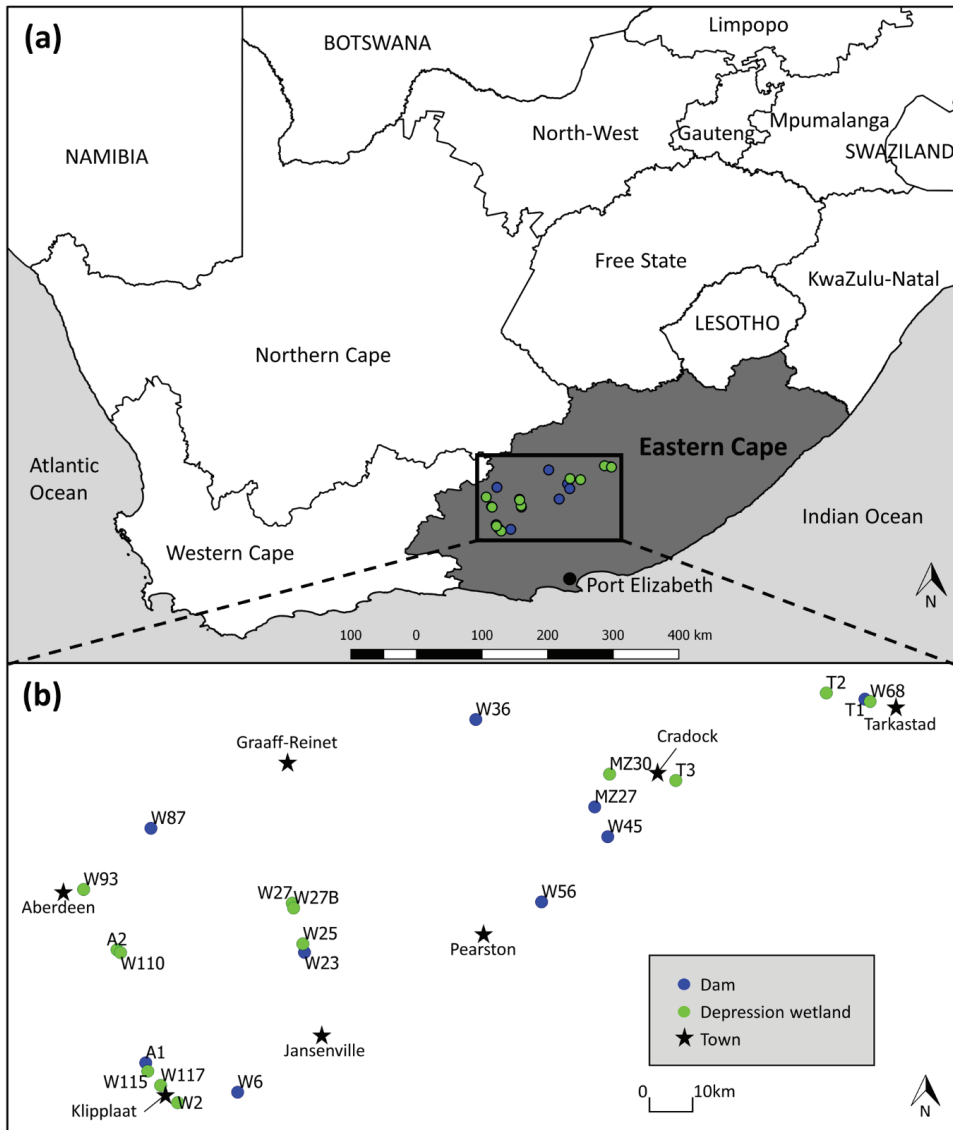


Figure 1. Location of the sites sampled for large branchiopods in the Eastern Cape Karoo region of South Africa (a), including a zoomed-in perspective of the 22 sites within the focal study area (b).

the three size categories. Small wetlands were swept for three minutes, medium-sized wetlands for six minutes and large wetlands for twelve minutes. The samples were preserved in a 10% formalin solution. Material was identified to lowest justifiable taxonomic level using keys by Day et al. (1999), the primary literature (Barnard 1924, 1929, Hamer and Appleton 1993, 1996, Hamer et al. 1994a, b) and through direct comparison with museum material.

Dissolved oxygen, pH, electrical conductivity, turbidity, temperature and salinity were measured *in situ* on both sampling occasions using a YSI 6600–V2 multi-probe system. Waterbody dimensions (surface area and maximum depth) were estimated at each site. Maximum depth was measured at the deepest point of each waterbody using a marked depth stick. The surface area was calculated using a handheld GPS device (Garmin eTrex Vista HCx, ~ 3 m point accuracy).

An integrated 2 L surface water sample was collected from the water column at each site and thoroughly mixed, before taking a 1 L sub-sample for laboratory analysis of nutrients, suspended solids and water column chlorophyll *a*. Water samples were immediately stored in the dark below 4 °C and analysed within 24 h in the laboratory. A 300 ml subsample was taken to determine total suspended solids (TSS) and particulate organic matter (POM) in the laboratory, using APHA method 2540 as described in Eaton et al. (2005). Another 300 ml subsample from the 1 L integrated sample was filtered through 0.7 µm glass-fibre filters (Whatman GF/F). The filtrand and filtrate from each sample were stored in the dark at -19 °C until further analysis within 14 days of sampling. Soluble reactive phosphorus and ammonium were determined from the filtrate spectrophotometrically, using standard spectrophotometric methods as described by Parsons et al. (1984). Total oxidised nitrogen (the sum of nitrate and nitrite) was measured using the reduced copper cadmium method as described by Bate and Heelas (1975). Three sediment core samples (3 cm³ each) were collected from each site to determine sediment chlorophyll *a*. The cores were immediately placed in 90% acetone and stored in the dark at 4°C in the field and then at -20°C within 8 hrs of collection. Sediment and water column chlorophyll *a* and phaeopigments were extracted using 90% acetone and measured using a Turner Designs (model 10-AU) fluorometer (Welschmeyer 1994), following the standard methods of Holm–Hansen and Riemann (1978).

The presence and extent of macrophyte habitat was visually assessed qualitatively. The total cover of macrophytes (emergent and submerged) in each waterbody was recorded on an ordinal scale: 0 (not present); 1 (sparse); 2 (moderate); 3 (extensive) and 4 (complete cover). The presence and extent of floating macroalgal mats was also recorded at each site on a scale of 0–4, as for the vegetation. An estimate of the degree of agricultural land use impact within 500 m of each waterbody was visually assessed using four nominal categories: 0 (none); 1 (low); 2 (moderate); and 3 (high). The presence at each site of animals, signs of grazing, dung, and trampling was noted in order to estimate the degree of impact and place a site into one of the above categories. The sampling sites were overlain on the South African lithological map in QGIS v2.2.0 software to assess the geology underlying each site.

Data analysis

Affinity between pairs of large branchiopod species was calculated from species co-occurrence data for both April and November samples using Fager's index of affinity.

This index (IF) indicates the likelihood that two species will co-occur in a species assemblage (Maeda-Martínez et al. 1997). The formula is as follows:

$$IF = \frac{2J}{(n_1 + n_2)}$$

Where J is the number of joint occurrences, n_1 is the total number of occurrences of species 1 and n_2 is the total number of occurrences of species 2. Results equal to or higher than 0.5 were considered to show affinity (Fager and McGowan, 1963, see Supplementary Appendix 2 for the original data used to perform this analysis). Species data from previous collections in the region were compiled together with the current collections to provide a distribution record for the Eastern Cape Province.

In order to investigate which environmental factors best explained branchiopod assemblage composition, we related the compositional data (presence–absence) to the various environmental variables measured using distance-based Redundancy Analysis (dbRDA, Legendre and Anderson 1999, McArdle and Anderson 2001). dbRDA is a non-parametric multivariate regression procedure based on any given dissimilarity measure, in this case the Bray–Curtis coefficient (see Supplementary Appendices 3 and 4 for the original data used to perform this analysis). Environmental predictor variables were \log_{10} transformed where appropriate, in order to achieve normality. For each separate sampling trip (November and April), assemblage composition as a multivariate response matrix was regressed separately either on individual environmental variables or on sets of environmental variables where appropriate (i.e. for sets of similar variables). Each environmental variable or variable set was regressed separately against assemblage composition expressed as the Bray–Curtis dissimilarity among sites. P values for dbRDA models were tested by 9999 permutations of residuals under the reduced model. Separate regressions were performed for each of the following variables or variable sets: wetland type (dam vs depression – categorical variables); underlying geology (Ecca shale, Beaufort Adelaide shale, Beaufort Tarkastad shale – categorical variables); spatial factors (latitude, longitude, altitude – continuous variables); in–wetland habitat structure (total vegetation cover, macroalgal cover – ordinal variables); surrounding land use impact (ordinal variable); dissolved oxygen, pH, temperature, turbidity (continuous variables); hydro–morphometry (depth, total surface area – continuous variables); nutrients (soluble reactive phosphorus, dissolved inorganic nitrogen – continuous variables); suspended material (total suspended solids, particulate organic matter – continuous variables); and chlorophyll a concentration (pelagic and benthic chlorophyll a – continuous variables).

Group average clustering was used to construct a dendrogram to depict the Bray–Curtis similarity of branchiopod assemblages among sites, sampling events and subregions/localities sampled. We then tested for a significant difference in species composition between the two sampling events and between subregions/localities of the East-

ern Cape Karoo using nonparametric permutational MANOVA (PERMANOVA, Anderson 2001). A two-way design was employed, which incorporated the factor ‘season’ (spring – November vs autumn – April) and ‘locality’ (Aberdeen, Cradock, Jansenville, Klipplaat, Tarkastad and Mountain Zebra National Park). Residuals were permuted under a reduced model (9999 permutations). We used the zero-adjusted Bray–Curtis measure of Clarke and Gorley (2006) for calculating compositional dissimilarity among sites, given that there were some joint species absences among sites in the compositional dataset. Given the small sample size to variable ratio for each of the dbRDA, multivariate regression tests provided residual degrees of freedom ranging between 7 and 12. This possible lack of power to detect effects was countered by interpreting P values < 0.10 as offering some evidence against the null hypothesis. A standard α level of 0.05 was used for the PERMANOVA test (29 degrees of freedom). Cluster analysis and dendrogram construction were performed using PRIMER v6 software (Clarke and Warwick 2001, Clarke and Gorley 2006). dbRDA and permutational MANOVA were performed using the DISTLM and PERMANOVA routines (respectively) of the PERMANOVA+ add-on package (Anderson et al. 2008) to PRIMER v6.

Results

Patterns of occurrence

The species collected at each site are listed in Table 1. Large branchiopods occurred in 15 out of the 22 waterbodies investigated (i.e. 68% of the total). Thirteen species were collected in total across the two sampling events. Seven of the thirteen species were collected on both sampling events. Regarding the anostracans, four species of *Streptocephalus* were recorded, while *Branchipodopsis* was represented by a single species (Table 1). Only one notostracan, *Triops granarius* (Lucas, 1864), was recorded from the Karoo waterbodies, being present at six of the sites. Six spinicaudatan species were also collected, three of the genus *Leptestheria* and one from each of the genera *Cyzicus*, *Eocyzicus* and *Eulimnadia*. Only one laevicaudatan, *Lynceus truncatus* Barnard, 1924, was recorded. This species was found in a single small depression wetland in the Mountain Zebra National Park near Cradock (Figure 1). *Eulimnadia* sp. and *Leptestheria inermis* Barnard, 1929 were only collected in November 2014, while the other three spinicaudatans (*E. obliquus* Sars, 1905, *L. rubidgei* Baird, 1862 and *L. striatoconcha* Barnard, 1924) and the single laevicaudatan (*L. truncatus* Barnard, 1924) were only collected in April 2015.

Fourteen waterbodies (i.e. 93% of the total 15) contained at least one anostracan species (Table 1). The anostracans and spinicaudatans were the most common, occurring across 14 and 13 of the sampled waterbodies respectively. However, three of these species were represented at only a single site (*S. spinicaudatus* Hamer & Appleton, 1993, *L. striatoconcha* and *Eulimnadia* sp.). Large branchiopod species co-occurred in

Table 1. Large branchiopod species collected from 15 waterbodies of the Eastern Cape Karoo sampled in November 2014 and April 2015. See Supplementary file 1: Appendix 1 for full locality information for each site code.

Site code	A1	A2	MZ30	T2	T3	W2	W23	W25	W27	W27B	W36	W68	W93	W110	W115
Notostraca															
<i>Triops granarius</i> Lucas, 1864	+	+					+		+	+				+	+
Anostraca															
<i>Streptocephalus spinicaudatus</i> Hamer & Appleton, 1993												+			
<i>Streptocephalus cafer</i> Lovén, 1847				+	+	+		+				+			+
<i>Streptocephalus indistinctus</i> Barnard, 1924		+							+	+	+		+		+
<i>Streptocephalus ovamboensis</i> Barnard, 1924		+	+				+		+					+	
<i>Branchipodopsis wolffi</i> Daday, 1910				+				+	+						+
Laevicaudata															
<i>Lynceus truncatus</i> Barnard, 1924			+												
Spinicaudata															
<i>Cyzicus australis</i> Loven, 1847		+	+	+		+	+	+	+	+		+		+	
<i>Eocyzicus obliquus</i> Sars, 1905				+		+	+	+						+	
<i>Leptestheria rubidgei</i> Baird, 1862	+						+			+					+
<i>Leptestheria striatochoncha</i> Barnard, 1924															+
<i>Leptestheria inermis</i> Barnard, 1929											+				
<i>Eulimnadia</i> sp.									+						
Total number of species per site	2	4	3	4	1	3	5	4	6	4	2	3	1	4	6

13 out of the 15 occupied sites (87%), with up to six species co-occurring within the same waterbody (sites W27 and W115, Table 1). An assemblage comprising *T. granarius*, *S. ovamboensis* and *C. australis* Lovén, 1847 was observed on four sites in total across both trips (Table 1) and was the most common assemblage. The Fager's index

of affinity of the different species collected is presented in Table 2. A relatively high affinity (> 0.50) with most of the species was observed for *T. granarius*. This species co-occurred most often with *L. rubidgei* (0.73), followed by *S. ovamboensis* (0.67), *S. indistinctus* (0.62) and *C. australis* (0.59), while the species had the lowest affinity with *S. cafer* (Table 2).

Current distribution records for the province

New and historical records for large branchiopods of the Eastern Cape are presented in Table 3. The spinicaudatan species *C. australis* is a widespread and common species in South Africa, and has previously been recorded in the Eastern Cape. During this study, the species was collected from 10 out of the 22 waterbodies (45%). The spinicaudatans *E. obliquus* and *L. rubidgei*, collected during this study, are both known to occur in the Eastern Cape region, having previously been recorded from Hanover, Grahamstown and Port Elizabeth (Table 3). *S. dregei* Sars, 1899, which is known to be common and widespread in the region (Hamer and Brendonck 1997), was not encountered during the current study. This was also the case with *S. gracilis* Sars, 1898, *S. dendyi* Barnard, 1929, *S. cirratus* Daday, 1908, *B. drakensbergensis* Hamer & Appleton, 1996, *B. hodgsoni* Sars, 1898, *B. scambus* Barnard, 1929, *E. dentatus* Barnard, 1929 and *L. triangularis* Daday, 1927, which have all been previously recorded in the region.

Table 2. Fager’s affinity indices between pairs of large branchiopod species in waterbodies in the Eastern Cape Karoo collected in November 2014 and April 2015.

Species	<i>T. granarius</i>	<i>S. spinicaudatus</i>	<i>S. cafer</i>	<i>S. indistinctus</i>	<i>S. ovamboensis</i>	<i>B. wolffi</i>	<i>L. truncatus</i>	<i>C. australis</i>	<i>E. obliquus</i>	<i>L. rubidgei</i>	<i>L. striatochoncha</i>	<i>L. inermis</i>	<i>Eulimnadia</i> sp.
<i>T. granarius</i>													
<i>S. spinicaudatus</i>	0												
<i>S. cafer</i>	0.15	0.29											
<i>S. indistinctus</i>	0.62	0	0.17										
<i>S. ovamboensis</i>	0.67	0	0	0.36									
<i>B. wolffi</i>	0.36	0	0.6	0.4	0.22								
<i>L. truncatus</i>	0	0	0	0	0.33	0							
<i>C. australis</i>	0.59	0.18	0.5	0.38	0.67	0.43	0.18						
<i>E. obliquus</i>	0.33	0	0.55	0	0.4	0.44	0	0.67					
<i>L. rubidgei</i>	0.73	0	0.2	0.4	0.22	0.25	0	0.29	0.22				
<i>L. striatochoncha</i>	0.25	0	0.29	0.29	0	0.4	0	0	0	0.4			
<i>L. inermis</i>	0	0	0	0.29	0	0	0	0	0	0	0		
<i>Eulimnadia</i> sp.	0.25	0	0	0.29	0.33	0.4	0	0.18	0	0	0	0	

Bold values indicate high species affinity.

Table 3. Large branchiopod distribution records for the Eastern Cape Province. Species previously recorded in the province are indicated with an asterisk. For collections made during the current study (November 2014 and April 2015), the site code is given (see Supplementary file 1: Appendix 1 for full locality information for each site code). EC = Eastern Cape.

Species	Locality & site code	Year collected	Reference
<i>Streptocephalus cafer</i> Lovén, 1847	T2; T3; W2; W25; W68; W115	2014; 2015	This study
<i>Streptocephalus cirratus</i> Daday, 1908*	Grahamstown	1998	Hamer and Martens 1998
<i>Streptocephalus dendyi</i> Barnard, 1929*	Port Elizabeth	1990	Hamer 1999
<i>Streptocephalus dregei</i> Sars, 1899*	EC	1993	Hamer 1999
<i>Streptocephalus gracilis</i> Sars, 1898*	Port Elizabeth	1898	Hamer 1999
<i>Streptocephalus indistinctus</i> Barnard, 1924	A2; W27; W27B; W36; W93; W115	2014; 2015	This study
<i>Streptocephalus ouamboensis</i> Barnard, 1924	A2; MZ30; W23; W110	2014; 2015	This study
<i>Streptocephalus spinicaudatus</i> Hamer & Appleton, 1993*	W68	2014	This study
	Indwe/Dordrecht; Glen Avis area	1993	Hamer and Martens 1998
	Dordrecht; Queenstown; Sterkstroom; Umtata Dam area	1998	Hamer 1999
	Prentjiesberg	1996	Hamer 1999
<i>Branchiopodopsis drakensbergensis</i> Hamer & Appleton, 1996*	Kenton-on-Sea; Port Elizabeth	1990	Hamer 1999
<i>Branchiopodopsis bodgoni</i> Sars, 1898*	Grahamstown	1989	Hamer 1999
<i>Branchiopodopsis scambus</i> Barnard, 1929*	T2; W27; W115	2014; 2015	This study
<i>Branchiopodopsis wolffi</i> Daday, 1910	A1; A2; MZ30; W2; W23; W25; W27; W27B; W68; W110	2014; 2015	This study
<i>Cyzicus australis</i> Lovén, 1847*	Port Elizabeth; Hanover; Queenstown; Molteno	1847	Brendonck 1999
<i>Eocyclus obliquus</i> Sars, 1905*	A2; MZ30; W23; W93	2015	This study
	Hanover	1905	Brendonck 1999
<i>Eulimnadia</i> sp.	W27	2014	This study
<i>Eulimnadia denitatus</i> Barnard, 1929*	Hanover	1929	Brendonck 1999
<i>Leptestheria inermis</i> Barnard, 1929	W36	2014	This study
<i>Leptestheria rubidgei</i> Baird, 1862*	A1; W23; W27B; W115	2015	This study
<i>Leptestheria striatochorcha</i> Barnard, 1924	Hanover; Grahamstown; Port Elizabeth	1862	Brendonck 1999
<i>Lynceus triangularis</i> Daday, 1927 *	W115	2015	This study
<i>Lynceus truncatus</i> Barnard, 1924	Port Elizabeth	1927	Brendonck 1999
<i>Triops granarius</i> Lucas, 1864*	MZ30	2015	This study
	A1; A2; W23; W27; W27B; W10; W115	2014; 2015	This study
	EC		Rayner 1999

Assemblage composition in relation to environmental factors

Habitat cover (macrophytes, macroalgae) was the only environmental predictor in the dbRDA regression models that was significantly related (albeit marginally) to large branchiopod assemblage composition across both seasons sampled (Table 4). Dissolved oxygen, turbidity, waterbody hydro-morphometry, suspended material and chlorophyll *a* all showed significant association with branchiopod assemblages in November 2014 only. The underlying geology of each waterbody, its position (spatial factors) and the electrical conductivity of its water were significant predictors of assemblage composition in April 2015 only. There was therefore little consistency in the environmental correlates of assemblage composition across the two seasons. The amounts of explained variation were moderate, with significant predictors explaining between 16.95% and 52.2% of the variation in branchiopod assemblage composition among sites (Table 4).

Compositional differences between seasons and localities

The dendrogram of Figure 2 depicts the similarity of sites in terms of assemblage composition between the two seasons sampled and between the various subregions of the Karoo. The sites do not appear to separate out according to either season or locality on the dendrogram, and there appears to be much assemblage variation even within

Table 4. Tests for relationships between the composition of large branchiopod assemblages and environmental predictor variables, either singular or in sets, using the dbRDA multivariate F-statistic. P values less than 0.10 are highlighted in bold. The column headed ‘% var’ indicates the percentage of multivariate assemblage variation (in terms of Bray-Curtis similarity) that is explained by the particular variables or sets of variables.

Variables	November 2014			April 2015		
	F	P	% var	F	P	% var
Wetland type	1.355	0.3441	13.12	1.093	0.3643	8.35
Geology	0.374	0.9074	8.56	2.014	0.0870	26.80
Spatial	0.767	0.6788	24.74	2.118	0.0608	38.85
Habitat cover	1.874	0.0992	31.91	1.928	0.0956	25.96
Land use	1.093	0.3768	10.83	0.455	0.7422	3.65
DO	4.519	0.0016	33.42	1.369	0.2758	10.24
pH	1.951	0.1492	17.81	0.058	0.9350	4.62
Temperature	0.512	0.7280	0.053	0.870	0.4813	6.76
Conductivity	1.584	0.2082	14.97	2.450	0.0647	16.95
Hydro-morphometry	1.775	0.1237	30.74	0.433	0.8488	7.29
Nutrients	1.031	0.4479	20.50	0.760	0.6299	12.14
Turbidity	4.359	0.0042	32.63	1.044	0.3999	8.01
Suspended material	4.369	0.0007	52.20	0.743	0.6692	11.91
Chlorophyll <i>a</i>	2.357	0.0389	37.07	0.778	0.6028	12.40

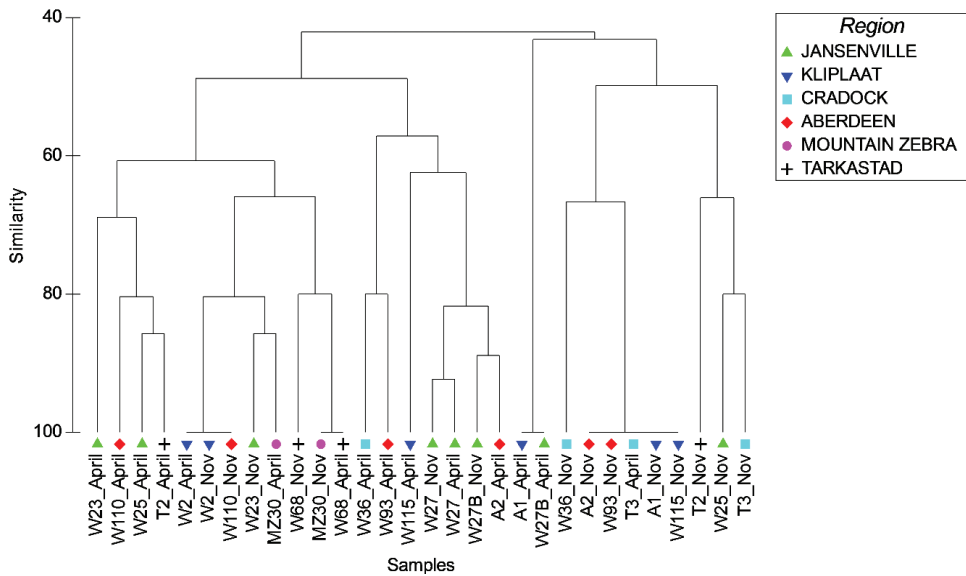


Figure 2. Dendrogram plot depicting the Bray-Curtis similarity of large branchiopod assemblages among sites sampled in the Eastern Cape Karoo. Sites are coded according to the season sampled (spring – November 2014 vs autumn – April 2015) and symbols indicate the sub region in which each site occurs, by reference to the nearest town name (with the exception of sites occurring within the Mountain Zebra National Park, coded as ‘Mountain Zebra’).

each locality/subregions. The PERMANOVA test, which tests for an overall difference in multivariate space between the group centroids of each season and each locality, showed no significant overall difference between large branchiopod assemblages sampled in spring and autumn or between the localities (season: $F_{1,18} = 2.262$, $P = 0.0807$; locality: $F_{5,18} = 1.707$, $P = 0.0632$; season \times locality: $F_{5,18} = 0.691$, $P = 0.7809$). The P values were low however ($0.05 < P < 0.10$) for both factors, suggesting some influence of these factors on assemblage composition, albeit non-significant.

Discussion

Large branchiopod species co-occurrence

Most study sites were inhabited by two or more species. Only two out of fifteen sites with large branchiopods contained a single *Streptocephalus* species each. The high incidence of co-occurrence is common in southern Africa. In KwaZulu-Natal, nine large branchiopod species co-occurred in a single pool (Hamer and Appleton, 1991), while eight species were collected in a small unvegetated pool in the Northern Cape (Hamer and Rayner 1996). Similar multispecies co-occurrences have been reported from Botswana (Brendonck and Riddoch 1997), the Namib Desert (Day 1990), Morocco

(Thiéry 1991), India (Padhye and Dahanukar 2015), USA and Mexico (Maeda–Martínez et al. 1997, Rogers 2014a, b). Large branchiopod co-occurrence has been attributed to different life history traits, availability of biotopes and abiotic factors (Thiéry 1991, Rogers 2014a, b, 2015). In South Africa, single species occurrences have been reported from the Drakensberg Mountains and the Western Cape Province (Hamer and Martens 1998, De Roeck et al. 2007). Rogers (2014b, c, 2015) demonstrated that these vagaries of distribution are directly related to species-specific geochemical tolerance ranges. Rogers' (2014b, c) statistical analyses demonstrated a strong correlation between pool substrate geochemistry and species or species assemblages, independent of temperature, hydroperiod, or other resources.

The only *Branchipodopsis* species found in our study, *B. wolffi*, always occurred together with *Streptocephalus* species. This is in contrast to the findings of Hamer and Appleton (1996), who reported that in most temporary aquatic habitats in southern Africa where *Streptocephalus* is present, *Branchipodopsis* are either absent, or are found in low numbers. There is only one known case of a multispecies *Branchipodopsis* occurrence (Barnard 1929). In that case, *B. drepane* Barnard, 1929, *B. tridens* Daday, 1910 and *B. wolffi* were all found in the same waterbody. The spinicaudatan *Eulimnadia* sp. was collected at a single location in our study (Table 1), co-occurring with other large branchiopod species.

Current distribution records for the Eastern Cape Province

Previous accounts for the Eastern Cape report 14 large branchiopod species (Hamer and Brendonck 1997). Our study increases that number to 22 species, including the first record of Laevicaudata. This diversity is high compared to other areas of southern Africa. For example, only 14 species were recorded from the Western Cape (De Roeck et al. 2007) and 16 in south-eastern Zimbabwe, although in this last case a much smaller survey area was involved (Nhiwatiwa et al. 2014). Precipitation seasonality is less pronounced in the Eastern Cape than in other parts of South Africa. Most Eastern Cape areas have spring and autumn annual rainfall maxima (Stone 1988), whereas other parts of the country receive only summer or winter rainfall maxima (Mucina and Rutherford 2006). Despite this survey having been conducted during the spring and autumn annual rainfall peaks, only 13 species were collected. The nine species that were previously recorded in the province but not encountered in our study were collected mostly in the coastal areas, or outside the Karoo region. They are therefore not likely to occur in the survey area. However, the limited number of sampling trips may also have restricted our encounter probability and thus it should be emphasised that more frequent visits both intra- and inter-annually would likely reveal a more accurate picture of total diversity in the region. Furthermore, some anostracan species previously reported from the Eastern Cape may in fact have become extinct, given that they are only known from type material collected over 100 years ago (Hamer and Appleton 1996).

The anostracan *Streptocephalus cafer*, collected in 38% of the waterbodies (Table 1), is a widespread and common species in South Africa and has previously been recorded in the Eastern Cape and throughout the Karoo, while *S. ovamboensis* is common in the arid southwest Karoo, extending north-eastwards to Groblerhoop in the Northern Cape, where rainfall is less than 300 mm per year. However, its distribution is known to vary, occurring also further east and south where rainfall is slightly higher (Hamer and Brendonck 1997; Hamer 1999). Six spinicaudatans were collected during this study, including *Eulimnadia* sp., which prior to this study had not been recorded in the Eastern Cape region. The genus is fairly widespread in South Africa, with distribution records for Heidelberg (Gauteng Province), Kimberley (Northern Cape), Greater Namaqualand (Northern Cape), Ovamboland and Kaokoveld (Namibia) (Brendonck 1999).

New distribution records for the Eastern Cape Province

S. spinicaudatus is a common species in the high altitude northern parts of the Eastern Cape (Dordrecht, Queenstown and Sterkstroom areas), where annual rainfall is higher than in the Karoo basin and peaks strongly during the summer months (Hamer 1999). Thus, our Tarkastad collection represents the westernmost distribution range for the species. Hamer and Brendonck (1997) reported that *S. indistinctus* distribution appeared to be restricted to areas where average rainfall is > 500 mm. Thus, the species was thought to be largely excluded from the Karoo where the less than 300 mm annual rainfall is unpredictable (Hamer 1999). This species has otherwise been recorded north of 29°S in Mpumalanga and the Limpopo provinces (Hamer 1999), with only one record outside this area (Brehm 1958). Our record is the second outside its typical distribution and indicates that this species may have a wider distribution than previously believed. *B. wolffi* is widespread in South Africa, with distribution records from the Northern Cape, Mpumalanga and KwaZulu-Natal provinces (Hamer 1999), although prior to this study the species had not been reported from the Eastern Cape. However, this is a variable taxon that may well represent several species. Thus, there is need for molecular investigations to resolve its true status.

The *Eulimnadia* sp. record needs further analysis to determine which species occurs here. Our specimen did not have any eggs and *Eulimnadia* is only identifiable to species based on egg morphology (Rogers et al. 2012). The genus is widespread globally (Rogers et al. 2012) and in Africa (Rabet et al. 2015), but distribution data for the Eastern Cape Province prior to this study have been deficient, with records only for Heidelberg (Gauteng), Kimberley and Greater Namaqualand (Northern Cape), as well as Ovamboland and Kaokoveld (Namibia) (Brendonck 1999). *Leptestheria striatoconcha* had previously only been recorded in Heidelberg (Gauteng) and Ovamboland (Namibia). *L. inermis* had previously been recorded only between Upington and Keimoes in the Northern Cape (Brendonck 1999). Our collection extends its distribution to the Eastern Cape region, which consequently becomes the easternmost distribution range for both *Leptestheria* species. There was no Eastern Cape record of Laevicaudata

prior to this study. *Lynceus truncatus* was only previously recorded in KwaZulu-Natal and Ovamboland (Barnard 1929, Rayner and Bowland 1985). We collected this species from a depressional wetland in the Mountain Zebra National Park (site MZ30).

Environmental factors affecting assemblage composition

There was little consistency in the environmental correlates of assemblage composition across the two seasons. Habitat cover was the only variable that was significantly associated with branchiopod distribution during both sampled periods, and even these relationships were marginal ($P \approx 0.9$). Thus, our data does not indicate any convincing or consistent environmental correlates of large branchiopod species composition in Eastern Cape Karoo waterbodies. This follows the prevailing sentiment of other authors, such as Rogers (2014b, 2015), who have argued that water quality data are generally of limited use in species distribution patterns, because in temporary wetlands physico-chemical parameters can fluctuate dramatically over a range of time scales. Furthermore, adaptations to highly fluctuating physico-chemical environments of arid-zone temporary wetlands may well allow some large branchiopod species to be habitat generalists, their distributions not being highly affected by local physico-chemistry. For instance, species such as *Triops granarius*, which was present at seven of our sites, is known to tolerate wide temperature and dissolved oxygen ranges. Although having some preference for warmer, muddier waters, this species appears to be a habitat generalist and can be found associated with a wide range of physico-chemical conditions (Barnard 1929, Meintjes et al. 1994).

Geology was significantly associated with species distribution during the April sampling trip. Geology underlying waterbodies affects their geochemistry and in this regard Rogers (2014b, c) found significant relationships between geochemical variables such as percent gypsum and calcium carbonate, salinity, cation type, and substrate type with anostracan species distribution across different bioregions in North America and Australia. Tuytens et al. (2015) did not find any strong effect of soil geology on the composition of anostracan and notostracan assemblages in pools in a tropical savannah habitat of south-east Zimbabwe. However, Tuytens et al. (2015) looked at a far smaller area and far fewer species than Rogers (2014b, c). During our study, large branchiopods were absent from six of the nine dams surveyed. This absence could be attributed to these systems not being sufficiently ephemeral (in fact some of the dams were semi-permanent), or perhaps they were not sampled when the active stages were present (the substrate was not sampled to determine if an egg bank was present). Large branchiopods are known for 'bet hedging' strategies, whereby egg banks do not necessarily hatch out on every inundation and thus could be absent from the surface water during a given sampling event, but remain in the substrate as eggs (Brendonck and De Meester 2003, Schwentner and Richter 2015, Rogers 2015a, b). Furthermore, they are known to suffer stochastic extinctions, or may not colonise successfully if the habitat is not suitable due to geochemistry, hydroperiod, natural or anthropogenic pollution, or a range of other factors (Rogers 2015).

Conclusions

Agricultural and mining activities pose a major threat to temporary wetlands in South Africa (Hamer and Brendonck 1997, Henri et al. 2014). In our study, only one depression wetland was found in the protected Mountain Zebra National Park, whilst the other waterbodies remain at risk to potential future developments. Hydraulic fracturing is known to produce large amounts of brine wastewater, which if mismanaged may contaminate surrounding surface water. High salinities are a limiting factor for propagule hatching and survival in temporary wetlands (Cancela da Fonseca et al. 2008, Waterkeyn et al. 2008). Thus, salinisation has the potential to destroy egg banks. Additionally, climate change is a potential risk to wetlands, particularly in areas where the frequency and extent of droughts is predicted to increase (Hewitson and Crane 1996, Cubasch et al. 2013). Temporary aquatic habitats are among the most threatened globally, given that their relatively small volume and shallow depth make them extremely vulnerable to pollution, drainage and infilling (Hamer and Brendonck 1997, Williams 2006, Darwall and Brooks 2011, Collen et al. 2014). Therefore, baseline data on the distribution and ecology of keystone temporary wetland species (e.g. large branchiopods) is required in lesser studied areas, such as the Eastern Cape Karoo. It is hoped that the large branchiopod data presented in this study will contribute towards a broader biodiversity database that can be used to help inform future sustainable development in the region.

Acknowledgements

A Mabidi was funded by a PhD grant from the South African Research Chairs Initiative, administered by the National Research Foundation (NRF), as well as a grant from the Africa Earth Observatory Network (AEON) at Nelson Mandela Metropolitan University (NMMU). M Bird was funded by a DST/NRF Scarce-Skills Postdoctoral Fellowship. We thank the staff and management of Mountain Zebra National Park for their logistical support and cooperation. We are grateful to the Eastern Cape Department of Economic Affairs, Environmental Affairs and Tourism (Cacadu Region) and AGRI Eastern Cape for granting permits and supporting this research project. In particular, we thank the various farmers involved for granting permission to work on their property. Special thanks go to Bradley Ah Yui, Daniel Lemley, Jacqueline Raw, Liza Rishworth, Lynette Clennell, Natasha Roussouw, Nicholas Galuszynski, Nuette Gordon, Shayla Tricam and Sourav Paul for their assistance during collection and analysis of samples. We also thank Michelle Hamer, Martin Schwentner and another anonymous reviewer for contributing valuable comments towards the improvement of the manuscript. Any findings and conclusion expressed in this material is that of the author(s) and the NRF does not accept any liability in this regard.

References

- Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26: 32–46.
- Anderson MJ, Gorley RN, Clarke KR (2008) PERMANOVA+ for PRIMER: Guide to software and statistical methods. PRIMER-E, Plymouth Marine Laboratory, Plymouth, UK.
- Barnard KH (1929) Contributions to the crustacean fauna of South Africa: A revision of the South African Branchiopoda (Phyllopoda). *Annals of the South African Museum* 29(10): 181–272.
- Barnard KH (1929) Contributions to the Crustacean fauna of South Africa 10: A revision of the South African Phyllopoda. *Annals of the South African Museum* 25: 181–272.
- Barnard KH (1924) Contributions to a knowledge of the fauna of South-West Africa II: Crustacea Entomostraca, Phyllopoda. *Annals of the South African Museum* 20: 213–227.
- Bate GC, Heelas BV (1975) Studies on the nitrate nutrition of two indigenous Rhodesian grasses. *Journal of Applied Ecology* 12(3): 941–952. doi: 10.2307/2402100
- Boven L, Stoks R, Forro L, Brendonck L (2008) Seasonal dynamics in water quality and vegetation cover in temporary pools with variable hydroperiods in Kiskunsag (Hungary). *Wetlands* 28: 401–410. doi: 10.1672/06-174.1
- Brehm V (1958) Crustacea. Phyllopoda und Copepoda Calanoida. In: Hanström B (Ed.) *South African animal life, results of the Lund University expedition, 1950–1951*. Almqvist & Wiksell, Uppsala, 10–39.
- Brendonck L (1999) Conchostraca. In: Day JA, Stewart BA, de Moor IJ, Louw AE (Eds) *Freshwater Invertebrates of Southern Africa. Crustacea I: Notostraca, Anostraca, Conchostraca and Cladocera*. WRC Report no. TT 121/00, Water Research Commission, Pretoria, South Africa, 59–80.
- Brendonck L, De Meester L (2003) Egg banks in freshwater zooplankton: evolutionary and ecological archives in the sediment. *Hydrobiologia* 491: 65–84. doi: 10.1023/A:1024454905119
- Brendonck L, Riddoch B (1997) Anostracans (Branchiopoda) of Botswana: morphology, distribution, diversity, and endemism. *Journal of Crustacean Biology* 17: 111–134. doi: 10.2307/1549468
- Brendonck L, Rogers DC, Olesen J, Weeks SRH (2008) Global diversity of large branchiopods (Crustacea: Branchiopoda) in fresh water. *Hydrobiologia* 595: 167–176. doi: 10.1007/s10750-007-9119-9
- Brendonck L, Williams WD (2000) Biodiversity in wetlands of dry regions (drylands). In: Gopal B, Junk WJ, Davis JA (Eds) *Biodiversity in Wetlands: Assessment, Function and Conservation*. Backhuys Publishers, Leiden, 181–194.
- Brock MA, Nielsen DL, Crossle K (2005) Changes in biotic communities developing from freshwater wetland sediments under experimental salinity and water regimes. *Freshwater Biology* 50(8): 1376–1390. doi: 10.1111/j.1365-2427.2005.01408.x
- Cancela da Fonseca L, Cristo M, Machado M, Sala J, Reis J, Alcazar R, Beja P (2008) Mediterranean temporary ponds in Southern Portugal: key faunal groups as management tools? *Pan-American Journal of Aquatic Sciences* 3(3): 304–320.
- Collen B, Whittton F, Dyer EE, Baillie JEM, Cumberlidge N, Darwall WRT, Pollock C, Richman NI, Soulsby A-M, Böhm M (2014) Global patterns of freshwater species diversity, threat and endemism. *Global Ecology and Biogeography* 23(1): 40–51. doi: 10.1111/geb.12096

- Clarke KR, Gorley RNP (2006) RIMER v6: User Manual/Tutorial. PRIMER-E, Plymouth Marine Laboratory, Plymouth.
- Clarke KR, Warwick RM (2001) Change in Marine Communities: An Approach to Statistical Analysis and Interpretation (2nd edn). PRIMER-E, Plymouth Marine Laboratory, Plymouth.
- Cubasch U, Wuebbles D, Chen D, Facchini MC, Frame D, Mahowald NWJG (2013) Introduction. In: Stocker TF, Qin DP, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM (Eds) Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, 119–158.
- Darwall W, Smith K, Allen D, Holland R, Harrison I, Brooks E (2011) The diversity of life in African freshwaters: underwater, under threat. IUCN, Cambridge and Gland.
- Day JA (1990) Environmental correlates of aquatic faunal distribution in the Namib Desert. In: Seely MK (Ed.) Namib ecology: 25 years of Namib research. Monograph no. 7. Transvaal Museum, Pretoria, 99–107.
- Day JA, Stewart BA, de Moor IJ, Louw AE (1999) Guides to the Freshwater Invertebrates of Southern Africa: Crustacea I -Notostraca, Anostraca, Conchostraca and Cladocera. WRC Report no. TT 121/00, Water Research Commission, Pretoria, South Africa, 126 pp.
- De Roeck ER, Vanschoenwinkel BJ, Day JA, Xu Y, Rait L, Brendonck L (2007a) Conservation status of large branchiopods in the Western Cape, South Africa. *Wetlands* 27(1): 162–173. doi: 10.1672/0277-5212(2007)27[162:CSOLBI]2.0.CO;2
- De Roeck ER, Vanschoenwinkel BJ, Day JA, Xu Y, Raitt L, Brendonck L (2007b) Conservation status of large branchiopods in the Western Cape, South Africa. *Wetlands* 27(1): 162–173. doi: 10.1672/0277-5212(2007)27[162:CSOLBI]2.0.CO;2
- De Roeck ER, Waterkeyn A, Brendonck L (2010) Life-history traits of *Streptocephalus purcelli* Sars, 1898 (Branchiopoda, Anostraca) from temporary waters with different phenology. *Water SA* 36(3): 323–328.
- Dean WRJ, Milton SJ (1999) Animal foraging and food. In: Dean WRJ, Milton SJ (Eds) The Karoo. Ecological patterns and processes. Cambridge University Press, Cambridge, 164–177. doi: 10.1017/CBO9780511541988.014
- Eaton AD, Clesceri LS, Rice EW, Greenberg AE (2005) Standard methods for the examination of water and wastewater. 21st edition, American Public Health Association, Washington DC.
- Econometrix (2012) Karoo shale gas report—Special report on economic considerations surrounding potential shale gas resources in the southern Karoo of South Africa. Econometrix (Pty), Johannesburg, 74 pp.
- Fager EW, McGowan JA (1963) Zooplankton species groups in the north Pacific. *Science* 140: 453–61. doi: 10.1126/science.140.3566.453
- Geel C, De Wit M, Booth MP, Schulz HM, Horsefield B (2015) Paleo-Environment, Diagenesis and Characteristics of Permian Black Shales in the Lower Karoo Supergroup flanking the Cape Fold Belt near Jansenville, Eastern Cape, South Africa: Implications for the shale gas potential of the Karoo Basin. *South African Journal of Geology* 118(3): 249–274. doi: 10.2113/gssajg.118.3.249
- Hamer M (1999) Anostraca. In: Day JA, Stewart BA, de Moor IJ, Louw AE (Eds) Guides to the Freshwater Invertebrates of Southern Africa: Crustacea I – Notostraca, Anostraca, Conchostraca and Cladocera. WRC Report no. TT 121/00, Water Research Commission, Pretoria, 14–58.

- Hamer ML, Martens K (1998) The large Branchiopoda (Crustacea) from temporary habitats of the Drakensberg region, South Africa. *Hydrobiologia* 384: 151–165. doi: 10.1023/A:1003251428494
- Hamer ML, Brendonck L (1997) Distribution, diversity and conservation of Anostraca (Crustacea: Branchiopoda) in southern Africa. In: Simovich M, Sassaman C, Belk D (Eds) *Studies on Large Branchiopod Biology and Conservation*. Springer, 1–12.
- Hamer M, Rayner NA (1996) A note on the unusual crustacean community of a temporary pool in the northern Cape. *South African Journal of Aquatic Science* 22(1): 100–104. doi: 10.1080/10183469.1996.9631376
- Hamer ML, Appleton CC (1996) The genus *Branchipodopsis* (Crustacea, Branchiopoda, Anostraca) in southern Africa. Morphology, distribution, relationships and the description of five new species. *Annals of The South African Museum* 104: 311–377.
- Hamer M, Appleton CC (1993) Four new *Streptocephalus* (Crustacea, Branchiopoda, Anostraca) species from south-east Africa. *Annals of the South African Museum* 103: 167–181.
- Hamer M, Brendonck L, Coomans A, Appleton CC (1994a) A review of the African Streptocephalidae (Crustacea: Branchiopoda: Anostraca) Part 1: south of Zambezi and Kunene rivers. *Archiv für Hydrobiologie* 99(suppl.): 235–277.
- Hamer M, Brendonck L, Coomans A, Appleton CC (1994b) A review of the African Streptocephalidae (Crustacea: Branchiopoda: Anostraca) Part 2: north of Zambezi and Kunene rivers. *Archiv für Hydrobiologie* 99(suppl.): 279–311.
- Hamer ML, Appleton CC (1991a) Physical and chemical characteristics and phyllopod fauna of temporary pools in northeastern Natal, Republic of South Africa. *Hydrobiologia* 212: 95–104. doi: 10.1007/BF00025992
- Hamer ML, Appleton CC (1991b) Life history adaptations of phyllopods in response to predators, vegetation, and habitat duration in north-eastern Natal. *Hydrobiologia* 212: 105–116. doi: 10.1007/BF00025993
- Henri AJ, Wepener V, Ferreira M, Malherbe W, van Vuren JHJ (2014) The effect of acid mine drainage on the hatching success of branchiopod egg banks from endorheic wetlands in South Africa. *Hydrobiologia* 738: 35–48. doi: 10.1007/s10750-014-1913-6
- Hewitson BC, Crane RG (1996) Climate downscaling: Techniques and application. *Climate Research* 7: 85–95. doi: 10.3354/cr007085
- Holm-Hansen O, Riemann B (1978) Chlorophyll a determination: improvements in methodology. *Oikos*, 438–447. doi: 10.2307/3543338
- King JL, Sirnovich MA, Brusca RC (1996) Species richness, endemism and ecology of crustacean assemblages in northern California vernal pools. *Hydrobiologia* 328: 85–116. doi: 10.1007/BF00018707
- Legendre P, Anderson MJ (1999) Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. *Ecological Monographs* 69: 1–24. doi: 10.1890/0012-9615(1999)069[0001:DBRATM]2.0.CO;2
- Maeda-Martínez MA, Belk D, Barboza OH, Dumont HJ (1997) Large branchiopod assemblages common to Mexico and the United States. *Hydrobiologia* 359: 45–62. doi: 10.1023/A:1003102601542

- Mauter MS, Alvarez PJ, Burton A, Cafaro DC, Chen W, Gregory KB, Jiang G, Li Q, Pittock J, Reible D et al. (2014) Regional variation in water-related impacts of shale gas development and implications for emerging international plays. *Environmental Science & Technology* 48(15): 8298–306. doi: 10.1021/es405432k
- McCordle BH, Anderson MJ (2001) Fitting multivariate models to community data: a comment on distance-based redundancy analysis. *Ecology* 82(1): 290–297. doi: 10.1890/0012-9658(2001)082[0290:FMMTCD]2.0.CO;2
- McBroom M, Thomas T, Zhang YL (2012) Soil Erosion and Surface Water Quality Impacts of Natural Gas Development in East Texas, USA. *Water* 4(4): 944–958. doi: 10.3390/w4040944
- Meadows ME, Watkeys JK (1999) Paleoenvironments. In: Dean WRJ, Milton SJ (Eds) *The Karoo. Ecological patterns and processes*. Cambridge University Press, Cambridge, 27–41. doi: 10.1017/CBO9780511541988.006
- Meintjes S, Seaman MT, Kok DJ (1994) Duration of inundation and change in physical and chemical characteristics of small temporary pans in South Africa. *Hydrobiologia* 281: 79–90. doi: 10.1007/BF00006437
- Mucina L, Rutherford MC, Milton SJ, Scott L, Lloyd WJ, van de Merwe B, Hoare DB, Bezuidenhout H, Vlok JHK, Euston-Brown DIW et al. (2006) Nama-Karoo Biome. In: Mucina L, Rutherford MC (Eds) *The Vegetation of South Africa, Lesotho and Swaziland*. Strelitzia 19. South African National Biodiversity Institute, Pretoria, 324–347.
- Mucina L, Rutherford MCE (2006) *The Vegetation of South Africa, Lesotho and Swaziland*. Strelitzia 19. South African National Biodiversity Institute, Pretoria, 807 pp.
- Murray R, Swana K, Miller J, Talma S, Tredoux G, Vengosh A, Darrah T (2015) *The Use of Chemistry, Isotopes and Gases as Indicators of Deeper Circulating Groundwater in the Main Karoo Basin*. WRC Report No. 2254/1/15, Water Research Commission, Pretoria, 215 pp.
- Nhiwatiwa T, Waterkeyn A, Riddoch BJ, Brendonck L (2014) A hotspot of large branchiopod diversity in south-eastern Zimbabwe. *African Journal of Aquatic Science* 39(1): 57–65. doi: 10.2989/16085914.2014.892869
- Nhiwatiwa T, Brendonck L, Waterkyn A, Vanschoenwinkel B (2011) The importance of habitat and landscape properties in explaining instantaneous and long-term distributions of large branchiopods in subtropical temporary pans. *Freshwater Biology* 56: 1992–2008. doi: 10.1111/j.1365-2427.2011.02630.x
- Ollis D, Ewart-Smith J, Day J, Job N, Macfarlane D, Snaddon C, Sieben E, Dini J, Mbona N (2015) The development of a classification system for inland aquatic ecosystems in South Africa. *Water SA* 41(5): 727–745.
- Padhye SM, Dahanukar N (2015) Distribution and assemblages of large branchiopods (Crustacea: Branchiopoda) of northern Western Ghats, India. *Journal of Limnology* 74(2): 371–380. doi: 10.4081/jlimnol.2015.1115
- Parsons TR, Maita Y, Lalli CM (1984) *A Manual of Chemical and Biological Methods for Seawater Analysis*. Pergamon Press, New York, 173 pp.
- Pennak RW (1978) *Freshwater invertebrates of the United States*. John Wiley and Sons, New York, 326–346.

- Rabet N, Clarac F, Lluch P, Gallerne E, Korn M (2015) Review of the *Eulimnadia* (Branchiopoda: Spinicaudata) from north Africa and adjacent regions, with two new species from Mauritania. *Journal of Crustacean Biology* 35: 461–472. doi: 10.1163/1937240X-00002340
- Rayner NA, Bowland AE (1985a) A note on *Triops granarius* Lucas, *Lynceus truncatus* Barnard and *Streptocephalus cafer* Loven from Umfolozi Game Reserve, South Africa. *Journal of the Limnological Society of Southern Africa* 11: 11–13. doi: 10.1080/03779688.1985.9633103
- Rayner NA, Bowland AE (1985b) Notes on the taxonomy and ecology of *Triops granarius* (Lucas) (Notostraca: Crustacea) in South Africa. *South African Journal of Science* 81: 500–505.
- Riato L, Van Ginkel C, Taylor JC (2014) Zooplankton and diatoms of temporary and permanent freshwater pans in the Mpumalanga Highveld region, South Africa. *African Zoology* 49(1): 113–127. doi: 10.3377/004.049.0113
- Rogers DC (2009) Branchiopoda (Anostraca, Notostraca, Laevicaudata, Spinicaudata, Cyclotherida). In: Likens GF (Ed.) *Encyclopedia of Inland Waters*. Elsevier, Boston, 242–249. doi: 10.1016/B978-012370626-3.00157-5
- Rogers DC, Rabet N, Weeks SC (2012) A revision of the extant genera of the clam shrimp family Limnadiidae. *Journal of Crustacean Biology* 32: 827–842. doi: 10.1163/193724012X637212
- Rogers DC (2013) Anostraca Catalogus (Crustacea: Branchiopoda). *Raffles Bulletin of Zoology* 61: 525–546.
- Rogers DC (2014a) Anostracan (Crustacea: Branchiopoda) Biogeography I. North American Bioregions. *Zootaxa* 3838: 251–275. doi: 10.11646/zootaxa.3838.3.1
- Rogers DC (2014b) Anostracan (Crustacea: Branchiopoda) Zoogeography II. Relating distribution to geochemical substrate properties in the USA. *Zootaxa* 3856(1): 1–49. doi: 10.11646/zootaxa.3856.1.1
- Rogers DC (2014c) Anostracan (Crustacea: Branchiopoda) Biogeography III. Australian Bioregions. *Zootaxa* 3881: 453–487. doi: 10.11646/zootaxa.3881.5.3
- Rogers DC (2015a) Hatching response to temperature along a latitudinal gradient by the fairy shrimp *Branchinecta lindahli* (Crustacea: Branchiopoda: Anostraca) in culture conditions. *Journal of Limnology* 74: 85–94.
- Rogers DC (2015b) A conceptual model for anostracan biogeography. *Journal of Crustacean Biology* 35: 686–699. doi: 10.1163/1937240X-00002369
- Schwentner M, Richter S (2015) Stochastic effects associated with resting egg banks lead to genetically differentiated active populations in large branchiopods from temporary water bodies. *Hydrobiologia* 760: 239–253. doi: 10.1007/s10750-015-2382-2
- Seaman MT, Kok DJ, Meintjes S (1995) The description and preliminary prediction of the inundation pattern in a temporary habitat of Anostraca, Notostraca and Conchostraca in South Africa. *Hydrobiologia* 298: 93–104. doi: 10.1007/BF00033804
- Seaman MT, Kok DJ, Von Schlichting BJ, Kruger AJ (1991) Natural growth and reproduction in *Triops granarius* (Lucas) (Crustacea: Notostraca). *Hydrobiologia* 212: 87–94. doi: 10.1007/BF00025991

- Semlitsch RD, Bodie JR (1998) Are small, isolated wetlands expendable? *Conservation Biology* 12: 1129–1133. doi: 10.1046/j.1523-1739.1998.98166.x
- StatSoft Incoporation (2015) STATISTICA (data analysis software system) version 12. <http://www.statsoft.com/>
- Thiéry A (1991) Multispecies coexistence of branchiopods (Anostraca, Notostraca and Spinicaudata) in temporary ponds of Chaouia plain (western Morocco): sympatry or syntopy between usually allopatric species. *Hydrobiologia* 212: 117–136. doi: 10.1007/978-94-011-3366-1_15
- Thiéry A, Puente L (2002) Crustacean assemblage and environmental characteristics of a man-made solar salt work in southern France, with emphasis on anostracan (Branchiopoda) population dynamics. *Hydrobiologia* 48: 191–200. doi: 10.1023/A:1021354903052
- Timms BV, Sanders PR (2002) Biogeography and ecology of Anostraca (Crustacea) in middle Paroo catchment of the Australian arid-zone. *Hydrobiologia* 486: 225–238. doi: 10.1023/A:1021363104870
- Tucker AR, van Tonder G (2015) The Karoo Fracking Debate: A Christian Contribution to the World Communities of Faith. *Science and Engineering Ethics* 21(3): 631–653. doi: 10.1007/s11948-014-9563-7
- Tuytens K, Vanschoenwinkel B, Clegg B, Nhiwatiwa T, Brendonck L (2015) Exploring links between geology, hydroperiod, and diversity and distribution patterns of anostracans and notostracans (branchiopoda) in a tropical savannah habitat in se Zimbabwe. *Journal of Crustacean Biology* 35(3): 309–318. doi: 10.1163/1937240X-00002319
- van Tonder GJ, de Lange F, Steyl G, Vermeulen D (2013) Potential impacts of fracking on groundwater in the Karoo Basin of South Africa. *Proceedings of the 13th Biennial Conference of the Groundwater Division, Durban (South Africa), September 2013. The Geological Society of South Africa, Cape Town, 1–10.*
- Warner NR, Christie CA, Jackson RB, Vengosh A (2013) Impacts of shale gas wastewater disposal on water quality in western Pennsylvania. *Environmental Science & Technology* 47(20): 11849–57. doi: 10.1021/es402165b
- Waterkeyn A, Grillas P, De Roeck ERM, Boven L, Brendonck L (2009) Assemblage structure and dynamics of large branchiopods in Mediterranean temporary wetlands: patterns and processes. *Freshwater Biology* 54: 1256–1270. doi: 10.1111/j.1365-2427.2009.02174.x
- Waterkeyn A, Grillas P, Vanschoenwinkel B, Brendonck L (2008) Invertebrate community patterns in Mediterranean temporary wetlands along hydroperiod and salinity gradients. *Freshwater Biology* 53: 1808–1822. doi: 10.1111/j.1365-2427.2008.02005.x
- Welschmeyer NA (1994) Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. *Limnology and Oceanography* 39(8): 1985–1992. doi: 10.4319/lo.1994.39.8.1985
- Wiggins GB, Mackay RJ, Smith IM (1980) Evolutionary and ecological strategies of animals in annual temporary pools. *Archiv fur Hydrobiologie Supplement* 58(1/2): 97–206.
- Williams DD (2006) *The biology of temporary waters*. Oxford University Press, Oxford, 347 pp.
- Yee SH, Willig MR, Moorhead DL (2005) Tadpole shrimp structure macro invertebrate communities in playa lake microcosms. *Hydrobiologia* 541: 139–148. doi: 10.1007/s10750-004-5033-6

Supplementary material I

Appendix 1–4

Authors: Annah Mabidi, Matthew S. Bird, Renzo Perissinotto, D. Christopher Rogers
Data type: species data

Explanation note: **Appendix 1.** Geographic position and underlying geology of the of 22 study sites. **Appendix 2.** Large branchiopod species found in November 2014 and April 2015 in the 15 study sites included in the survey. **Appendix 3.** Environmental characteristics of the 22 waterbodies investigated in November 2014. **Appendix 4.** Environmental characteristics of the 22 waterbodies investigated in April 2015.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Five new species of the genus *Ischnothyreus* Simon, 1893 from Singapore

Yanfeng Tong^{1,2}, Joseph K. H. Koh³, Xiujiao Tong¹, Shuqiang Li⁴

1 Life Science College, Shenyang Normal University, Shenyang 110034, China **2** Southeast Asia Biodiversity Research Institute, Chinese Academy of Sciences, Menglun, Mengla, Yunnan 666303, China **3** National Biodiversity Centre, National Parks Board, 259598, Singapore **4** Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China

Corresponding author: Shuqiang Li (lisq@ioz.ac.cn)

Academic editor: Cor Vink | Received 6 June 2016 | Accepted 1 September 2016 | Published 19 September 2016

<http://zoobank.org/D471637D-9FCA-475A-AA93-44C6FF96AA0F>

Citation: Tong Y, Koh JKH, Tong X, Li S (2016) Five new species of the genus *Ischnothyreus* Simon, 1893 from Singapore. ZooKeys 618: 39–66. doi: 10.3897/zookeys.618.9451

Abstract

Five new species of the genus *Ischnothyreus* are reported from Singapore: *I. an* Tong & Li, **sp. n.**, *I. brunneus* Tong & Li, **sp. n.**, *I. dactylinus* Tong & Li, **sp. n.**, *I. poculum* Tong & Li, **sp. n.** and *I. tectorius* Tong & Li, **sp. n.** Morphological descriptions and illustrations are given for all new species.

Keywords

Diagnosis, goblin spider, morphology, taxonomy, type

Introduction

With a population of 5.5 million people packed in a total land area measuring only 719 km², the Republic of Singapore is one of the most urbanized countries in the world. Yet, Singapore projects itself as a “city in a garden”, with 9,704 hectares or 13.5% of Singapore still covered with greenery, including 3,375 hectares (4.7%) fully protected as Nature Reserves (National Parks Board 2015). It is thus not surprising that Singapore is still home to a surprising diversity of flora and fauna, with many new species discovered even in recent years.

Out of the 1,628 described species in 113 oonopid genera worldwide, only 129 valid species, currently assigned under 12 genera, have been described from south-east Asia (Li and Lin 2016; World Spider Catalog 2016). The foundation was laid by pioneer arachnologists such as Koch (1873), Simon (1893, 1905, 1907, 1909), and Thorell (1887, 1890, 1895, 1897). After a hiatus of almost a century, the knowledge has been augmented in recent studies including those by Baehr et al. (2012), Eichenberger et al. (2012), Eichenberger and Kranz-Baltensperger (2011), Kranz-Baltensperger (2011, 2012), Thoma et al. (2014), and Tong and Li (2013a, b, c). Among the total of 12 species of Singapore that has been documented in published records, four species were described with Singapore as their type locality. Two of them were described by Simon, viz., *Gamasomorpha camelina* Simon, 1893, and *Xyphinyx hystrix* Simon, 1893; and another two by Thoma, viz., *Aposphragisma salweskii* Thoma, 2014 and *A. stannum* Thoma, 2014.

The genus *Ischnothyreus* Simon, 1893 can be recognized by the presence of leg spines, the usually small abdominal scutum, the strongly sclerotized male palps, the heavily sclerotized male endites and the winding genital tube in the females (Kranz-Baltensperger 2011). There are currently 84 valid specific names assigned to *Ischnothyreus*, but the presently recognized species may represent only a small fraction of the actual biodiversity (Edward and Harvey 2014). Among these 84 species are 28 recorded from Southeast Asia (World Spider Catalog 2016). Only two of these were recorded in Singapore itself, viz., *I. flagellichelis* Xu, 1989, previously described in China; and a pantropical species *I. peltifer* (Simon, 1891) whose type locality is St. Vincent (Murphy and Murphy 2000; Song et al. 2002).

As no oonopids have been deposited at the Lee Kong Chian Natural History Museum in Singapore, a concerted survey of the oonopid spiders was carried out in Singapore in August 2015, with the support and encouragement of the Singapore National Parks Board. From the many specimens of *Ischnothyreus* collected, neither of the two species of previously recorded from Singapore was recognized. However, we have been able to add five new species of *Ischnothyreus* to the Singapore Oonopidae inventory.

Material and methods

All the specimens were collected by sifting leaf litter. The specimens were examined using a Leica M205C stereomicroscope. Details were studied under an Olympus BX51 compound microscope. All illustrations were made using a drawing tube and inked on ink jet plotter paper. Photos were made with a Canon EOS 550D zoom digital camera (18 mega pixels) mounted on an Olympus BX51 compound microscope. Vulvae were cleared in lactic acid. Male palps and chelicerae were mounted in Kaiser's glycerol gelatin. All measurements were taken using an Olympus BX51 compound microscope and are in millimeters.

The following abbreviations are used in the text: **ALE** = anterior lateral eyes; **PLE** = posterior lateral eyes; **PME** = posterior median eyes.

All types of the new species are deposited in Lee Kong Chian Natural History Museum, National University of Singapore (**LKCNHM**). Other material studied is deposited in Shenyang Normal University (**SYNU**) in Shenyang, China.

Taxonomy

Ischnothyreus **an** Tong & Li, sp. n.

<http://zoobank.org/ACEC0E27-0783-4364-9D15-7EACAEBBC161F>

Figs 1–3

Type material. Holotype: male (LKCNHM), Singapore: Central Catchment Nature Reserve, Alt. 60 m, 1°21'21.7"N, 103°48'3.8"E, August 26, 2015, S. Li and Y. Tong leg. **Paratypes:** 1 male, 5 females (LKCNHM), same data as holotype.

Other material studied. 4 females (SYNU-60), Singapore: Central Catchment Nature Reserve, near Mandai Agrotechnology Park, Alt. 46 m, 1°24'53.7"N, 103°47'56.2"E, Sep 1, 2015, S. Li and Y. Tong leg.; 8 females (SYNU-61), Singapore: Central Catchment Nature Reserve, Alt. 46 m, 1°21'13.3"N, 103°48'29.4"E, August 27, 2015, S. Li and Y. Tong leg.; 6 males, 6 females (SYNU-62), Singapore: Central Catchment Nature Reserve, treetop walk, 1°21'13.3"N, 103°48'29.4"E, August 28, 2015, S. Li and Y. Tong leg.; 1 male, 1 female (SYNU-63), Singapore: Central Catchment Nature Reserve, Alt. 39 m, 1°21'17.9"N, 103°47'50.7"E, August 25, 2015, S. Li and Y. Tong leg.; 2 males, 1 female (SYNU-64), Singapore: Central Catchment Nature Reserve, Alt. 39 m, 1°21'17.9"N, 103°47'50.7"E, August 25, 2015, S. Li and Y. Tong leg.

Etymology. The species's name is derived from the Chinese Pinyin "an", meaning dark, which refers to the color of the palp; term in apposition.

Diagnosis. The new species is similar to *I. tekek* Kranz-Baltensperger, 2012 in having similar thorn-like protrusion (tlp) on the proximal part of the paturon (Figs 1G, H, 3G and Kranz-Baltensperger 2012: fig. 3D) in male, but can be distinguished from it by the finger-shaped sclerotized process (fsp) at base of fangs (Fig. 3H) in male, and the triangular shaped atrium (tsa) in the female epigastric region (Fig. 2G–J)

Description. Male (holotype). Total length 1.18; carapace 0.65 length, 0.48 width; abdomen 0.57 length, 0.34 width. Habitus as in Fig. 1A, C, E. *Carapace:* pale orange, with brown, egg-shaped patches behind eyes, ovoid in dorsal view, strongly elevated in lateral view, surface of elevated portion of pars cephalica smooth, sides finely reticulate, fovea absent, lateral margin straight, smooth (Fig. 1B, D). *Chypeus:* straight in frontal view, vertical in lateral view, ALE separated from edge of carapace by their radius or more. *Eyes:* six, well developed, ALE largest, ALE circular, PME and PLE oval, posterior eye row procurved from both above and front, ALE separated by less than their radius, ALE-PLE separated by less than ALE radius, PME touching, PLE-PME touching (Fig. 1I). *Sternum:* longer than wide, pale orange, uniform, not fused to carapace, surface smooth, setae sparse. *Mouthparts:* chelicerae, endites and labium

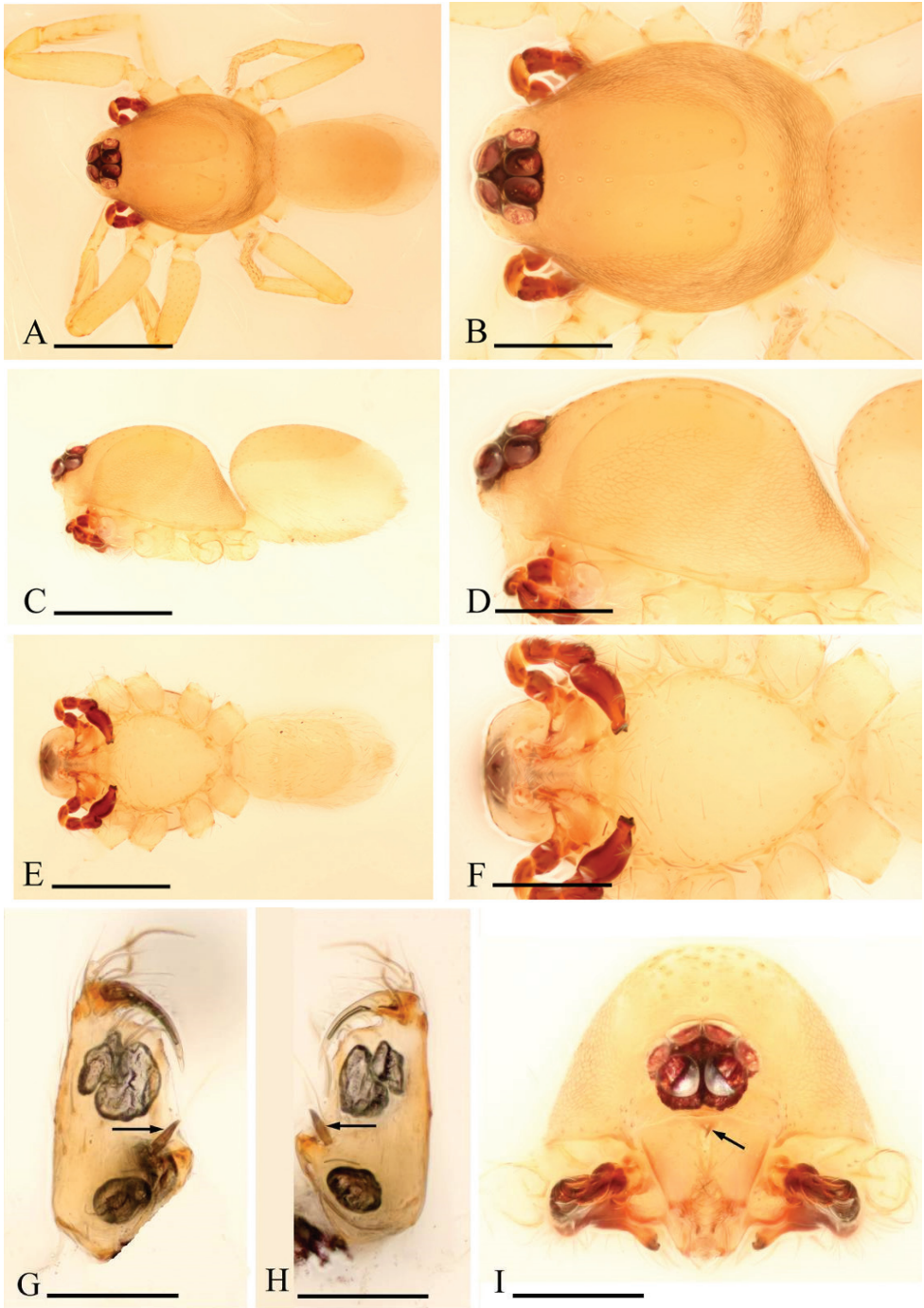


Figure 1. *Ischnothyreus an* sp. n., male. **A, C, E** habitus, dorsal, lateral and ventral views **B, D, F, I** prosoma, dorsal, lateral, ventral and anterior views **G, H** left chelicera, anterior and posterior views. Arrows show the thorn-like protrusion. Scale bars: **A, C, E** = 0.4 mm; **B, D, F, I** = 0.2 mm; **G, H** = 0.1 mm.

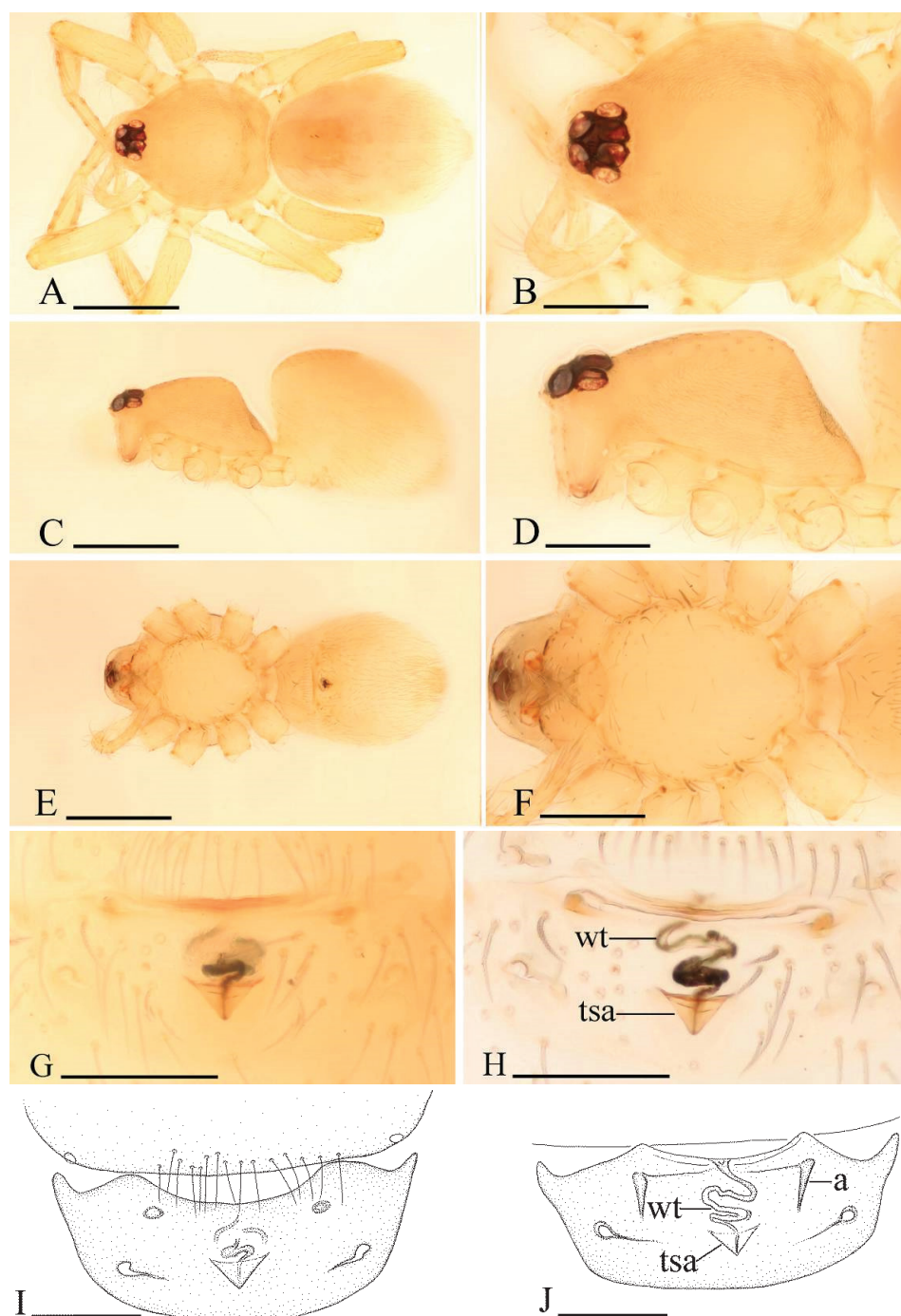


Figure 2. *Ischnothyreus an* sp. n., female. **A, C, E** habitus, dorsal, lateral and ventral views **B, D, F** prosoma, dorsal, lateral and ventral views **G, I** epigastric region, ventral view **H** epigastric region, ventral view (cleared in lactic acid) **J** epigastric region, dorsal view. Abbreviations: a = apodeme; tsa = triangular shaped atrium; wt = winding tube. Scale bars: **A, C, E** = 0.4 mm; **B, D, F** = 0.2 mm; **G-J** = 0.1 mm.

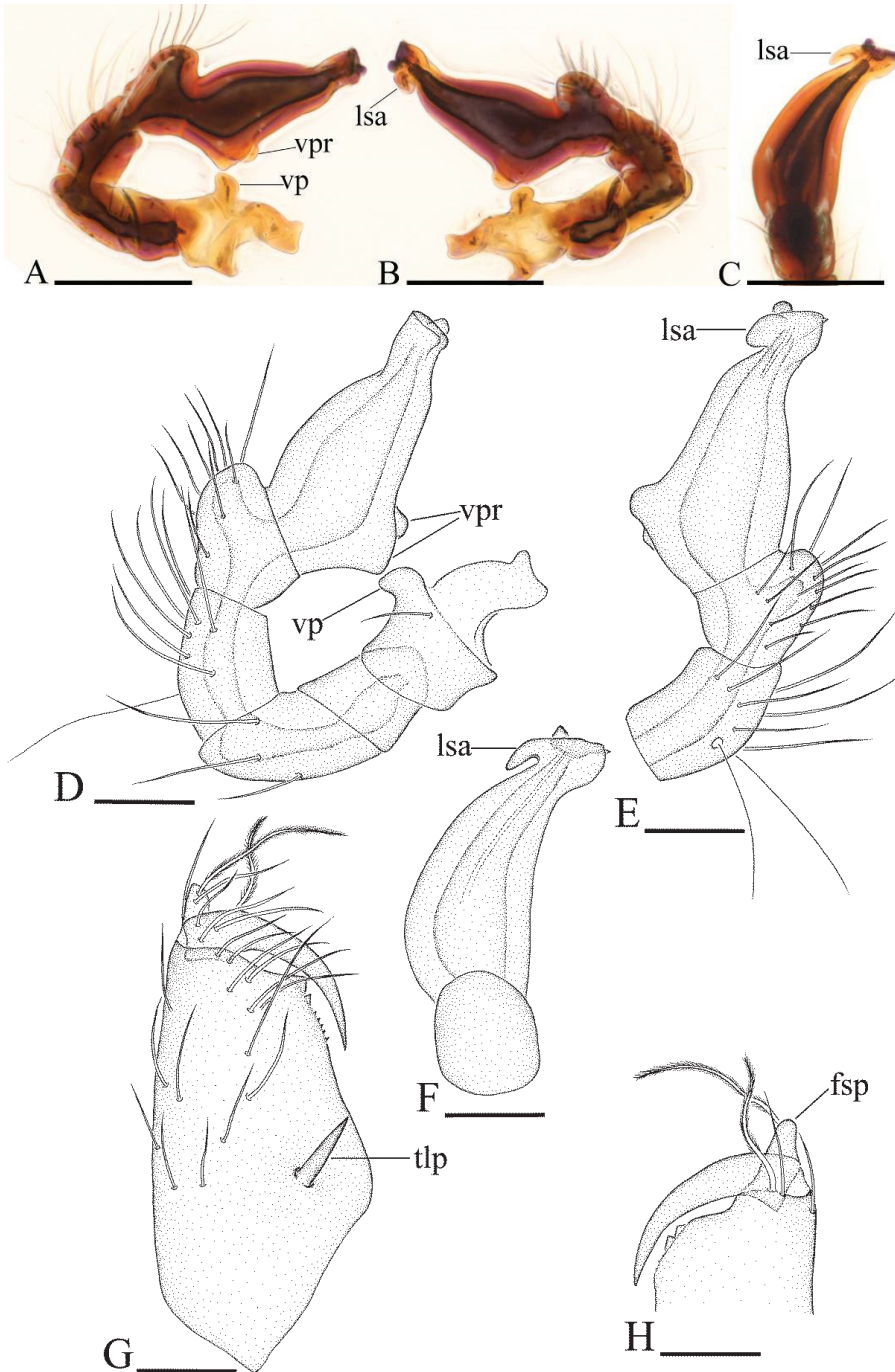


Figure 3. *Ischnothyreus an* sp. n., male. **A, D** left palp, prolateral view **B, E** left palp, retrolateral view **C, F** left palp, dorsal view **G, H** left chelicera, anterior and posterior views. Abbreviations: fsp = finger-shaped sclerotized process; lsa = leaf-shaped apophysis; tlp = thorn-like protrusion; vp = ventral projection; vpr = ventral protuberance. Scale bars: **A–C** = 0.1 mm; **D–H** = 0.05 mm.

orange. Chelicerae straight, with finger-shaped sclerotized process (fsp) at base of fangs (Fig. 3H), proximal part of paturon with a thorn-like protrusion (tlp) (Figs 1G, H, 3G), fang groove with a few small and two larger denticles. Labium rectangular, fused to sternum, anterior margin not indented at middle. Anteromedian tip of endites with one strong, tooth-like projection (Fig. 1E, F). *Abdomen*: ovoid, rounded posteriorly. Posterior spiracles not connected by groove. Pedicel tube short, ribbed, scutum not extending far dorsal of pedicel. Dorsal scutum well sclerotized, pale orange, covering whole abdomen width and approximately 4/5 of abdomen length, fused to epigastric scutum, middle surface and sides smooth. Epigastric and postepigastric scutum well sclerotized, pale orange, fused, without posteriorly directed lateral apodemes. Dorsum setae present, light, needle-like. *Legs*: pale orange, femur I with two prolateral and two small retrolateral spines, tibia I with four pairs, metatarsus I with two pairs of long ventral spines. Leg II spination is similar to leg I except femur with only one prolateral and one retrolateral spine. Legs III and IV spineless. *Genitalia*: epigastric region with sperm pore middle sized, circular, situated at level of anterior spiracles. Palp strongly sclerotized, right and left palps symmetrical, trochanter with ventral projection (vp) (Fig. 3A, D), cymbium brown, fused with bulb, bulb brown, more than two times as long as cymbium, tapering apically, with two small ventral protuberances (vpr) (Fig. 3D), distal part elongated, with membranous leaf-shaped apophyses (lsa) (Fig. 3B, C, E, F).

Female (paratype). Total length 1.37; carapace 0.64 length, 0.51 width; abdomen 0.76 length, 0.53 width. Habitus as in Fig. 2A, C, E. As in male except as noted. *Carapace*: without any pattern. *Mouthparts*: chelicerae and endites unmodified. *Abdomen*: dorsal scutum covering less than 1/2 of abdomen length, less than 1/3 of abdomen width. Postepigastric scutum rectangular. *Genitalia*: the posterior margin of the epigastric scutum is lined with numerous needle-like setae. The epigastric groove is narrow. From the middle of the slightly thickened margin of the postepigastric scutum runs a dark, winding tube posteriorly (wt) (Fig. 2G, I), ending in an equilateral triangular shaped atrium (tsa) (Fig. 2H, J).

Distribution. Singapore.

***Ischnothyreus brunneus* Tong & Li, sp. n.**

<http://zoobank.org/14137BDE-A1CF-4315-8BF0-3C405C84C437>

Figs 4–6

Type material. Holotype: male (LKC�HM), Singapore: Central Catchment Nature Reserve (off Mandai Lake Road), Alt. 39 m, 1°24'30.7"N, 103°46'51.3"E, August 31, 2015, S. Li and Y. Tong leg. **Paratypes:** 7 males, 8 females (LKC�HM), same data as holotype.

Other material studied. 8 males, 14 females (SYNU-65), Singapore: Central Catchment Nature Reserve (off Mandai Lake Road), Alt. 39 m, 1°24'30.7"N, 103°46'51.3"E, August 31, 2015, S. Li and Y. Tong leg.; 3 females (SYNU-66), Singapore: Pulau Ubin, Alt. 2 m, 1°25'18.0"N, 103°56'25.4"E, August 22, 2015, S. Li and

Y. Tong leg.; 1 female (SYNU-67), Singapore: Central Catchment Nature Reserve (off Mandai Lake Road), Alt. 39 m, 1°24'30.7"N, 103°46'51.3"E, August 31, 2015, S. Li and Y. Tong leg.

Etymology. The specific epithet means “brown” in Latin, and refers to the body color of this species; adjective.

Diagnosis. Males of the new species is similar to those of *I. dactylinus* sp. n., but can be distinguished from it by the larger eyes and the unmodified chelicerae. Furthermore the distal part of the male palpal bulb lacks the finger-like apophyses present in *I. dactylinus* (Fig. 6A–F). Females of the new species is similar to those of *I. barus* Kranz-Baltensperger, 2011, but can be distinguished from it by the brown body color and the dark brown pattern on leg IV, and the small bell-shaped atrium (bsa) in the epigastric region (Fig. 5J).

Description. Male (holotype). Total length 1.36; carapace 0.73 length, 0.57 width; abdomen 0.67 length, 0.48 width. Habitus as in Fig. 4A, C, E. *Carapace*: yellow, dark brown on lateral and posterior surfaces, with brown egg-shaped patches behind eyes, ovoid in dorsal view, slightly elevated in lateral view, surface of elevated portion of pars cephalica smooth, sides finely reticulate, fovea absent, lateral margin straight, smooth (Fig. 4B, D). *Chypeus*: straight in frontal view, vertical in lateral view, high, ALE separated from edge of carapace by more than their radius. *Eyes*: six, well developed, ALE largest, ALE circular, PME and PLE oval, posterior eye row straight from above, procurved from front, ALE separated by less than their radius, ALE-PLE separated by less than ALE radius, PME touching, PLE-PME touching (Fig. 4J). *Sternum*: longer than wide, pale yellow, uniform, not fused to carapace, surface smooth, setae sparse. *Mouthparts*: chelicerae, endites, and labium yellow. Chelicerae straight, base of fangs unmodified, fang groove with many small denticles (Fig. 6G, H). Labium rectangular, fused to sternum, anterior margin not indented at middle. Anteromedian tip of endites with one strong, tooth-like projection (Fig. 4E, F). *Abdomen*: ovoid, rounded posteriorly. Posterior spiracles not connected by groove. Pedicel tube short, ribbed, scutum not extending far dorsal of pedicel. Dorsal scutum well sclerotized, dark brown, covering whole abdomen width and approximately 5/6 of abdomen length, fused to epigastric scutum, middle surface and sides smooth. Epigastric and postepigastric scutum well sclerotized, fused, upper pedicel tube region dark brown, the other part yellow, without posteriorly directed lateral apodemes. Dorsum setae present, light, needle-like. *Legs*: yellow, with dark brown pattern on distal part of femur IV and middle part of tibia IV (Fig. 4G), femur I with two prolateral and two small retrolateral spines, tibia I with four pairs, metatarsus I with two pairs of long ventral spines. Leg II spination is similar to leg I except femur with only one prolateral and one retrolateral spine. Legs III and IV spineless. *Genitalia*: epigastric region with sperm pore large, circular, situated at level of anterior spiracles. Palp strongly sclerotized, right and left palps symmetrical, trochanter with ventral projection (vp) (Fig. 6A, D), cymbium brown, fused with bulb, bulb brown, more than two times as long as cymbium, tapering apically, with two small ventral protuberances (vpr) (Fig. 6E), distal part elongated, with membranous outgrowth (Fig. 6B, C).

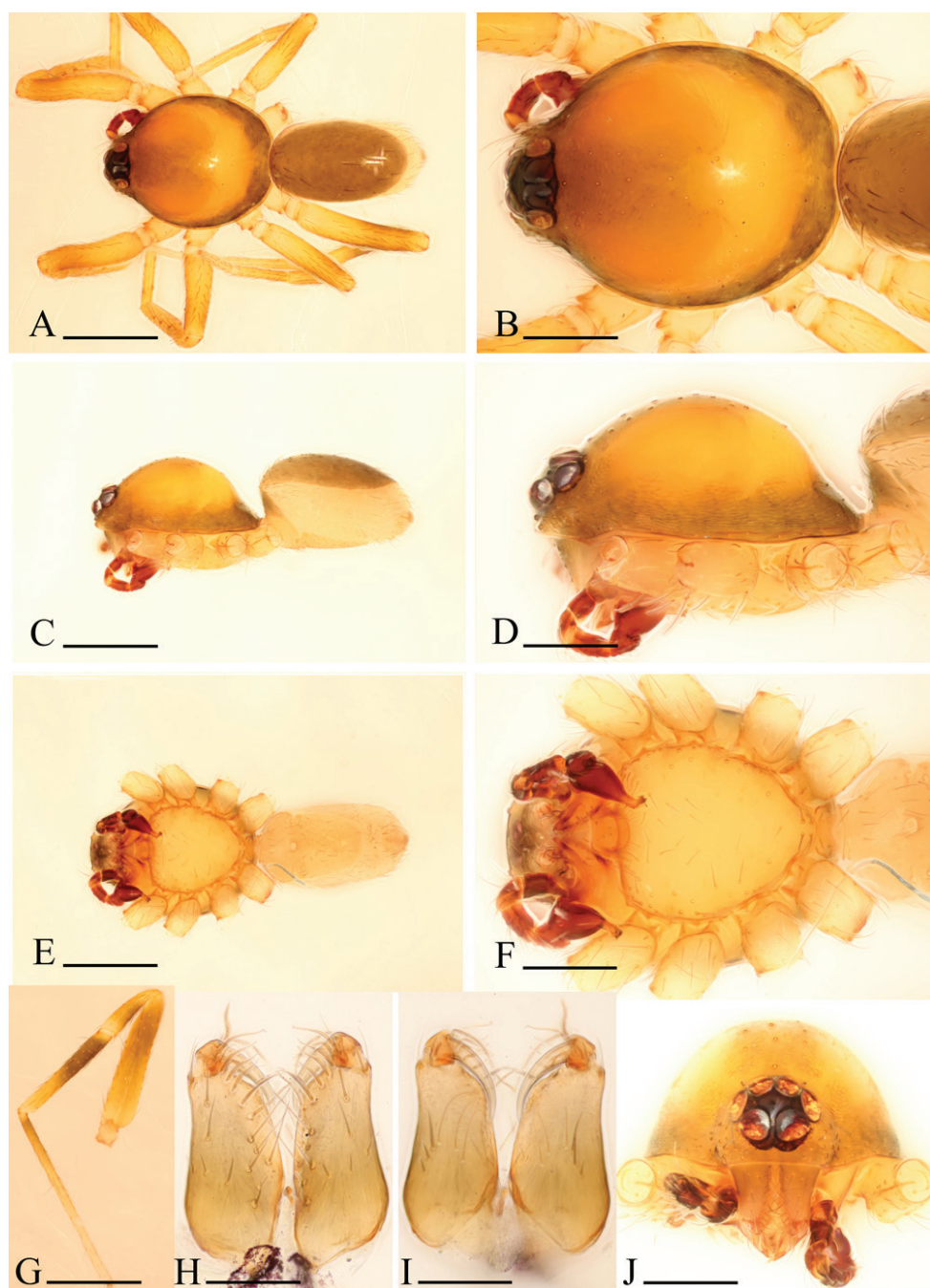


Figure 4. *Ischnothyreus brunneus* sp. n., male. **A, C, E** habitus, dorsal, lateral and ventral views **B, D, F, J** prosoma, dorsal, lateral, ventral and anterior views **G**, left leg IV, retrolateral view **H, I** chelicerae, anterior and posterior views. Scale bars: **A, C, E, G** = 0.4 mm; **B, D, F, J** = 0.2 mm; **H, I** = 0.1 mm.

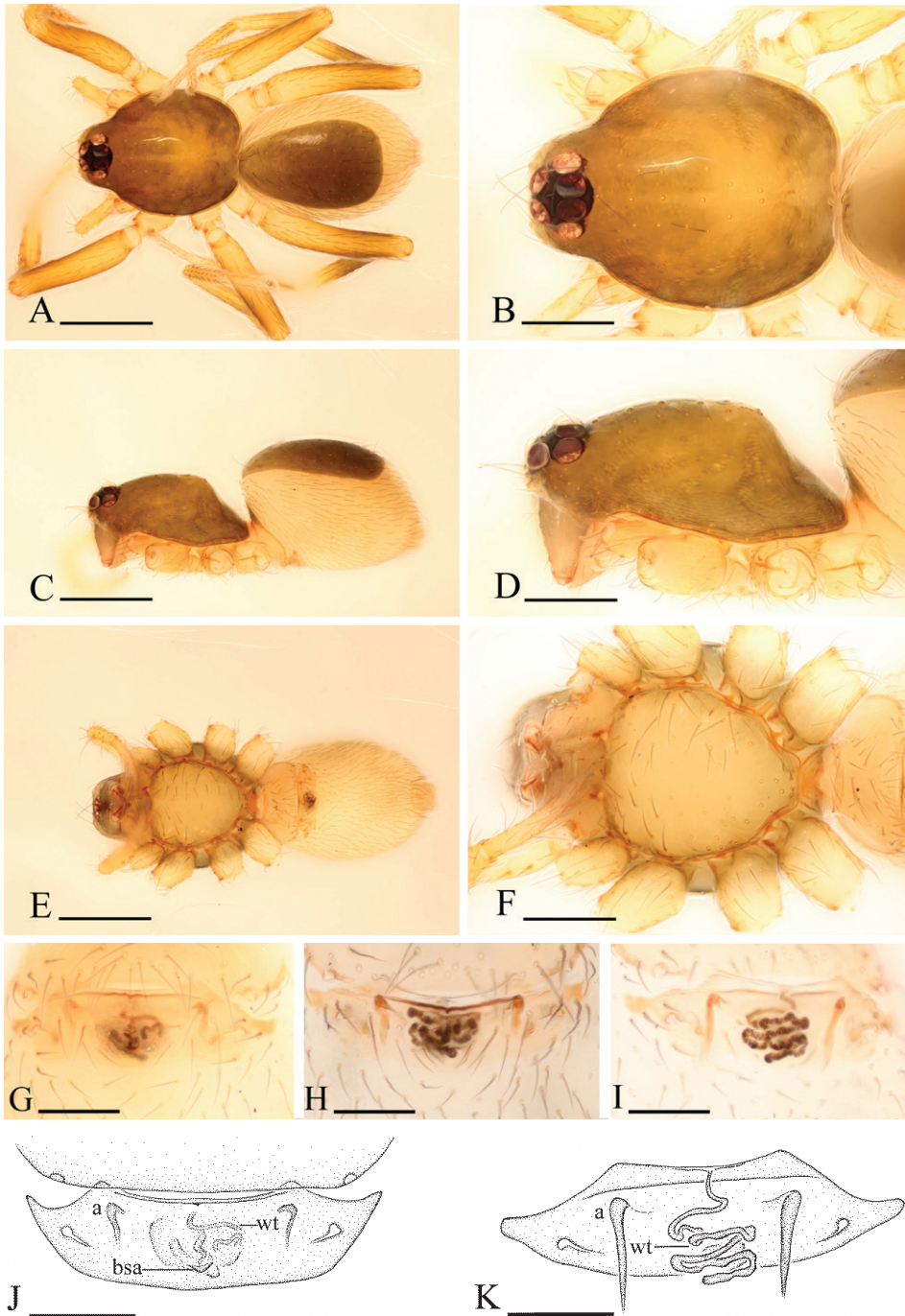


Figure 5. *Ischnothyreus brunneus* sp. n., female. **A, C, E** habitus, dorsal, lateral and ventral views **B, D, F** prosoma, dorsal, lateral and ventral views **G, H, J** epigastric region, ventral view **I, K** epigastric region, dorsal view (H, I cleared in lactic acid). Abbreviations: a = apodeme; bsa = bell-shaped atrium; wt = winding tube. Scale bars: **A, C, E** = 0.4 mm; **B, D, F** = 0.2 mm; **G–K** = 0.1 mm.

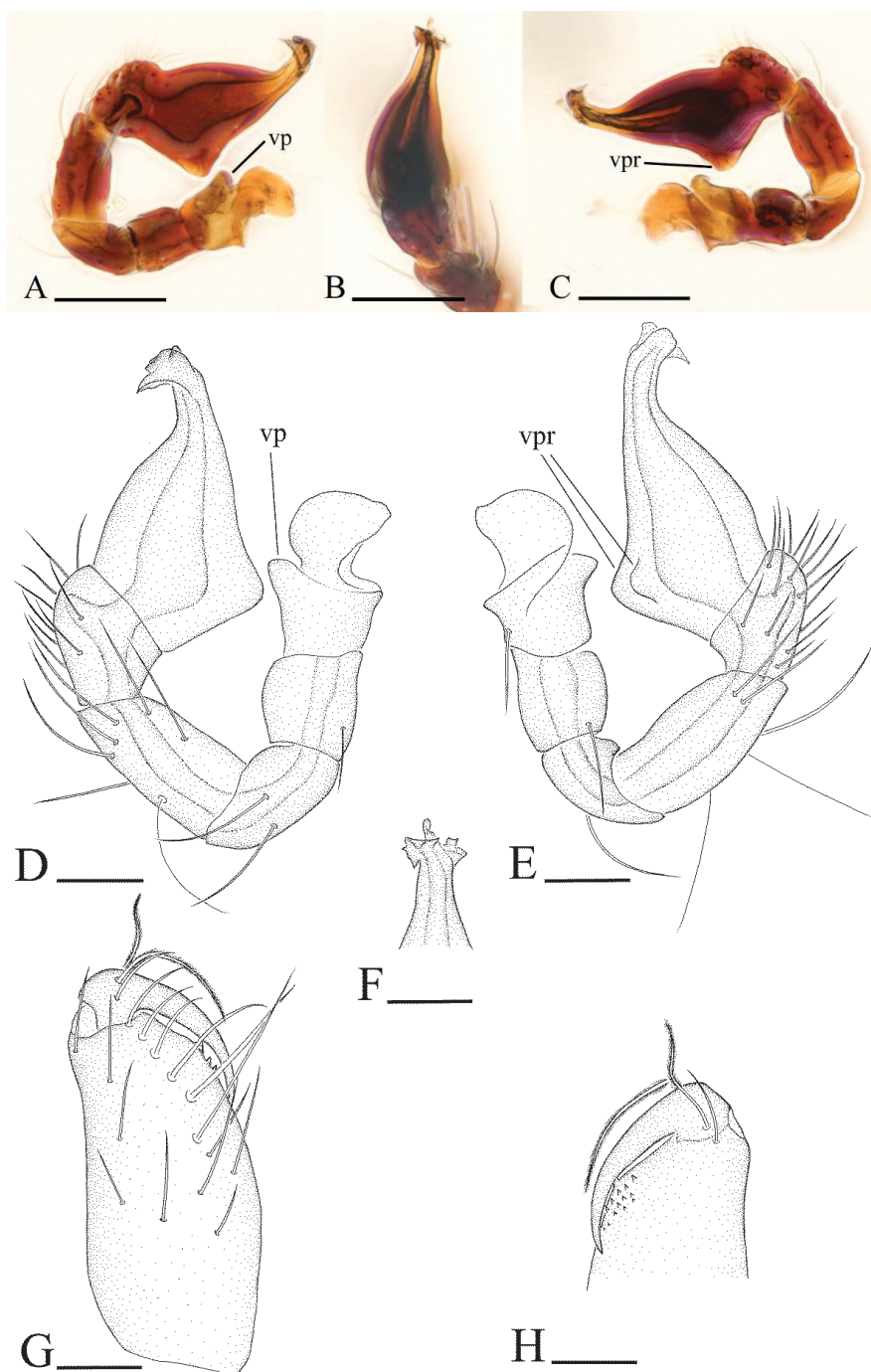


Figure 6. *Ischnothyreus brunneus* sp. n., male. **A, D** left palp, prolateral view **B** left palp, dorsal view **C, E** left palp, retrolateral view **F** distal part of palpal bulb, dorsal view **G, H** left chelicera, anterior and posterior views. Abbreviations: vp = ventral projection; vpr = ventral protuberance. Scale bars: **A–C** = 0.1 mm; **D–H** = 0.05 mm.

Female (paratype). Total length 1.47; carapace 0.76 length, 0.56 width; abdomen 0.79 length, 0.52 width. Habitus as in Fig. 5A, C, E. As in male except as noted. *Carapace*: dark brown, without any pattern. *Mouthparts*: endites unmodified. *Abdomen*: dorsal scutum covering approximately 2/3 of abdomen length, 1/2 of abdomen width. *Genitalia*: from the middle of the slightly thickened margin of the postepigastriac scutum runs a dark, winding tube posteriorly (wt) (Fig. 5G, H), ending in a small bell-shaped atrium (bsa) (Fig. 5J).

Distribution. Singapore.

***Ischnothyreus dactylinus* Tong & Li, sp. n.**

<http://zoobank.org/FA51E4D1-06C2-413E-A767-8B380A779DAF>

Figs 7–9

Type material. Holotype: male (LKCNHM), Singapore: Central Catchment Nature Reserve, near Singapore Zoo, Alt. 50 m, 1°24'22.3"N, 103°47'7.4"E, August 30, 2015, S. Li and Y. Tong leg. **Paratypes:** 5 males, 8 females (LKCNHM), same data as holotype.

Other material studied. 1 male, 1 female (SYNU-85), Singapore: Bukit Timah Nature Reserve, Alt. 86 m, 1°21'37.4"N, 103°46'30.0"E, August 24, 2015, S. Li and Y. Tong leg.; 7 males, 9 females (SYNU-86), Singapore: Central Catchment Nature Reserve, near Singapore Zoo, Alt. 50 m, 1°24'22.3"N, 103°47'7.4"E, August 30, 2015, S. Li and Y. Tong leg.; 1 male, 8 females (SYNU-87), Singapore: Central Catchment Nature Reserve (off Mandai Lake Road), Alt. 39 m, 1°24'30.7"N, 103°46'51.3"E, August 31, 2015, S. Li and Y. Tong leg.; 1 female (SYNU-88), Singapore: Central Catchment Nature Reserve, near Mandai Agrotechnology Park, Alt. 46 m, 1°24'53.7"N, 103°47'56.2"E, Sep 1, 2015, S. Li and Y. Tong leg.; 1 male, 1 female (SYNU-89), Singapore: Central Catchment Nature Reserve, Alt. 46 m, 1°21'13.3"N, 103°48'29.4"E, August 27, 2015, S. Li and Y. Tong leg.; 1 male, 1 female (SYNU-91), Singapore: Central Catchment Nature Reserve, Alt. 39 m, 1°21'17.9"N, 103°47'50.7"E, August 25, 2015, S. Li and Y. Tong leg.; 1 male (SYNU-92), Singapore: Central Catchment Nature Reserve, Alt. 60 m, 1°21'21.7"N, 103°48'3.8"E, August 26, 2015, S. Li and Y. Tong leg.; 2 females (SYNU-93), Singapore: Bukit Timah Nature Reserve, Bukit Timah Summit, Alt. 163 m, 1°21'16.65"N, 103°46'34.95"E, August 19, 2015, S. Li and Y. Tong leg.; 1 male, 1 female (SYNU-94), Singapore: Bukit Timah Nature Reserve, Bukit Timah Summit, Alt. 163 m, 1°21'16.65"N, 103°46'34.95"E, August 19, 2015, S. Li and Y. Tong leg.; 4 females (SYNU-95), Singapore: Bukit Timah Nature Reserve, Catchment Path, Alt. 107 m, 1°21'12.5"N, 103°46'50.6"E, August 20, 2015, S. Li and Y. Tong leg.; 1 male, 1 female (SYNU-96), Singapore: Bukit Timah Nature Reserve, Bukit Timah Summit, Alt. 163 m, 1°21'16.65"N, 103°46'34.95"E, August 19, 2015, S. Li and Y. Tong leg.; 2 males, 5 females (SYNU-97), Singapore: Central Catchment Nature Reserve, near Singapore Zoo, Alt. 50 m, 1°24'22.3"N, 103°47'7.4"E, August 30, 2015, S. Li and Y. Tong leg.; 1 male, 3 females (SYNU-

98), Singapore: Bukit Timah Nature Reserve, Seraya Loop, Alt. 118 m, 1°21'25.4"N, 103°46'25.3"E, August 17, 2015, S. Li and Y. Tong leg.

Etymology. The specific epithet means “finger-like” in Greek, and refers to the long apophysis on the distal part of the male palpal bulb (Fig. 9D, E); adjective.

Diagnosis. The new species is similar to *I. browni* Chickering, 1968 (Platnick et al. 2012), but can be distinguished from it by the dark brown body color, the flake-like dorsal process (fdp) on the male chelicerae (Fig. 9F, G), the finger-like apophysis on the distal part of the male palpal bulb (Fig. 9D, E) and the fan-shaped atrium (fsa) in the female epigastric region (Fig. 8H). The female epigastric region of the new species is also similar to that of *I. balu* Kranz-Baltensperger, 2011, but can be distinguished from it by the larger abdominal scutum and the color patterns on legs and abdomen.

Description. Male (holotype). Total length 1.43; carapace 0.78 length, 0.57 width; abdomen 0.65 length, 0.39 width. Habitus as in Fig. 7A, C, E. *Carapace*: yellow, dark brown on lateral and posterior surfaces, with brown egg-shaped patches behind eyes, ovoid in dorsal view, slightly elevated in lateral view, surface of elevated portion of pars cephalica smooth, sides strongly reticulate, fovea absent, lateral margin straight, smooth (Fig. 7B, D). *Chelyeus*: straight in frontal view, vertical in lateral view, high, ALE separated from edge of carapace by more than twice of their diameter. *Eyes*: six, very small, ALE largest, ALE circular, PME and PLE oval, posterior eye row procurved from both above and front, ALE separated by less than their radius, ALE-PLE separated by less than ALE radius, PME touching, PLE-PME touching (Fig. 7I). *Sternum*: longer than wide, pale yellow, uniform, not fused to carapace, surface smooth, setae sparse. *Mouthparts*: chelicerae, endites and labium yellow. Chelicerae straight, base of fangs with a flake-like dorsal process (fdp) (Fig. 9F, G), fang groove with a few small and one larger denticles. Labium rectangular, fused to sternum, anterior margin not indented at middle. Anteromedian tip of endites with one strong, tooth-like projection (Fig. 7E, F). *Abdomen*: ovoid, rounded posteriorly. Posterior spiracles not connected by groove. Pedicel tube short, ribbed, scutum not extending far dorsal of pedicel. Dorsal scutum well sclerotized, yellow, except dark brown on posterior part, covering whole abdomen width and approximately 5/6 of abdomen length, not fused to epigastric scutum, middle surface and sides smooth. Epigastric and postepigastric scutum well sclerotized, fused, the upper part of the pedicel tube is dark brown, the other part pale yellow, without posteriorly directed lateral apodemes. Dorsum setae present, light, needle-like. *Legs*: yellow, with dark brown pattern on subbasal part of femur, trochanter and basal half part of tibia of leg IV, femur I with two prolateral and two small retrolateral spines, tibia I with four pairs, metatarsus I with two pairs of long ventral spines. Leg II spination is similar to leg I except femur with only one prolateral and one retrolateral spine. Legs III and IV spineless. *Genitalia*: epigastric region with sperm pore large, circular, situated at level of anterior spiracles. Palp strongly sclerotized, right and left palps symmetrical, proximal segments brown, trochanter with ventral projection (vp) (Fig. 9A), cymbium brown, fused with bulb, bulb brown, more than two times as long as cymbium, tapering apically, with one large ventral protuberance (vpr) (Fig. 9B, C), distal part elongated, with two long apophyses, one sclerotized, finger-like (sfa), one membranous, triangle-shaped (mta) (Fig. 9D, E).

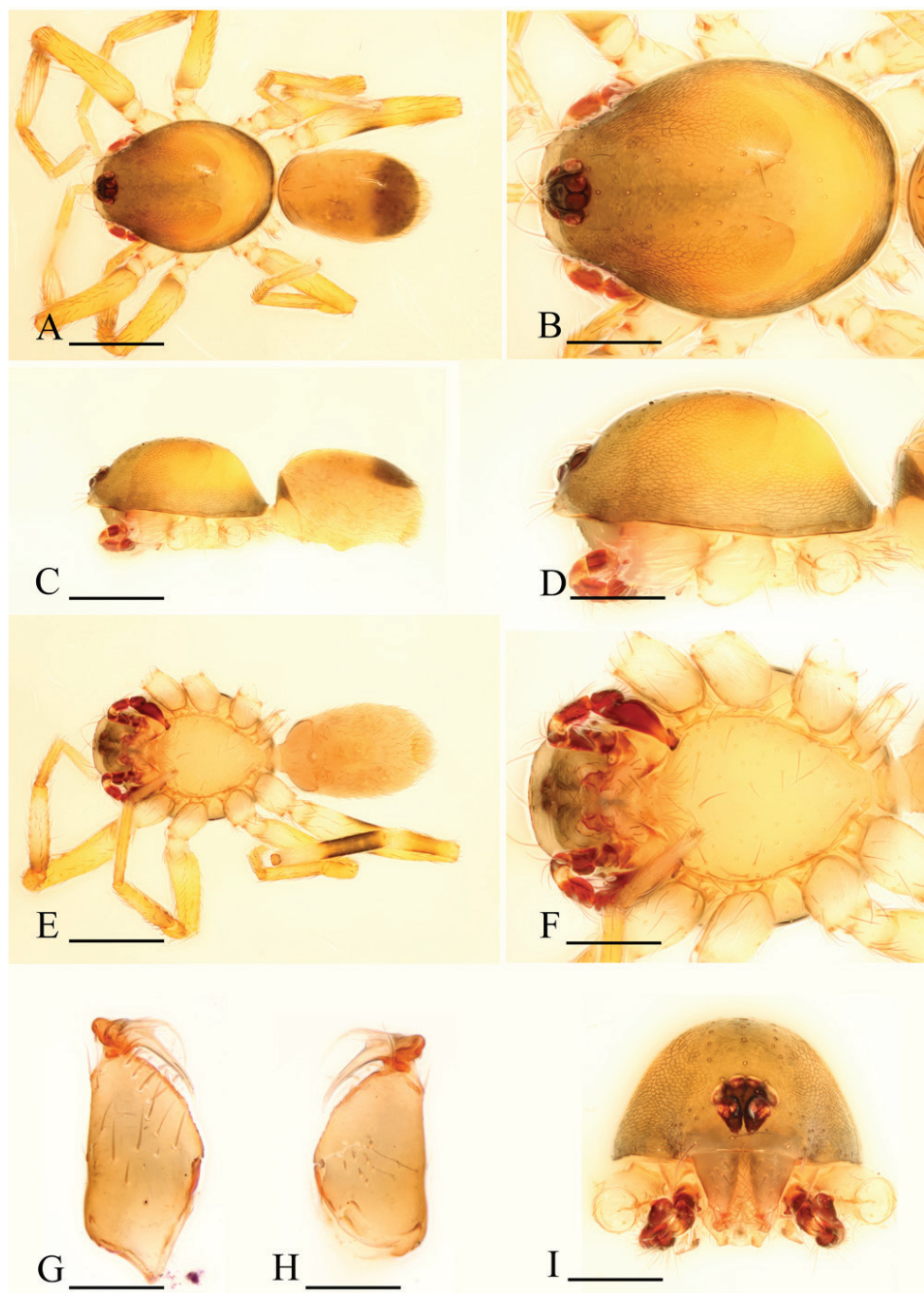


Figure 7. *Ischnothyreus dactylinus* sp. n., male. **A, C, E** habitus, dorsal, lateral and ventral views **B, D, F, I** prosoma, dorsal, lateral, ventral and anterior views **G, H** left chelicera, anterior and posterior views. Scale bars: **A, C, E** = 0.4 mm; **B, D, F, I** = 0.2 mm; **G, H** = 0.1 mm.

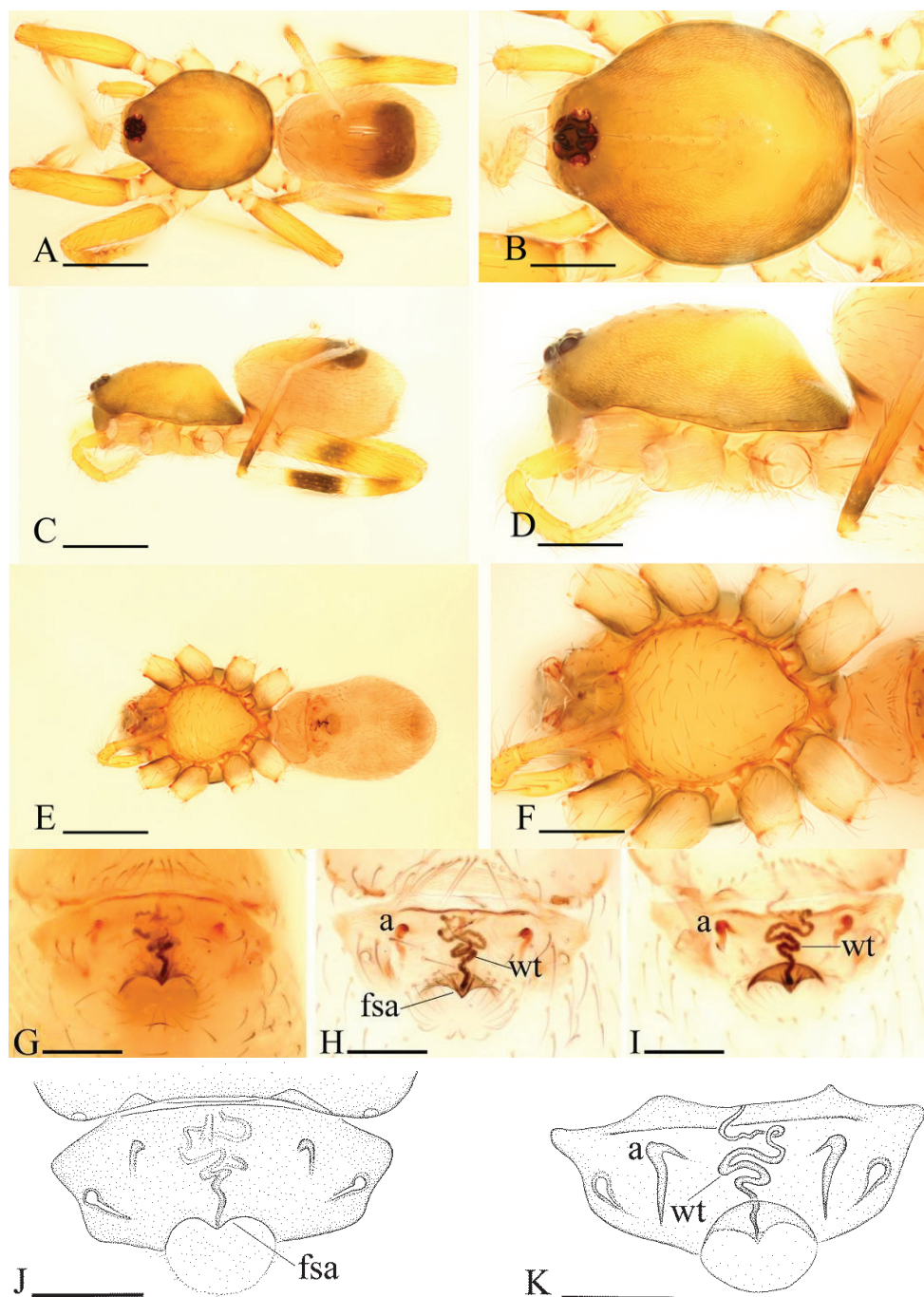


Figure 8. *Ischnothyreus dactylinus* sp. n., female. **A, C, E** habitus, dorsal, lateral and ventral views **B, D, F** prosoma, dorsal, lateral and ventral views **G, H, J** epigastric region, ventral view **I, K** epigastric region, dorsal view (H, I cleared in lactic acid). Abbreviations: a = apodeme; fsa = fan-shaped atrium; wt = winding tube. Scale bars: **A, C, E** = 0.4 mm; **B, D, F** = 0.2 mm; **G-K** = 0.1 mm.

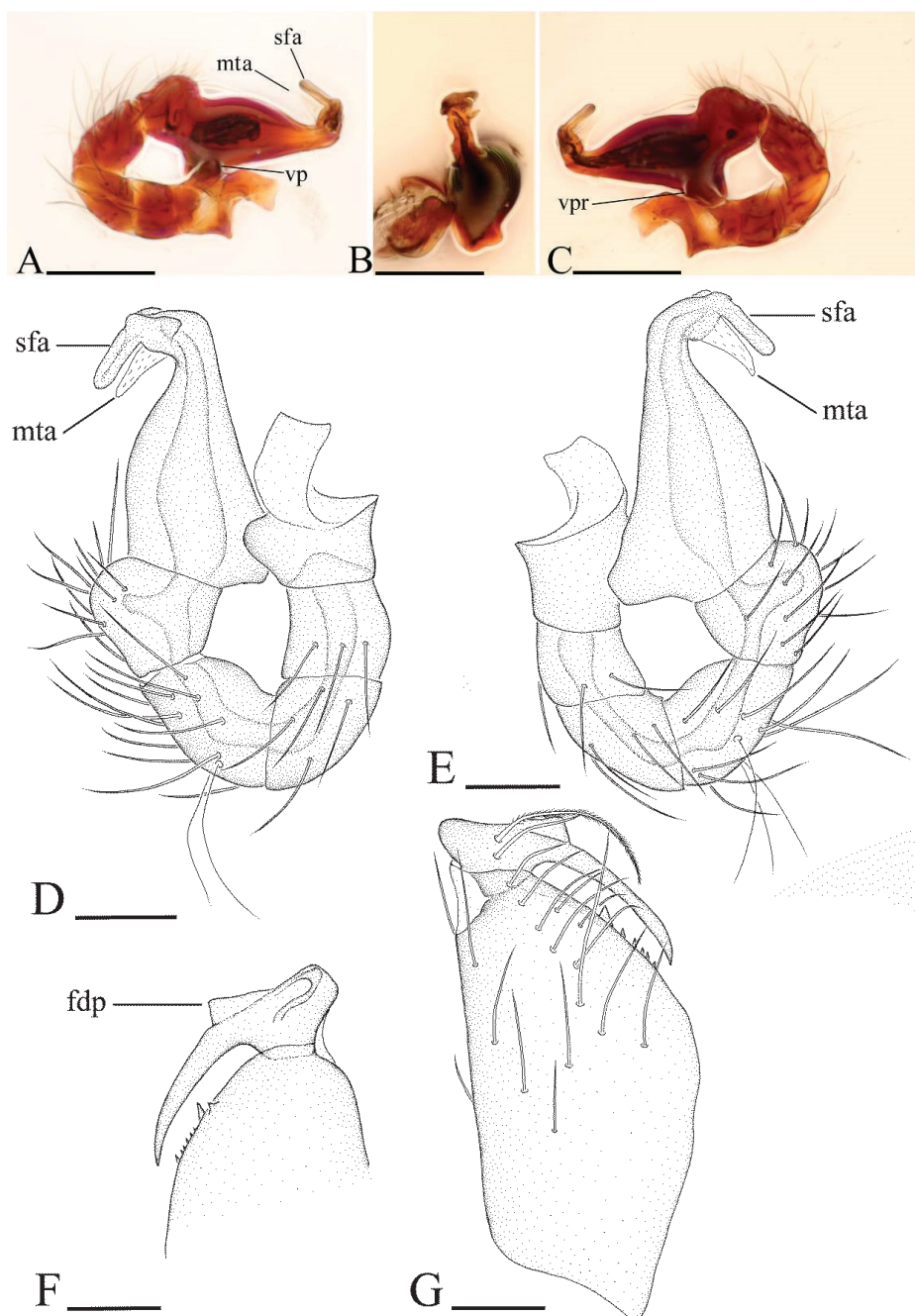


Figure 9. *Ischnothyreus dactylinus* sp. n., male. **A, D** left palp, prolateral view **B** distal part of palpal bulb, apical view **C, E** left palp, retrolateral view **F, G** left chelicera, posterior and anterior views. Abbreviations: fdp = flake-like dorsal process; mta = membranous, triangle-shaped apophysis; sfa = sclerotized, finger-like apophysis; vp = ventral projection; vpr = ventral protuberance. Scale bars: **A–C** = 0.1 mm; **D–G** = 0.05 mm.

Female (paratype). Total length 1.51; carapace 0.73 length, 0.58 width; abdomen 0.76 length, 0.49 width. Habitus as in Fig. 8A, C, E. As in male except as noted. *Carapace*: without any pattern. *Mouthparts*: endites unmodified. *Abdomen*: dorsal scutum covering less than 5/6 of abdomen length, 2/3 of abdomen width. Postepigastric scutum widely hexagonal. *Genitalia*: the posterior margin of the epigastric scutum is lined with numerous needle-like setae. The epigastric groove is narrow. From the middle of the slightly thickened margin of the postepigastric scutum runs a dark, winding tube posteriorly (wt) (Fig. 8G, J), ending in a fan-shaped atrium (fsa) (Fig. 8H).

Distribution. Singapore.

***Ischnothyreus poculum* Tong & Li, sp. n.**

<http://zoobank.org/352FE6DD-AD01-432A-8ED0-5822A77DB4D6>

Figs 10–12

Type material. Holotype: male (LKCNHM), Singapore: Central Catchment Nature Reserve, near Singapore Zoo, Alt. 50 m, 1°24'22.3"N, 103°47'7.4"E, August 30, 2015, S. Li and Y. Tong leg. **Paratypes:** 1 female (LKCNHM), same data as holotype; 1 female (LKCNHM), Singapore: Bukit Timah Nature Reserve, Alt. 86 m, 1°21'37.4"N, 103°46'30.0"E, August 24, 2015, S. Li and Y. Tong leg.

Other material studied. 2 females (SYNU-71), Singapore: Central Catchment Nature Reserve, near Mandai Agrotechnology Park, Alt. 46 m, 1°24'53.7"N, 103°47'56.2"E, Sep 1, 2015, S. Li and Y. Tong leg.; 1 female (SYNU-72), Singapore: Central Catchment Nature Reserve, Alt. 46 m, 1°21'13.3"N, 103°48'29.4"E, August 27, 2015, S. Li and Y. Tong leg.

Etymology. The specific epithet means “bowl” in Latin, and refers to the bowl-shaped atrium in the female epigastric region; noun.

Diagnosis. The new species is similar to *I. campanaceus* Tong & Li, 2008, but can be distinguished from it by the small abdominal dorsal scutum, the long sclerotized process (lsp) and small sclerotized triangular-shaped apophysis (sta) at base of fangs in male (Fig. 10H), and the bowl-shaped atrium in the female epigastric region (Fig. 11G–K). Males of the new species is also similar to those of *I. jojo* Kranz-Baltensperger, 2011 by the long sclerotized process (lsp) on the cheliceral fang (Figs 10G, H, 12G), but can be distinguished from it by the small sclerotized triangular-shaped apophysis (sta) at base of fangs (Fig. 10H) and the membranous outgrowth on distal part of male palp (Fig. 12A–E).

Description. Male (holotype). Total length 1.64; carapace 0.83 length, 0.67 width; abdomen 0.80 length, 0.46 width. Habitus as in Fig. 10A, C, E. *Carapace*: pale orange, with brown egg-shaped patches behind eyes, ovoid in dorsal view, strongly elevated in lateral view, surface of elevated portion of pars cephalica smooth, sides finely reticulate, fovea absent, lateral margin straight, smooth (Fig. 10B, D). *Chelyeus*: straight in frontal view, vertical in lateral view, ALE separated from edge of carapace by their radius or more. *Eyes*: six, well developed, ALE largest, ALE circular, PME and PLE oval,

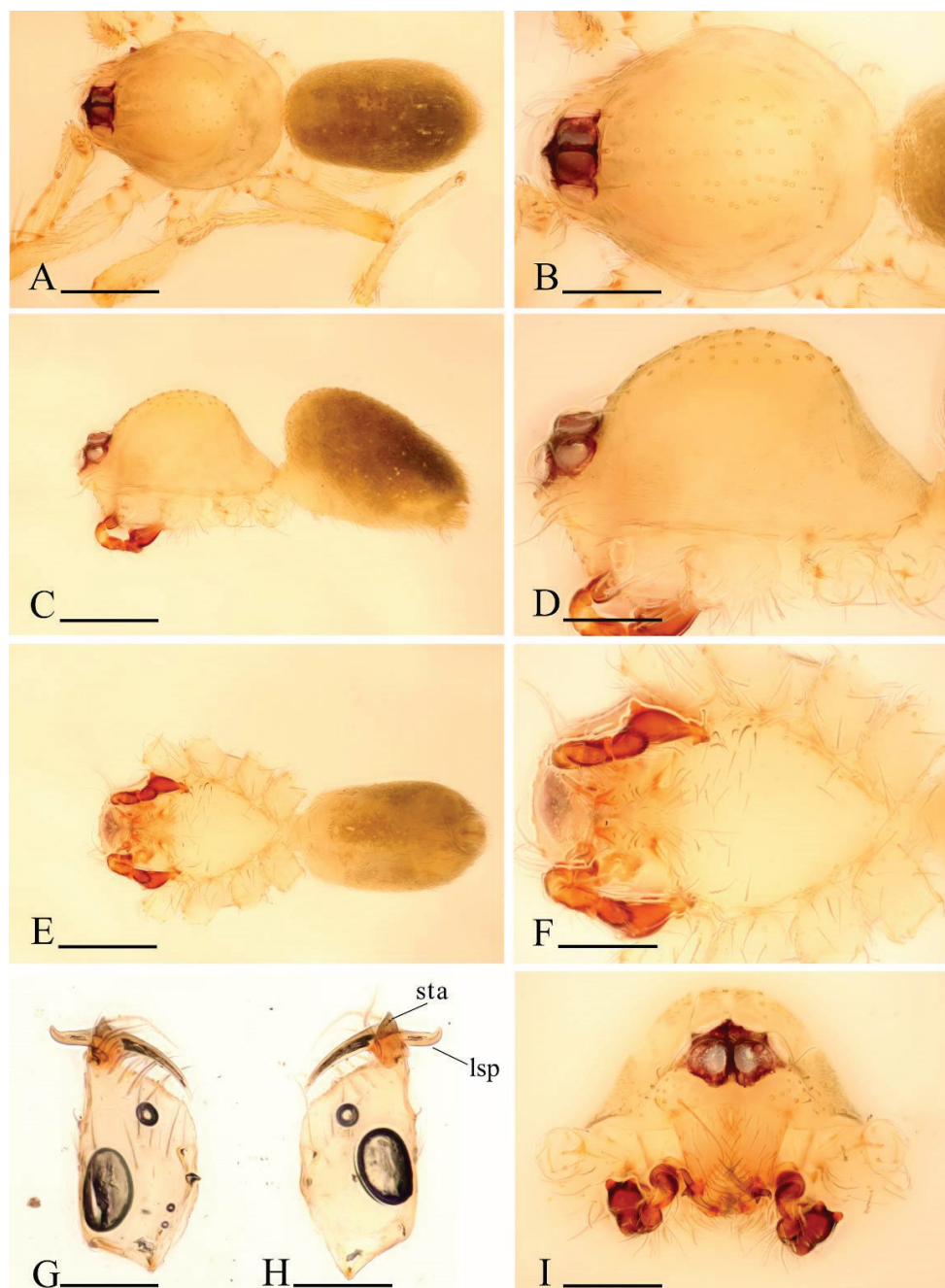


Figure 10. *Ischnothyreus poculum* sp. n., male. **A, C, E** habitus, dorsal, lateral and ventral views **B, D, F, I** prosoma, dorsal, lateral, ventral and anterior views **G, H** left chelicerae, anterior and posterior views. Abbreviations: lsp = long sclerotized process; sta = sclerotized triangular-shaped apophysis. Scale bars: **A, C, E** = 0.4 mm; **B, D, F, I** = 0.2 mm; **G, H** = 0.1 mm.

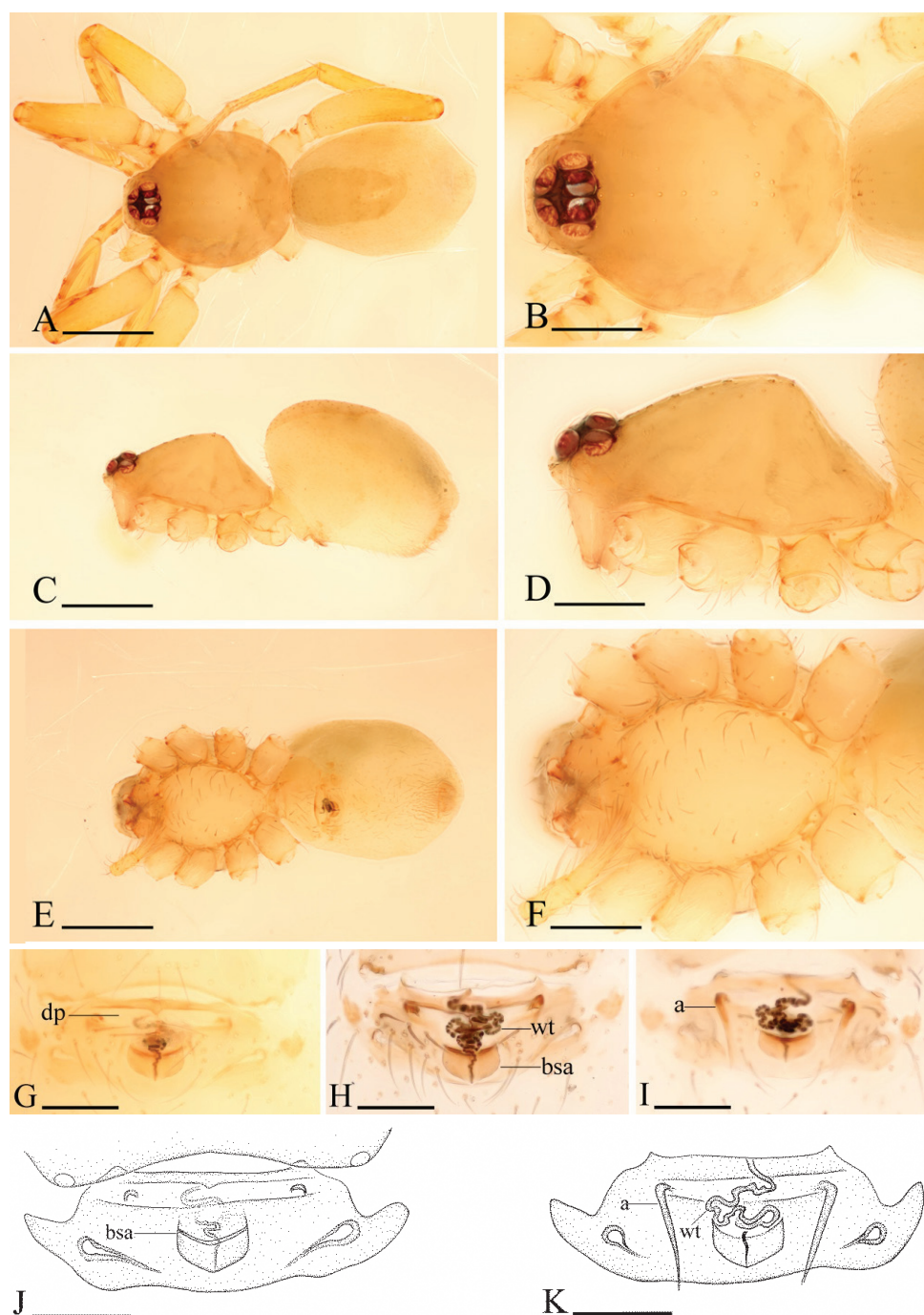


Figure 11. *Ischnothyreus poculum* sp. n., female. **A, C, E** habitus, dorsal, lateral and ventral views **B, D, F** prosoma, dorsal, lateral and ventral views **G, H, J** epigastric region, ventral view **I, K** epigastric region, dorsal view (H, I cleared in lactic acid). Abbreviations: a = apodeme; bsa = bowl-shaped atrium; dp = depression; wt = winding tube. Scale bars: **A, C, E** = 0.4 mm; **B, D, F** = 0.2 mm; **G-K** = 0.1 mm.

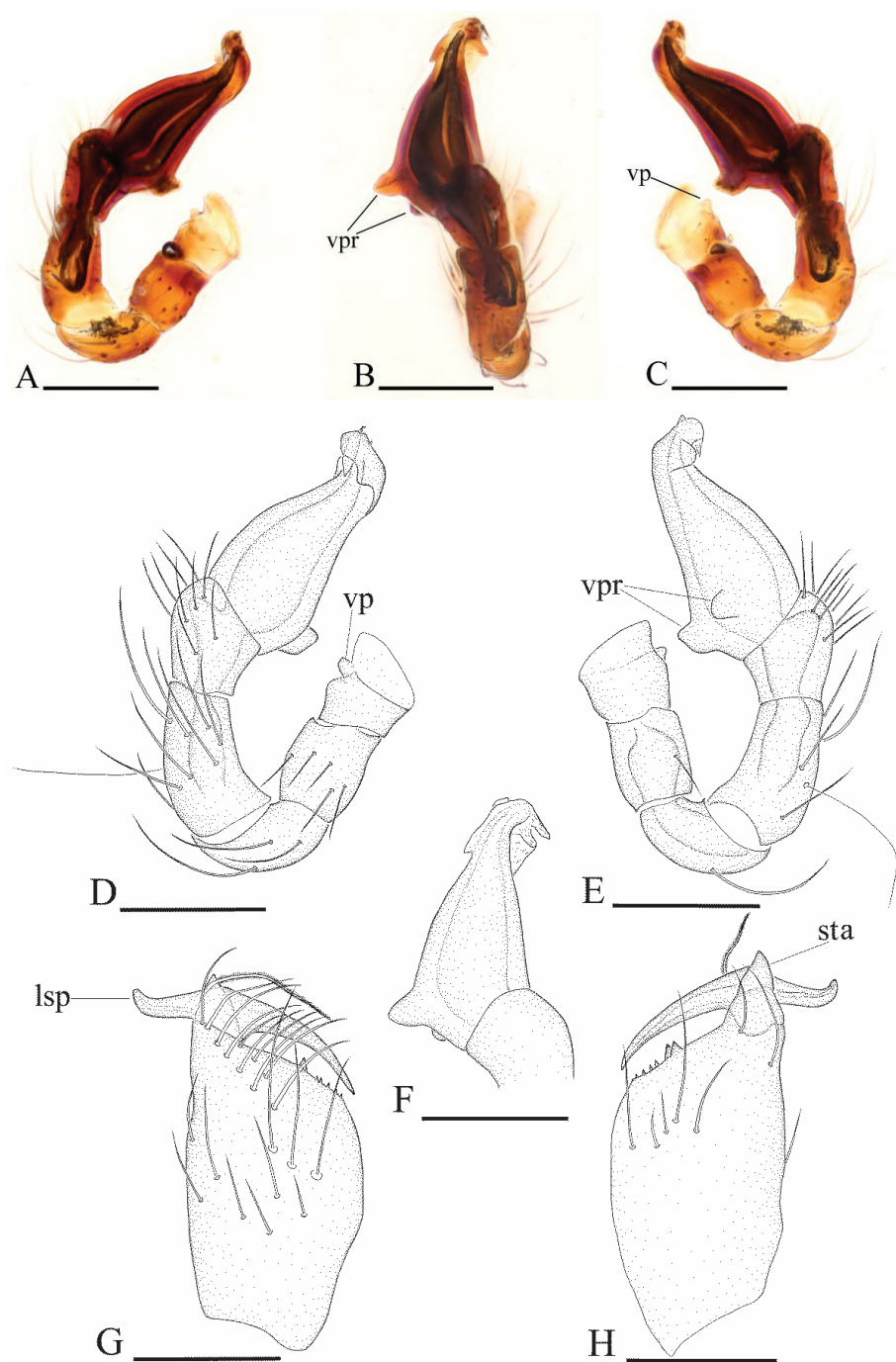


Figure 12. *Ischnothyreus poculum* sp. n., male. **A, D** left palp, prolateral view **B, F** left palpal bulb, dorsal view **C, E** left palp, retrolateral view **G, H** left chelicerae, anterior and posterior views. Abbreviations: lsp = long sclerotized process; sta = sclerotized triangular-shaped apophysis; vp = ventral projection; vpr = ventral protuberance. Scale bars: 0.1 mm.

posterior eye row straight from above, procurved from front, ALE separated by less than their radius, ALE-PLE separated by less than ALE radius, PME touching, PLE-PME touching (Fig. 10I). *Sternum*: longer than wide, pale orange, uniform, not fused to carapace, surface smooth, setae sparse. *Mouthparts*: chelicerae, endites and labium orange. Chelicerae straight, with long sclerotized process (lsp) and small sclerotized triangular-shaped apophysis (sta) at base of fangs (Fig. 12G, H), fang groove with a few small and one larger denticles. Labium rectangular, fused to sternum, anterior margin not indented at middle. Anteromedian tip of endites with one strong, tooth-like projection (Fig. 10E, F). *Abdomen*: ovoid, rounded posteriorly. Posterior spiracles not connected by groove. Pedicel tube short, ribbed, scutum not extending far dorsal of pedicel. Dorsal scutum weakly sclerotized, pale orange, covering approximately 1/2 of abdomen length, 1/2 of abdomen width, fused to epigastric scutum. Epigastric and postepigastric scutum weakly sclerotized, pale orange, fused, without posteriorly directed lateral apodemes. Dorsum setae present, light, needle-like. *Legs*: pale orange, femur I with two prolateral and two small retrolateral spines, tibia I with four pairs, metatarsus I with two pairs of long ventral spines. Leg II spination is similar to leg I except femur with only one prolateral and one retrolateral spine. Legs III and IV spineless. *Genitalia*: epigastric region with sperm pore middle sized, circular, situated at level of anterior spiracles. Palp strongly sclerotized, right and left palps symmetrical, trochanter with ventral projection (vp) (Fig. 12C, D), cymbium brown, fused with bulb, bulb brown, more than two times as long as cymbium, tapering apically, with two ventral protuberances (vpr) (Fig. 12B), distal part elongated, with membranous outgrowth (Fig. 12D, E, F).

Female (paratype). Total length 1.55; carapace 0.73 length, 0.61 width; abdomen 0.82 length, 0.59 width. Habitus as in Fig. 11A, C, E. As in male except as noted. *Carapace*: without any pattern. *Mouthparts*: chelicerae and endites unmodified. *Abdomen*: dorsal scutum well sclerotized, postepigastric scutum boat-shaped, very narrow. *Genitalia*: anterior margin of the postepigastric scutum slightly sclerotized, behind the anterior margin is a depression (dp); the winding tube runs posteriorly (wt), ending in a bowl-shaped atrium (bsa) (Fig. 11G–K).

Distribution. Singapore.

***Ischnothyreus tectorius* Tong & Li, sp. n.**

<http://zoobank.org/8044453E-C913-4CB4-A9C0-4179733324F6>

Figs 13–15

Type material. Holotype: male (LKCNHM), Singapore: Central Catchment Nature Reserve, near Mandai Agrotechnology Park, Alt. 46 m, 1°24'53.7"N, 103°47'56.2"E, Sep 1, 2015, S. Li and Y. Tong leg. **Paratypes:** 7 males, 7 females (LKCNHM), same data as holotype.

Other material studied. 2 males, 1 female (SYNU-73), Singapore: Bukit Timah Nature Reserve, Alt. 86 m, 1°21'37.4"N, 103°46'30.0"E, August 24, 2015, S. Li and

Y. Tong leg.; 7 males, 10 females (SYNU-74), Singapore: Central Catchment Nature Reserve, near Singapore Zoo, Alt. 50 m, 1°24'22.3"N, 103°47'7.4"E, August 30, 2015, S. Li and Y. Tong leg.; 12 females (SYNU-75), Singapore: Bukit Timah Nature Reserve, Seraya Loop, Alt. 118 m, 1°21'25.4"N, 103°46'25.3"E, August 17, 2015, S. Li and Y. Tong leg.; 6 males, 9 females (SYNU-76), Singapore: Central Catchment Nature Reserve (off Mandai Lake Road), Alt. 39 m, 1°24'30.7"N, 103°46'51.3"E, August 31, 2015, S. Li and Y. Tong leg.; 2 males, 5 females (SYNU-77), Singapore: Bukit Timah Nature Reserve, Catchment Path, Alt. 107 m, 1°21'12.5"N, 103°46'50.6"E, August 20, 2015, S. Li and Y. Tong leg.; 6 males, 11 females (SYNU-78), Singapore: Central Catchment Nature Reserve, near Singapore Zoo, Alt. 50 m, 1°24'22.3"N, 103°47'7.4"E, August 30, 2015, S. Li and Y. Tong leg.; 4 males, 3 females (SYNU-79), Singapore: Bukit Timah Nature Reserve, Jungle Fall Stream, Alt. 118 m, 1°21'25.4"N, 103°46'25.3"E, August 18, 2015, S. Li and Y. Tong leg.; 3 males, 3 females (SYNU-80), Singapore: Bukit Timah Nature Reserve, Jungle Fall Stream, Alt. 118 m, 1°21'25.4"N, 103°46'25.3"E, August 18, 2015, S. Li and Y. Tong leg.; 2 males, 2 females (SYNU-81), Singapore: Bukit Timah Nature Reserve, Jungle Fall Stream, Alt. 118 m, 1°21'25.4"N, 103°46'25.3"E, August 18, 2015, S. Li and Y. Tong leg.; 2 males, 2 females (SYNU-82), Singapore: Bukit Timah Nature Reserve, Bukit Timah Summit, Alt. 163 m, 1°21'16.65"N, 103°46'34.95"E, August 19, 2015, S. Li and Y. Tong leg.; 1 female (SYNU-83), Singapore: Bukit Timah Nature Reserve, Catchment Path, Alt. 107 m, 1°21'12.5"N, 103°46'50.6"E, August 20, 2015, S. Li and Y. Tong leg.; 2 females (SYNU-84), Singapore: Central Catchment Nature Reserve (off Mandai Lake Road), Alt. 39 m, 1°24'30.7"N, 103°46'51.3"E, August 31, 2015, S. Li and Y. Tong leg.; 2 males, 1 female (SYNU-90), Singapore: Pulau Ubin, Alt. 2 m, 1°25'18.0"N, 103°56'25.4"E, August 22, 2015, S. Li and Y. Tong leg.

Etymology. The specific epithet means “usable to cover a roof” in Latin, and refers to the long protruding extension on male clypeus; adjective.

Diagnosis. The new species can be distinguished from the congeneric species by the strongly protruding extension (spe) on male clypeus (Fig. 13I), the modifications on male chelicerae (Figs 13G, H, 15F) and the large, plate like sclerite (pls) in the female epigastric region (Fig. 14J, K).

Description. Male (holotype). Total length 1.26; carapace 0.73 length, 0.57 width; abdomen 0.65 length, 0.35 width. Habitus as in Fig. 13A, C, E. *Carapace*: pale orange, with brown egg-shaped patches behind eyes, ovoid in dorsal view, strongly elevated in lateral view, surface of elevated portion of pars cephalica smooth, sides strongly reticulate, fovea absent, lateral margin straight, smooth (Fig. 13B, D). *Clypeus*: anterior margin with strongly protruding extension (spe) (Fig. 13I). Carapace anterolateral corners with strongly sclerotized, triangular extension (ste) (Fig. 13D). *Eyes*: six, well developed, ALE largest, ALE circular, PME and PLE oval, posterior eye row procurved from both above and front, ALE separated by less than their radius, ALE-PLE separated by less than ALE radius, PME touching, PLE-PME touching. *Sternum*: longer than wide, pale orange, uniform, not fused to carapace, surface smooth, setae

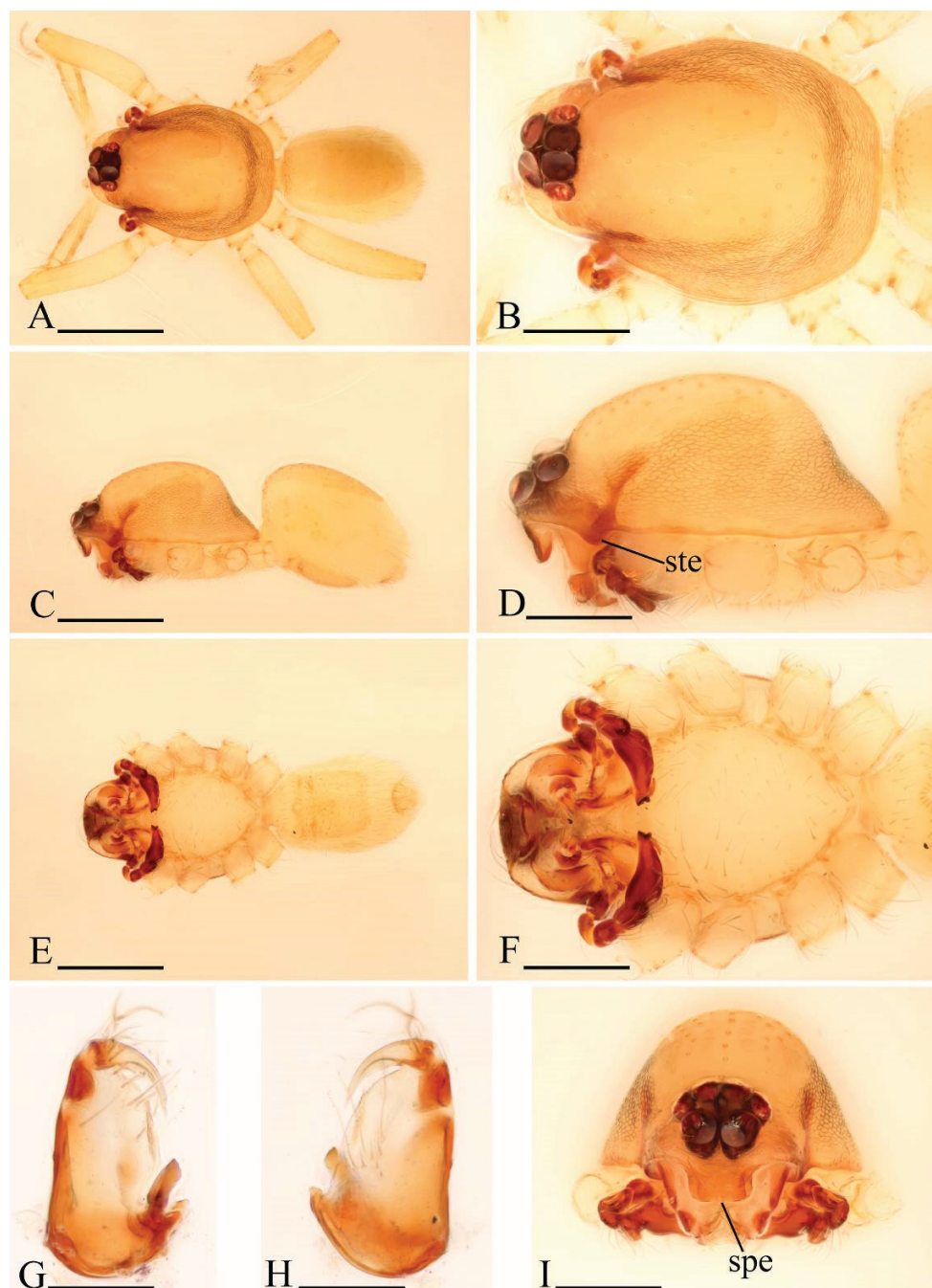


Figure 13. *Ischnothyreus tectorius* sp. n., male. **A, C, E** habitus, dorsal, lateral and ventral views **B, D, F, I** pro-soma, dorsal, lateral, ventral and anterior views **G, H** left chelicera, anterior and posterior views. Abbreviations: spe = strongly protruding extension; ste = sclerotized, triangular extension. Scale bars: **A, C, E** = 0.4 mm; **B, D, F, I** = 0.2 mm; **G, H** = 0.1 mm.

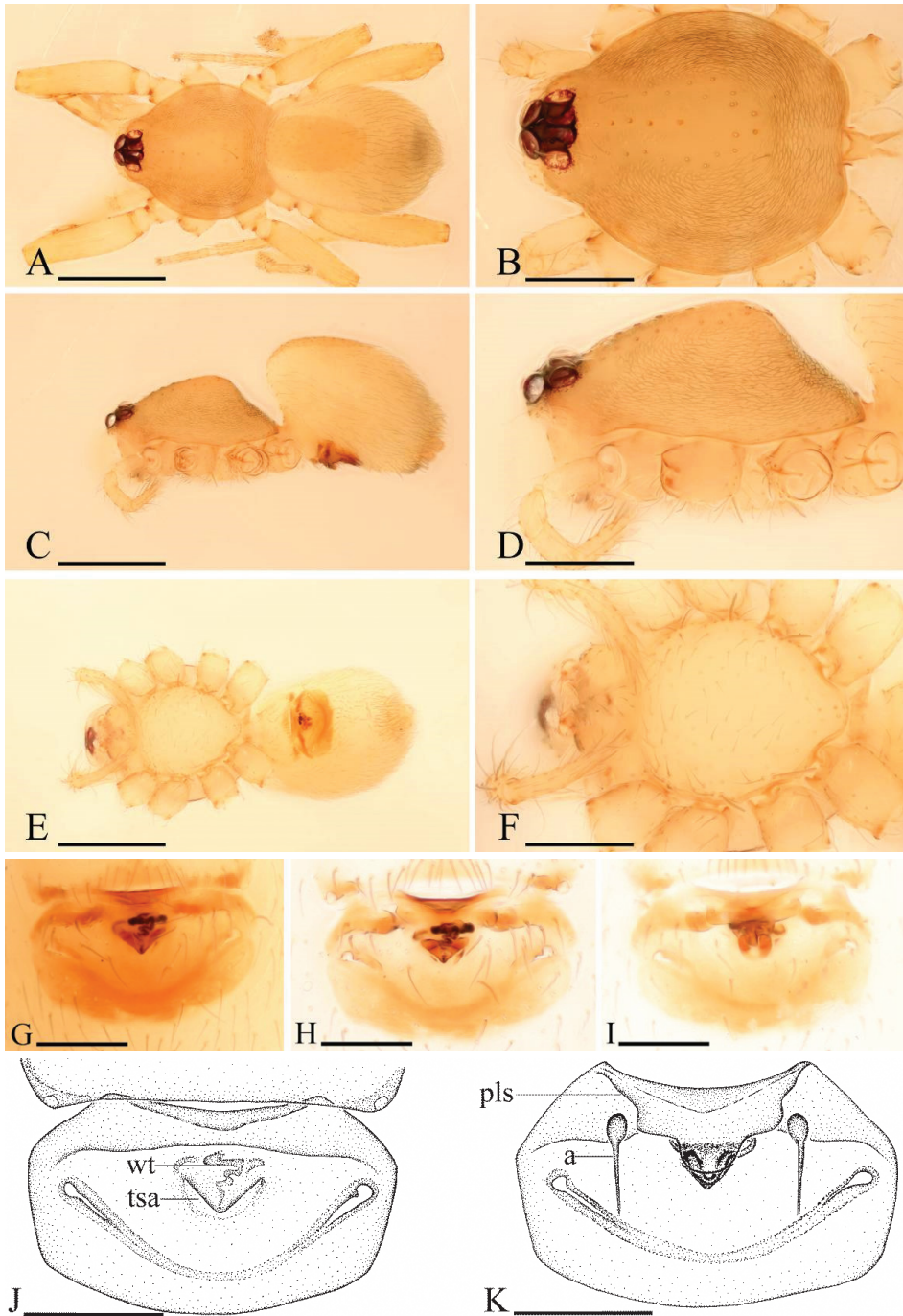


Figure 14. *Ischnothyreus tectorius* sp. n., female. **A, C, E** habitus, dorsal, lateral and ventral views **B, D, F** pro-soma, dorsal, lateral and ventral views **G, H, J** epigastric region, ventral view **I, K** epigastric region, dorsal view (**H, I** cleared in lactic acid). Abbreviations: a = apodeme; pls = plate-like sclerite; tsa = triangular-shaped atrium; wt = winding tube. Scale bars: **A, C, E** = 0.4 mm; **B, D, F** = 0.2 mm; **G–K** = 0.1 mm.

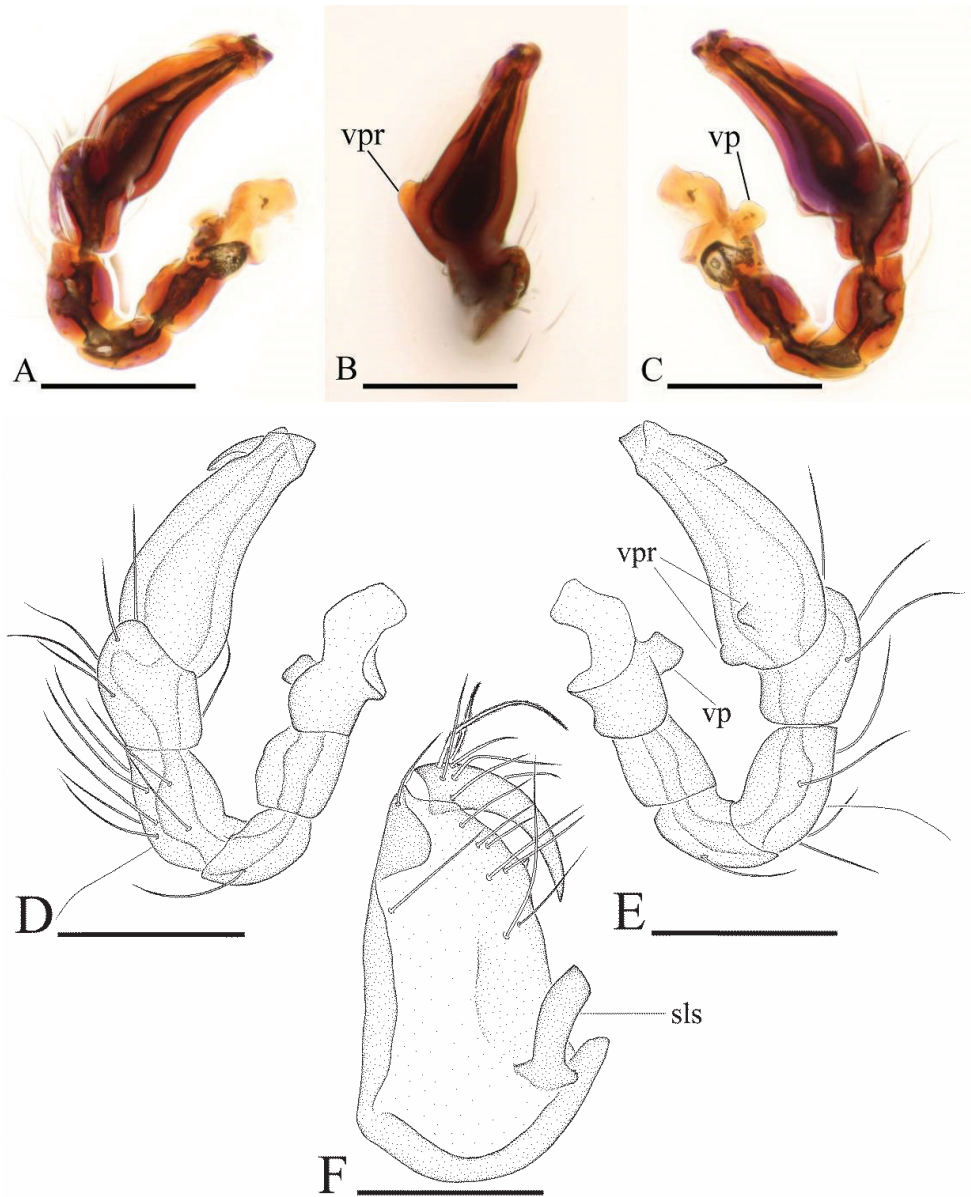


Figure 15. *Ischnothyreus tectorius* sp. n., male. **A, D** left palp, prolateral view **B** left palpal bulb, dorsal view **C, E** left palp, retrolateral view **F** left chelicerae, anterior view. Abbreviations: sls = scape-like sclerite; vp = ventral projection; vpr = ventral protuberance. Scale bars: 0.1 mm.

sparse. *Mouthparts*: chelicerae, endites and labium orange. Chelicerae straight, base of fang unmodified, strongly sclerotized at lateral margin of paturon, proximal part of paturon with a scape-like sclerite (sls) (Fig. 15F), fang groove with a small denticle. Labium rectangular, fused to sternum, anterior margin not indented at middle. Antero-

median tip of endites with one strong, tooth-like projection (Fig. 13E, F). *Abdomen*: ovoid, rounded posteriorly. Posterior spiracles connected by groove. Pedicel tube short, ribbed, scutum not extending far dorsal of pedicel. Dorsal scutum well sclerotized, pale orange, covering approximately 4/5 of abdomen length, 2/3 of abdomen width, fused to epigastric scutum, middle surface and sides smooth. Epigastric and postepigastric scutum well sclerotized, pale orange, fused, without posteriorly directed lateral apodemes. Dorsum setae present, light, needle-like. *Legs*: pale orange, femur I with two prolateral and two small retrolateral spines, tibia I with four pairs, metatarsus I with two pairs of long ventral spines. Leg II spination is similar to leg I except femur with only one prolateral and one retrolateral spine. Legs III and IV spineless. *Genitalia*: epigastric region with sperm pore large, circular, situated at level of anterior spiracles, anterior margin of sperm pore with a fringe of needle-like setae. Palp strongly sclerotized, right and left palps symmetrical, proximal segments brown, trochanter with ventral projection (vp) (Fig. 15C), cymbium brown, fused with bulb, bulb brown, more than two times as long as cymbium, tapering apically, with two small ventral protuberance (vpr) (Fig. 15E), distal part elongated, end stout (Fig. 15A, C, D, E).

Female (paratype). Total length 1.24; carapace 0.72 length, 0.44 width; abdomen 0.77 length, 0.56 width. Habitus as in Fig. 14A, C, E. As in male except as noted. *Carapace*: without any pattern, broadly oval in dorsal view. *Clypeus*: margin unmodified, ALE separated from edge of carapace by less than their radius. *Mouthparts*: chelicerae and endites unmodified. *Abdomen*: dorsal scutum covering less than 1/2 of abdomen length, less than 1/3 of abdomen width. Postepigastric scutum rectangular, strongly sclerotized. *Genitalia*: the posterior margin of the epigastric scutum is lined with numerous needle-like setae. The epigastric groove is narrow. From the middle of the strongly thickened margin of the postepigastric scutum runs a dark, winding tube posteriorly (wt), ending in a triangular-shaped atrium (tsa) (Fig. 14J); from dorsal view, a large, plate like sclerite (pls) covers the internal structures (Fig. 14K).

Distribution. Singapore.

Acknowledgements

We are indebted to Dr. Lena Chan of the National Biodiversity Centre, National Parks Board, Singapore (NParks) for supporting this joint project between Singapore and PRC arachnologists. We are grateful to NParks for permission to collect oonopids in the nature reserves and other protected areas in Singapore (NP/PR12-070 & NP/PR15-45a). We are also grateful to Cor Vink (Canterbury Museum, Christchurch, New Zealand), Karen Edward (The University of Western Australia, Perth, Australia), Miguel Richard (Natural History Museum, Bern, Switzerland), and Yvonne Kranz-Baltensperger (Natural History Museum, Bern, Switzerland) for their comments on the manuscript. This study was supported by the National Natural Science Foundation of China (NSFC-31372157, 31572237) and the State Key Laboratory of Forest and Soil Ecology (Grant No. LFSE2015-11) to Yanfeng Tong, by the National Natural Science Foundation of

China (NSFC-31272280, 31471960, 31530067) and the Southeast Asia Biodiversity Research Institute, Chinese Academy of Sciences (2015CASEABRI005, Y4ZK111B01) to Shuqiang Li, and by a Research Fellowship awarded in 2015 by National Biodiversity Centre, National Parks Board, Singapore, to Joseph K H Koh.

References

- Baehr BC, Harvey MS, Burger M, Thoma M (2012) The new Australasian goblin spider genus *Prethopalpus* (Araneae, Oonopidae). *Bulletin of the American Museum of Natural History* 369: 1–113. doi: 10.1206/763.1
- Edward KL, Harvey MS (2014) Australian goblin spiders of the genus *Ischnothyreus* (Araneae, Oonopidae). *Bulletin of the American Museum of Natural History* 389: 1–144. doi: 10.1206/865.1
- Eichenberger B, Kranz-Baltensperger Y (2011) New *Trilacuna* species from Thailand, Malaysia and Sumatra (Araneae, Oonopidae). *Zootaxa* 2823: 1–31.
- Eichenberger B, Kranz-Baltensperger Y, Ott R, Graber W, Nentwig W, Kropf C (2012) Morphology of new Indian/Indonesian *Gamasomorpha* and *Xestaspis* species (Araneae: Oonopidae). *Zootaxa* 3160: 1–68.
- Koch L (1873) *Die Arachniden Australiens*. Nürnberg 1: 369–472.
- Kranz-Baltensperger Y (2011) The oonopid spider genus *Ischnothyreus* in Borneo (Oonopidae, Araneae). *Zootaxa* 2939: 1–49.
- Kranz-Baltensperger Y (2012) Three new species of the oonopid spider genus *Ischnothyreus* (Araneae: Oonopidae) from Tioman Island (Malaysia). *Zootaxa* 3161: 37–47.
- Li S, Lin Y (2016) *Species Catalogue of China*. Volume 2. Animals. Invertebrates (I), Arachnida: Araneae. Science Press, Beijing, 549 pp.
- Murphy F, Murphy J (2000) *An introduction to the Spiders of Southeast Asia*. Malaysian Nature Society, Kuala Lumpur, 625 pp.
- National Parks Board (2015) Annual Report. <https://www.nparks.gov.sg/about-us/annual-reports> [accessed on 1 August, 2016]
- Platnick NI, Berniker L, Kranz-Baltensperger Y (2012) The goblin spider genus *Ischnothyreus* (Araneae, Oonopidae) in the New World. *American Museum Novitates* 3759: 1–32. doi: 10.1206/3759.2
- Simon E (1891) On the spiders of the island of St. Vincent. Part 1. *Proceedings of the Zoological Society of London* 1891: 549–575.
- Simon E (1893) *Histoire naturelle des araignées* (2nd eds). Vol. 1(2), Encyclopédie Roret, Paris, 257–488.
- Simon E (1905) Arachnides de Java, recueillis par le Prof. K. Kraepelin en 1904. *Mitteilungen aus dem Naturhistorischen Museum in Hamburg* 22: 49–73.
- Simon E (1907) Etude sur les araignées de la sous-section des Haplogynes. *Annales de la Société Entomologique de Belgique* 51: 246–264. doi: 10.5962/bhl.part.1584
- Simon E (1909) Etude sur les arachnides du Tonkin (1^{re} partie). *Bulletin Scientifique de la France et de la Belgique* 42: 69–147. doi: 10.5962/bhl.part.24151

- Song DX, Zhang JX, Li D (2002) A checklist of spiders from Singapore (Arachnida: Araneae). *Raffles Bulletin of Zoology* 50: 359–388.
- Thoma M, Kranz-Baltensperger Y, Kropf C, Graber W, Nentwig W, Frick H (2014) The new Southeast Asian goblin spider genus *Aposphragisma* (Araneae, Oonopidae): diversity and phylogeny. *Zootaxa* 3798: 1–86. doi: 10.11646/zootaxa.3798.1.1
- Thorell T (1887) Viaggio di L. Fea in Birmania e regioni vicine. II. Primo saggio sui ragni birmani. *Annali del Museo Civico di Storia Naturale di Genova* 25: 5–417.
- Thorell T (1890) Studi sui ragni Malesi e Papuani. IV, 1. *Annali del Museo Civico di Storia Naturale di Genova* 28: 1–419.
- Thorell T (1895) Descriptive catalogue of the spiders of Burma. London, 406 pp.
- Thorell T (1897) Viaggio di Leonardo Fea in Birmania e regioni vicine. LXXIII. Secondo saggio sui Ragni birmani. I. Parallelodontes. Tubitelariae. *Annali del Museo Civico di Storia Naturale di Genova* (2) 17[=37]: 161–267.
- Tong Y, Li S (2008) The oonopid spiders (Araneae: Oonopidae) from Hainan Island, China. *The Raffles Bulletin of Zoology* 56: 55–66.
- Tong Y, Li S (2013a) A new genus and species of oonopid spiders from Laos (Araneae, Oonopidae). *Acta Zootaxonomica Sinica* 38: 784–789.
- Tong Y, Li S (2013b) Six new species of oonopid spiders from Champasak, Laos (Araneae, Oonopidae). *Zootaxa* 3709: 71–88. doi: 10.11646/zootaxa.3709.1.3
- Tong Y, Li S (2013c) The first goblin spiders of the genus *Trilacuna* from Vietnam (Araneae, Oonopidae). *Zootaxa* 3709: 277–284. doi: 10.11646/zootaxa.3709.3.6
- World Spider Catalog (2016) World Spider Catalog. Natural History Museum Bern, online at <http://wsc.nmbe.ch> version 17.5 [accessed on 1 August, 2016]
- Xu YJ (1989) Key to Chinese Oonopidae and a new species of the genus *Ischnothyreus* from China. *Journal of the Huizhou Teachers College* 1: 17–21.

Lilioceris groehni sp. n.: the first authentic species of Criocerinae (Coleoptera, Chrysomelidae) from Baltic amber

Andris Bukejs¹, Michael Schmitt²

¹ Institute of Life Sciences and Technologies, Daugavpils University, Vienības Str. 13, Daugavpils, LV-5401, Latvia ² Ernst-Moritz-Arndt-Universität, Allgemeine & Systematische Zoologie, Soldmannstr. 14, 17489 Greifswald, Germany

Corresponding author: Michael Schmitt (michael.schmitt@uni-greifswald.de)

Academic editor: J. Santiago-Blay | Received 4 August 2016 | Accepted 9 September 2016 | Published 19 September 2016

<http://zoobank.org/8648005D-9B75-4905-A6A5-027830B5E95E>

Citation: Bukejs A, Schmitt M (2016) *Lilioceris groehni* sp. n.: the first authentic species of Criocerinae (Coleoptera, Chrysomelidae) from Baltic amber. ZooKeys 618: 67–77. doi: 10.3897/zookeys.618.10085

Abstract

Based on a single well-preserved specimen from Eocene Baltic amber, *Lilioceris groehni* sp. n. is described and illustrated using phase-contrast X-ray microtomography. It is the first described species of Criocerinae (Coleoptera: Chrysomelidae) from Baltic amber. A check-list of fossil Criocerinae is provided. Placement of *Crioceris pristiana* (Germar, 1813) is discussed, this species is removed from Criocerinae and placed in Coleoptera *incertae sedis*.

Keywords

Taxonomy, palaeontology, shining leaf beetles, new species, *Crioceris pristiana*, fossil resin, Tertiary, Eocene

Introduction

The subfamily Criocerinae (shining leaf beetles) contains ca. 1500 extant species (Schmitt 1996) in 20 genera (Seeno and Wilcox 1982), of which 211 species in 6 genera are recorded from the Palaearctic region (Schmitt 2010). The genus *Lilioceris* Reitter, 1913 comprises ca. 170 extant species distributed over the temperate, subtropical and tropical regions of the Palaearctis, Orientalis, Aethiopsis including Madagascar, and the Australis, and was introduced to North America by man (Monrós 1960, plus records from the Zoological Record up to present as taken from the Index to Organism Names).

Shining leaf beetles are rarely represented in fossil material and especially in Baltic amber (Table 1). Two fossil species from Baltic amber were mistakenly described within Criocerinae. *Electrolema baltica* Schaufuss, 1892 was described as member of Criocerinae but later transferred to Hispinae (Korschevsky 1939). According to modern classification (Staines 2012), it is placed in Gonophorini Chapuis, 1875 within Cassidinae. Another species, *Crioceris pristina* (Germar, 1813) originally described as *Criocerina* (Germar 1813) was mentioned within Criocerinae (e.g. Giebel 1856a, 1856b; Spahr 1981; Santiago-Blay 1994). In our opinion it is not a member of the Criocerinae (see Discussion).

Crioceris sp. and *Lema* sp. were mentioned from Eocene Baltic amber without detailed species descriptions (Hope 1836; Giebel 1856; Menge 1856; Scudder 1885, 1886, 1891; Handlirsch 1908; Klebs 1910; Bachofen-Echt 1949; Larsson 1978; Spahr 1981; Santiago-Blay 1994; Poinar 1999). In the current paper, the first extinct species of Criocerinae from Baltic amber is described, figured, and compared with extant species using phase-contrast X-ray microtomography.

Material and methods

The specimen is included in an amber piece that was polished by hand and faceted on their sides, allowing improved views of the included specimens. The material examined is deposited in the collection of the Geological-Palaeontological Institute of the University of Hamburg, Germany [GPIH], as part of the collection of Carsten Gröhn.

Observations were made using a Nikon SMZ 745T stereomicroscope. Photographs were taken using a Canon EOS 70D with a 100 mm macro lens, and a Canon EOS 5D with the Canon MP E 65 mm macro lens in a visionary digital bk plus lab system by Dun Inc. The microCT-images were produced by means of an Xradia Micro XCT-200 (Carl Zeiss X-ray Microscopy Inc.), using the 4x object lens units, at 30kV and 4W, with a pixel size of 5.36 µm. Tomography projections were reconstructed using the reconstruction software provided by XRadia. Volume rendering of image stacks was performed by using Amira 5.6.0 (FEI Visualization Science Group, Burlington, USA) using the “Volren” or “Votex” function.

Systematic Palaeontology

Chrysomelidae Latreille, 1802

Criocerinae Latreille, 1804

Criocerini Latreille, 1804

***Lilioceris* Reitter, 1913**

The specimen considered here was assigned to the family Chrysomelidae based on the pseudoteramorous tarsi and the lack of a rostrum and of antennae not inserted on

pronounced tubercles, to the subfamily Criocerinae because the prothorax does not bear side borders and the frons has distinct diverging grooves behind the antennal insertions, and to the genus *Lilioceris* based on (1) free tarsal claws and (2) divided vertex separated from the neck by a dorsal constriction.

***Lilioceris groehni* sp. n.**

<http://zoobank.org/FD228756-DF55-47E3-BDE4-F0D3937A8A1E>

Figs 1–5

Type material. Holotype: Nr. “C 8130” [GPIH]; female. A rather complete beetle (missing apical antennomere of left antenna, and tarsomeres 4 and 5 of left meso- and metatarsi) is included in a small, transparent yellow amber piece (length about 20 mm, width 12 mm, and maximum thickness 5 mm). Syninclusions: one specimen of *Nematocera* (Diptera), and few stellate Fagaceae trichomes (Figs 1 and 2).

Type strata. Baltic amber, mid-Eocene to Upper Eocene.

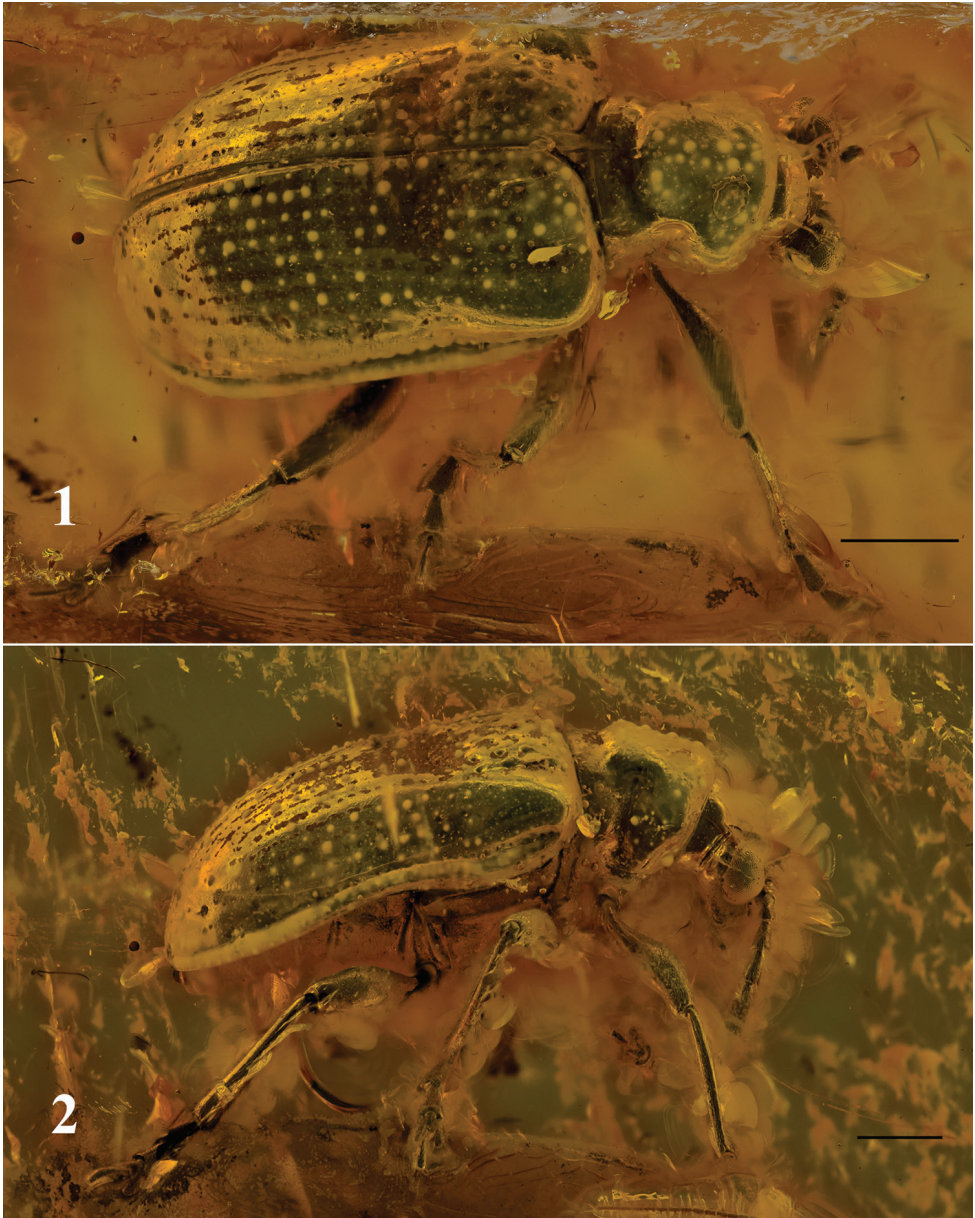
Type locality. Yantarny settlement (formerly Palmnicken), Sambian (Samland) Peninsula, the Kaliningrad region, Russia.

Differential diagnosis. Head, body, and elytra of *Lilioceris groehni* sp. n. appear unicolorous black and thus similar to the extant species *L. hitam* Mohamedsaid, 1990 from Borneo, which differs from the new species in (1) the shape of the pronotum (distinctly longer than wide with its constriction at the middle), (2) metaventrite glabrous in the middle, (3) pubescent scutellum, (4) impunctate elytra (with few moderately large punctures at base only), (5) vertex with sparse pubescence, (6) a distinct conical neck between head and pronotum, and (7) a larger body (10 mm).

Additionally, the extant species *L. lilii* Scopoli, 1763 and *L. merdigera* Linnaeus, 1758 from Baltic region differ from *L. groehni* sp. n. in having (1) a pronotum with a longitudinal row of punctures medially, (2) metaventrite, metepisternum and ventrites of abdomen almost glabrous or with very sparse pubescence, and (3) pronotum and elytra rufous to red.

Description. Holotype. Body length 7.1 mm, maximum width 4.1 mm; elongate, subparallel, moderately convex dorsally and ventrally, unicolorous black, glabrous dorsally.

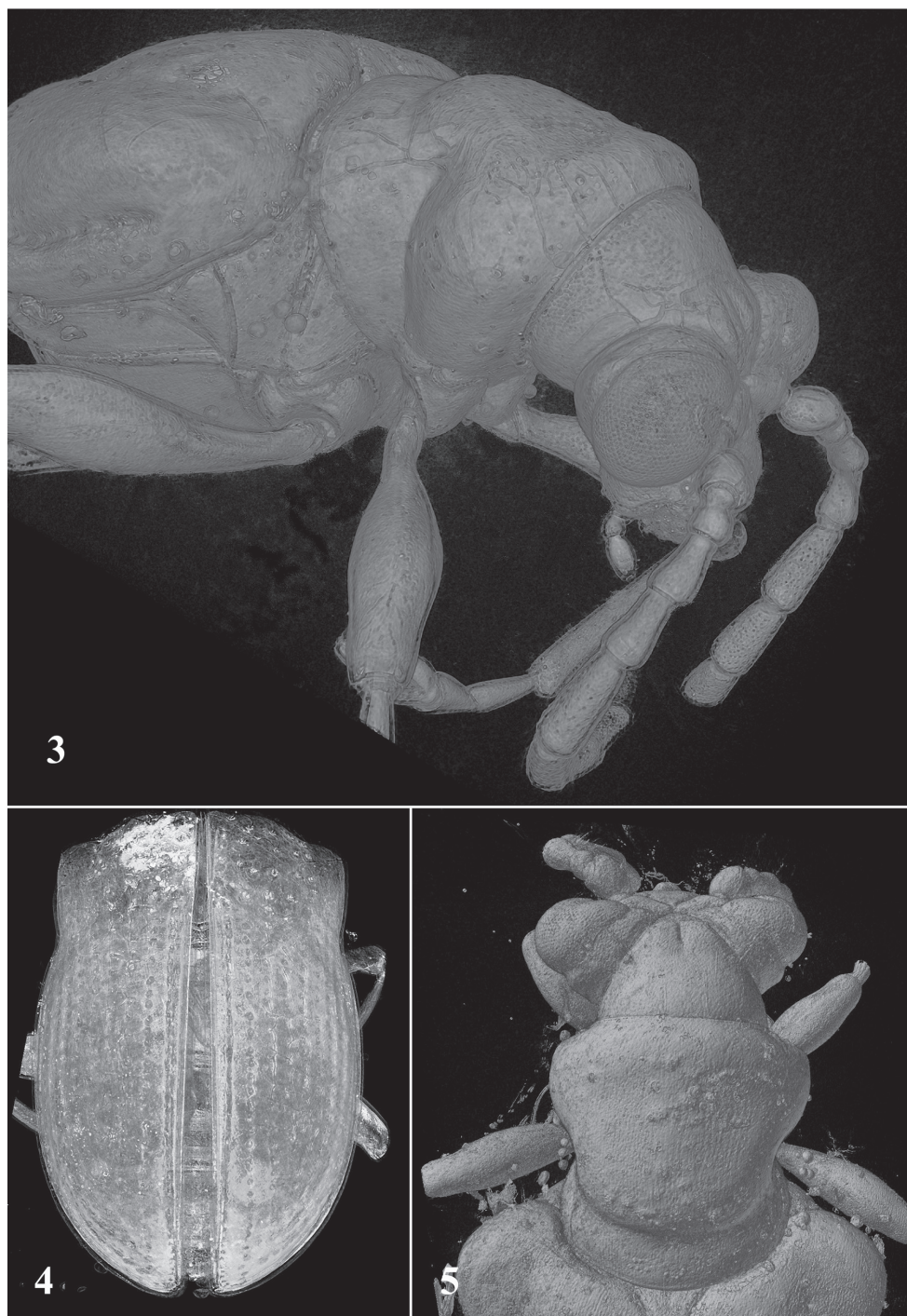
Head hypognathous, transverse, widest across eyes, together with eyes nearly as wide as pronotum, strongly constricted behind the eyes forming a neck (Fig. 3); shiny, hairless and without distinct punctures dorsally. Compound eyes large, strongly convex, deeply and acutely notched at antennal insertions; distance between eyes nearly as wide as transverse diameter of one eye. Frontal grooves deep, crossed forming X. Vertex convex, hairless, with median longitudinal groove. Genae large, with sparse pubescence. Antennae poorly visible because of a beetle location in amber piece. Antennae robust, covered with fine pubescence, moderately long, extending nearly to basal one-fourth of elytra, slightly widened apically; antennomere 2 shortest, about 0.4 times as long as antennomere 3, antennomere 4 slightly longer than antennomere



Figures 1–2. *Liliocerus groehni* sp. n., holotype: **1** habitus, dorsal view **2** habitus, lateral view. Scale bars: 1 mm.

3, antennomeres 5–7 subequal in length, antennomere 5 about 1.2 times as long as antennomere 4.

Pronotum nearly as long as wide, deeply constricted medially, distinctly narrower than elytra, widest in anterior one-third; impunctate, shiny; disc flattened, with an arcuate transverse depression subbasally (Figs 3 and 4). Anterior margin straight me-



Figures 3–5. *Liliocerus groehni* sp. n., holotype, microCT images: **3** habitus, fronto-lateral view, showing the pronounced arcuate constriction behind the disk of the pronotum **4** elytra, dorsal view **5** details of head and prothorax, dorsal view. Not reproduced to the same scale.

dially; posterior margin convex; lateral margins rounded anteriorly and strongly constricted just behind middle; all margins not bordered. Anterior and posterior angles obtusely rounded.

Scutellum large, triangular; apparently hairless and impunctate. Elytra subparallel, widest in the middle, about 1.5 times as long as wide; humeri prominent. Elytral punctures small and dense (in basal one-third deeper), arranged in rows; scutellar row present, short; intervals flat, only at apices weakly convex.

Metaventrite with sparse, fine pubescence; metepisternum and metepimeron densely covered with fine, short, semierect pubescence. Abdomen with sparse, fine pubescence.

Legs moderately long, covered with fine pubescence. Femora spindle-shaped; tibiae slightly curved, dilated apically. Tarsi long, about 0.7 times as long as tibia; metatarsomeres 1–2 subequal in length, distinctly dilated apically, metatarsomere 3 deeply bilobed, metatarsomere 4 subcylindrical, narrow. Tarsal claws free, not fused at base.

The interior of the abdomen does not contain any identifiable structure, as revealed by the microCT-analysis. No traces of an aedeagus could be found, and none of the smaller particles – all covered with homogeneous material – could be addressed as the spermatheca.

Derivatio nominis. This new species is named after Carsten Gröhn (Glinde, Germany) – he enabled us to study this specimen.

Discussion

The specimen of *Liliocerus groehni* sp. n. appears externally complete. However, the fact that we found no traces of internal structures in the abdomen, especially of an aedeagus, does most probably mean that (1) the specimen was a female, and (2) that it remained openly accessible for scavengers and/or detritivores before it was covered by resin. This could also provide a possible reason for its black appearance as the dead individual might have been exposed to humic acids before being fossilised. If this should be the case, the live animal had most probably a habitus similar to the extant lily beetles. Actually, *L. groehni* sp. n. is hardly distinguishable from extant *Liliocerus*-species. In this respect, the new species is quite normal. Hennig (1966) wrote that it is „a long known fact” [„eine altbekannte Tatsache”] that the morphological differences between fossils from Baltic amber and their extant relatives are only minute”.

Santiago-Blay (1994) mistakenly listed two fossil species as members of Criocerinae: *Lema pervetusta* Cockerell, 1921 and *Lema pulchella* Förster, 1891. *Lema pervetusta* was described from Bridgerian lacustrine shale (Eocene, 50.3–48.6 Ma) of the Green River Formation of Colorado, USA (Cockerell 1921), but according to Linsley (1942) this species belongs to the longhorn beetle genus *Clytus* Laicharting, 1784 (Cerambycidae). *Lema pulchella* was described from Oligocene lacustrine (33.9–28.4 Ma) of Riedisheim, Mulhouse, France (Förster 1891), but according to Théobald (1937) it belongs to the weevil genus *Phyllobius* Germar, 1824 (Curculionidae).

Few Quaternary sub-fossil records contain specimens of the extant species: *Lema cyanella* (Linnaeus, 1758) from La Taphanel, Massif Central, France (Ponel and Coope

Table 1. Check-list of records of fossil and sub-fossil Criocerinae.

Taxon	References	Fossil Type	Locality	Age
Criocerinae	Bachofen-Echt 1949; Handlirsch 1925; Spahr 1981	Baltic amber	Kaliningrad region (Russia)	37.2–33.9 Ma
Criocerinae	Hayashi et al. 2002	poorly lithified peat	Mizozono Formation, Yoshimatsusho, Kagoshima Prefecture (Japan)	0.1–0.0 Ma
<i>Crioceridea dubia</i>	Wickham 1912, 1913, 1914a, 1920; Santiago-Blay 1994	lacustrine shale	Florissant, Colorado (USA)	37.2–33.9 Ma
<i>Crioceris margarum</i>	Oustalet 1874; Handlirsch 1908; Thébald 1937; Santiago-Blay 1994	lacustrine shale	Aix-en-Provence (France)	28.4–23.0 Ma
<i>Crioceris vetusta</i>	Heer 1865 (<i>Lema</i>); Handlirsch 1908 (<i>Lema</i>); Cockerell 1921	lacustrine shale	Oeningen (Germany)	12.7–11.6 Ma
<i>Crioceris</i> sp.	Hope 1836; Giebel 1856a; Menge 1856; Scudder 1885, 1886, 1891; Handlirsch 1908; Klebs 1910; Bachofen-Echt 1949; Spahr 1891	Baltic amber	Kaliningrad region (Russia)	37.2–33.9 Ma
<i>Lema evanescens</i>	Wickham 1910, 1913, 1914a, 1920	lacustrine shale	Florissant, Colorado (USA)	37.2–33.9 Ma
<i>Lema fortior</i>	Wickham 1914a, 1920	lacustrine shale	Florissant, Colorado (USA)	37.2–33.9 Ma
<i>Lema lesqueruixi</i>	Wickham 1914b, 1920	lacustrine shale	Florissant, Colorado (USA)	37.2–33.9 Ma
<i>Lema tumulata</i>	Heyden and Heyden 1865; Handlirsch 1908	terrestrial siliclastic	Salzhäusen (Germany)	15.9–11.6 Ma
<i>Lema</i> sp.	Scudder 1885, 1886, 1891; Helm 1896; Handlirsch 1908; Larsson 1978; Spahr 1981; Poinar 1999	Baltic amber	Kaliningrad region (Russia)	37.2–33.9 Ma
<i>Lema</i> sp.	Pearson 1962	lacustrine shale	West Cumberland (England)	0.1–0.0 Ma
(?) <i>Lema</i> sp.	Kiselev and Nazarov 2009	unlithified siliclastic sediments	Achchagyai-Allaikha Yana–Indigirka Lowland, nord-east Siberia (Russia)	0.1–0.0 Ma
<i>Lilioceris groehni</i>	present paper	Baltic amber	Kaliningrad region (Russia)	37.2–33.9 Ma
<i>Coleoptera incertae sedis</i>				
<i>Crioceris pristina</i>	Gernar 1813 (<i>Criocerina</i>); Giebel 1856a, 1856b; Schlechrendal (<i>Criocerina</i>); Handlirsch 1908; Spahr 1981; Santiago-Blay 1994; Poinar 1999	Baltic amber	Kaliningrad region (Russia)	37.2–33.9 Ma

1990); *Lema trilinea* White, 1981 from late Quaternary Kaetan Cave, Colorado Plateau, Colorado, USA (Elias and Van Devender 1992); and *Oulema obscura* (Stephens, 1831) from the Holocene of Belarus (Nazarov 1984). These records are not mentioned in the current list (Table 1). None of the records of “Criocerinae”, “*Crioceris* sp.”, or “*Lema* sp.” from Baltic amber listed in Table 1 can be assigned to a certain species of shining leaf beetles.

Germar (1813) described *Criocerina pristina* from Baltic amber. Later this species was mentioned as *Crioceris pristina* (Germar, 1813) within Criocerinae (e.g. Giebel 1856a, 1856b; Spahr 1981; Santiago-Blay 1994). The correct subfamily and family placement of *Crioceris pristina* is doubtful in our opinion. According to the original description (Germar 1813: 14), this fossil species has antennae with a club (similar as in members of the genus *Anobium*) [“... Die Fühler von etwas mehr als halber Körperlänge, roth, and der Spitze dunkler, das erste Glied kurz und dick, vor den Augen auf der Stirn eingesetzt, das folgende Glied klein, kugelförmig, die nun folgenden sechs Glieder sehr klein und dicht zusammengedrängt, dass sie als blosse Ringe erscheinen, die drey letzten Glieder lang und dicker, fast wie bey *Anobium* gebaut, sie machen zusammen zwei Drittheil der ganzen Fühlerlänge aus ... ”]. In addition, Germar gave the length of this specimen as $1\frac{1}{8}$ lines = 2.54 mm. This would be an extremely low value for a species of *Lilioceris*. All extant species are described as being longer than 5 mm. Germar mentioned that his *Criocerina pristina* resembled „*Crioceris testacea* Fabr.”, of which he said it were six times larger – i.e. ca. 1.5 cm. The species Fabricius described as *Crioceris testacea* (Fabricius 1787: 87/88) is currently listed under *Aulacophora indica* (Gmelin, 1790), Galerucinae (Mohamedsaid 2009), and its lectotype is depicted in Lee and Beenen (2015, Figs 42 & 43). We conclude that „*Crioceris pristina*” is actually not a criocerine beetle nor a member of the family Chrysomelidae. Instead, we suggest that it should be better placed as Coleoptera *incertae sedis*. This conclusion leaves *Lilioceris groehni* sp. n. as the first beetle species from Baltic amber that we can classify with certainty as a member of the Coleoptera Chrysomelidae Criocerinae.

Acknowledgements

The authors are grateful to Carsten Gröhn (Glinde, Germany) for the loan of interesting fossil material, to Vitalii I. Alekseev (Kaliningrad, Russia) for valuable suggestions and constructive advice, to Peter Michalik (Greifswald, Germany) for invaluable help with macro-imaging and the microCT-analysis, and the German Science Foundation (DFG) for financial support (INST 292/119-1 FUGG, INST 292/120-1 FUGG).

References

Bachofen-Echt A (1949) Der Bernstein und seine Einschlüsse. Springer, Wien, 204 pp. doi: 10.1007/978-3-7091-2303-4

- Cockerell TDA (1921) Eocene Insects from the Rocky Mountains. Proceedings of the United States National Museum 57: 233–260. doi: 10.5479/si.00963801.57-2313.233
- Elias SA, Van Devender TR (1992) Insect fossil evidence of Late Quaternary environments in the northern Chihuahuan desert of Texas and New Mexico: comparisons with the paleobotanical record. The southwestern Naturalist 37(2): 101–116. doi: 10.2307/3671658
- Fabricius JC (1787) Mantissa insectorum sistens eorum species nuper detectas adiectis characteribus genericis, differentiis specificis, emendationibus, observationibus, vol. 2. C.G. Proft, Copenhagen, 382 pp. doi: 10.5962/bhl.title.36471
- Förster B (1891) Die Insekten der “Plattigen Steinmergels” von Brunstatt. Abhandlungen zur Geologischen Specialkarte von Elsass-Lothringen 3: 335–593.
- Germar EF (1813) Insekten in Bernstein eingeschlossen, beschrieben aus dem akademischen Mineralien—Cabinet zu Halle. Magazin der Entomologie 1: 11–18.
- Giebel CGA (1856a) Fauna der Vorwelt mit steter Berücksichtigung der lebenden Thiere. Gliederthiere. Zweite Band: Insecten und Spinnen. F.A. Brockhaus, Leipzig, XVII + 511 pp.
- Giebel CGA (1856b) Geologische Übersicht der vorweltlichen Insecten. Zeitschrift für die gesammten Naturwissenschaften 8: 174–188.
- Handlirsch A (1908) Die fossilen Insekten und die Phylogenie der rezenten Formen. Ein Handbuch für Paläontologen und Zoologen. Wilhelm Engelmann, Leipzig, IX + 1430 pp.
- Handlirsch A (1925) Paläontologie. In: Schröder CWM (Ed.) Handbuch der Entomologie, Volume 3. Gustav Fischer, Jena, 117–306.
- Hayashi M, Yahiro K, Kitabayashi E (2002) Late Pleistocene insects from the Mizozono Formation in Yoshimatsu-cho, Kagoshima Prefecture, Japan. Bulletin of the Mizunami Fossil Museum 29: 161–168.
- Heer O (1865) Die Urwelt der Schweiz. Zürich, Friedrich Schultheß, 622 pp.
- Helm O (1896) Beiträge zur Kenntniss der Insecten des Bernsteins. Schriften der Naturforschenden Gesellschaft in Danzig (Anlage C) N. S. 9: 220–231.
- Hennig W (1966) *Fannia scalaris* Fabricius, eine rezente Art im Baltischen Bernstein? (Diptera: Muscidae). Stuttgarter Beiträge zur Naturkunde Serie A (Biologie) 150: 1–12.
- Heyden C, Heyden L (1865) Fossile Insekten aus der Braunkohle von Salzhausen. Palaeontographica 14(1): 31–35.
- Hope FW (1836) Observations on succinic insects. The Transactions of the Royal Entomological Society of London 1: 133–147. doi: 10.1111/j.1365-2311.1838.tb00157.x
- Index to Organism Names: <http://www.organismnames.com/> [accessed August 1, 2016]
- Kiselev SV, Nazarov VI (2009) Late Cenozoic insects of northern Eurasia. Paleontological Journal 43(7): 732–850. doi: 10.1134/S0031030109070016
- Klebs R (1910) Über Bernsteineinschlüsse in allgemeinen und die Coleopteren meiner Bernsteinsammlung. Schriften der Physikalisch-ökonomischen Gesellschaft zu Königsberg 51: 217–242.
- Korschensky R (1939) Abbildungen und Bemerkungen zu vier Schaufuß’schen Coleopteren aus dem deutschen Bernstein. Arbeiten über morphologische und taxonomische Entomologie aus Berlin-Dahlem 6(1): 11–12.
- Larsson SG (1978) Baltic Amber – a Palaeobiological Study. Volume 1. Scandinavian Science Press Ltd., Klampenborg, 192 pp.

- Lee C-F, Beenen R (2015) Revision of the genus *Aulacophora* from Taiwan (Coleoptera: Chrysomelidae: Galerucinae). *Zootaxa* 3949(2): 151–190. doi: 10.11646/zootaxa.3949.2.1
- Linsley EG (1942) A review of the fossil Cerambycidae of North America. *Proceedings of the New England Zoölogical Club* 21: 17–42.
- Menge A (1856) Lebenszeichen vorweltlicher, im Bernstein eingeschlossener Thiere. Programm der öffentlichen Prüfung der Schüler der Petrischule. A.W. Kafemann, Danzig, 32 pp.
- Mohamedsaid MS (1990) A New Species of *Lilioceris* from Sabah, Malaysia (Chrysomelidae: Criocerinae). *Entomological Review of Japan* 45(2): 93–95.
- Monrós F (1960) Los Géneros de Chrysomelidae, *Opera Lilloana* III (1959), Tucumán, 337 pp.
- Nazarov VI [Назаров ВИ] (1984) Реконструкция ландшафтов Белоруссии по палеоэнтомологическим данным (антропоген) [Reconstruction of the landscapes of Byelorussia on the basis of palaeoentomological data]. *Труды Палеонтологического института Академии наук СССР* [Trudy paleontologicheskogo instituta Akademii Nauk SSSR] 205: 1–104. [In Russian]
- Oustalet ME (1874) Recherches sur les insectes fossiles des terraines Tertiaires de la France. Deuxième partie. Insectes fossiles d'Aix en Provence. *Annales des Sciences Géologiques* 5(2): 1–347.
- Pearson RG (1962) The Coleoptera from a late-glacial deposit at St. Bees, West Cumberland. *Journal of Animal Ecology* 31: 129–150. doi: 10.2307/2335
- Poinar GO Jr. (1999) Chrysomelidae in fossilized resin: behavioural inferences. In: Cox ML (Ed.) *Advances in Chrysomelidae Biology*. Backhuys Publishers, Leiden, the Netherlands, 1–16.
- Ponel P, Coope GR (1990) Late glacial and Early Flandrian Coleoptera from La Taphanel, Massif Central. France: climatic and ecological interpretations. *Journal of Quaternary Science* 5: 235–249. doi: 10.1002/jqs.3390050306
- Santiago-Blay JA (1994) Paleontology of leaf beetles. In: Jolivet PHA, Cox ML, Petitpierre E (Eds) *Novel Aspects of the Biology of Chrysomelidae*. Kluwer Academic Publishers, the Netherlands, 1–68. doi: 10.1007/978-94-011-1781-4_1
- Schaufuss C (1892) Preussens Bernstein-Käfer. Neue Formen aus der Helm'schen Sammlung im Danziger Provinzialmuseum. *Berliner Entomologische Zeitschrift* 36(1): 53–64. doi: 10.1002/mmnd.18910360111
- Schlechtendal D (1888) Mittheilungen über die in der Sammlung aufbewahrten Originale zu Germar's: "Insekten in Bernstein eingeschlossen" mit Rücksicht auf Giebels "Fauna der Vorwelt". *Zeitschrift für Naturwissenschaften* 61: 473–491.
- Schmitt M (1996) The phylogenetic system of the Chrysomelidae - history of ideas and present state of knowledge. In: Jolivet PHA, Cox ML (Eds) *Chrysomelidae Biology, Volume 1: The Classification, Phylogeny and Genetics*. SPB Publishing, the Netherlands, 57–96.
- Schmitt M (2010) Criocerinae. In: Löbl I, Smetana A (Eds) *Catalogue of Palaearctic Coleoptera, Volume 6*. Appollo Books, Stenstrup, 359–368.
- Scudder SH (1885) Insecta. Insekten. In: Zittel KA (Ed.) *Handbuch der Palaeontologie, I Abtheilung. Palaeozoologie, II Band. Mollusca und Arthropoda*. Oldenbourg, München & Leipzig, 747–831.

- Scudder SH (1886) Systematic review of our present knowledge of fossil insects, including Myriapods and Arachnids. Bulletin of the United States geological Survey 31: 1–128.
- Scudder SH (1891) Index to the known fossil insects of the world including Myriapods and Arachnids. Bulletin of the United States geological Survey 71: 1–744.
- Seeno TN, Wilcox JA (1982) Leaf beetle genera (Coleoptera: Chrysomelidae). Entomograph 1: 1–221.
- Spahr U (1981) Systematischer Katalog der Bernstein und Kopalkäfer (Coleoptera). Stuttgarter Beiträge zur Naturkunde, Serie B (Geologie und Paläontologie) 80: 1–107.
- Staines CL (2012) Catalog of the hispines of the world (Coleoptera: Chrysomelidae: Cassidinae). http://entomology.si.edu/Collections_Coleoptera-Hispines.html
- Théobald N (1937) Les insectes fossiles des terrains oligocènes de France. PhD thesis, Imprimerie George Thomas, University of Nancy, France.
- Wickham HF (1910) New fossil Coleoptera from Florissant, with notes on some already described. American Journal of Science 29: 47–51. doi: 10.2475/ajs.s4-29.169.47
- Wickham HF (1912) A report of some recent collections of fossil Coleoptera from the Miocene Shale of Florissant. Bulletins from the Laboratory of Natural History of the State University of Iowa 6(3): 1–38.
- Wickham HF (1913) Fossil Coleoptera from the Wilson Ranch near Florissant, Colorado. Bulletins from the Laboratory of Natural History of the State University of Iowa 6(4): 3–29.
- Wickham HF (1914a) New Miocene Coleoptera from Florissant. Bulletin of the Museum of Comparative Zoology, Harvard College 53: 423–494.
- Wickham HF (1914b) Twenty new Coleoptera from the Florissant Shales. Transactions of the American Entomological Society 40: 257–270.
- Wickham HF (1920) Catalogue of the North American Coleoptera described as fossils. In: Leng CW (Ed.) Catalogue of the Coleoptera of America North of Mexico. John D. Sherman Jr., Mount Vernon, N.Y., 349–365.

***Lenomyrmex hoelldobleri*: a new ant species discovered in the stomach of the dendrobatid poison frog, *Oophaga sylvatica* (Funkhouser)**

Christian Rabeling¹, Jeffrey Sosa-Calvo¹, Lauren A. O'Connell², Luis A. Coloma^{3,4},
Fernando Fernández⁵

1 Department of Biology, University of Rochester, Rochester, NY 14627, USA **2** FAS Center for Systems Biology, Harvard University, Cambridge, MA 02138, USA **3** Centro Jambatu de Investigación y Conservación de Anfibios, Fundación Otonga, San Rafael, Quito, Ecuador **4** Universidad Regional Amazónica Ikiam, Muyuna, Tena, Ecuador **5** Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Apartado 7495, Bogotá D.C., Colombia

Corresponding author: Christian Rabeling (crabeling@gmail.com)

Academic editor: B. Fisher | Received 28 June 2016 | Accepted 31 August 2016 | Published 19 September 2016

<http://zoobank.org/78EE84EC-2B15-4059-9050-73D3A90BA725>

Citation: Rabeling C, Sosa-Calvo J, O'Connell LA, Coloma LA, Fernández F (2016) *Lenomyrmex hoelldobleri*: a new ant species discovered in the stomach of the dendrobatid poison frog, *Oophaga sylvatica* (Funkhouser). ZooKeys 618: 79–95. doi: 10.3897/zookeys.618.9692

Abstract

The ant genus *Lenomyrmex* was recently discovered and described from mid to high elevation rainforests in southern Central and northwestern South America. *Lenomyrmex* currently consists of six described species, which are only rarely collected. Here, we add a new species, *Lenomyrmex hoelldobleri* **sp. n.**, which was discovered in a stomach content sample of the dendrobatid frog, *Oophaga sylvatica*, from northwestern Ecuador. *Lenomyrmex hoelldobleri* can be distinguished from other species in the genus by the presence of a well-developed petiolar node, whereas in all other species the node of the petiole is ill-defined. In addition to the shape of the petiolar node, *L. hoelldobleri* can be distinguished from the morphologically similar *L. costatus* by (i) the presence of the metanotal suture, (ii) the direction of the striae on dorsum of propodeum (concentrically transverse in *L. hoelldobleri*, longitudinal in *L. costatus*), (iii) the finely striate dorsum of postpetiole, (iv) its larger size, and (v) distinctly darker coloration. We also describe the gyne of *Lenomyrmex foveolatus*. This collection record from northwestern Ecuador extends the geographic distribution of *L. foveolatus* 400 km south from its previous record in Colombia. A revised taxonomic key to the workers and gynes of all described *Lenomyrmex* species is provided. We discuss the taxonomic relationship of *L. hoelldobleri* to other species in the genus and its biology based on the limited information that is currently available. Finally, we briefly discuss the feeding ecology of dendrobatid poison frogs in the context of providing a valuable source of rarely collected and cryptic new ant species.

Resumen

El género de hormigas *Lenomyrmex* fue recientemente descubierto y descrito de bosques lluviosos tropicales de mediana a gran altitud en el sur de Centro América y del noroeste de Sur América. El género *Lenomyrmex* está actualmente compuesto de seis especies, las cuales son raramente colectadas. En este artículo, agregamos una especie nueva, *Lenomyrmex hoelldobleri* **sp. n.**, que fue descubierta en una muestra de contenido estomacal de la rana dendrobátida, *Oophaga sylvatica*, colectada en el noroeste de Ecuador. *Lenomyrmex hoelldobleri* se puede distinguir de las otras especies del género por la presencia del nodo del pecíolo bien desarrollado, mientras que en todas las demás especies del género el nodo del pecíolo está mal definido o ausente. Además de la forma del nodo peciolar, *L. hoelldobleri* se puede distinguir de *L. costatus* por (i) la presencia de la sutura metanotal, (ii) la dirección de las estrías en el dorso del propodeo (concéntricamente transversal, en *L. hoelldobleri*, longitudinal en *L. costatus*), (iii) el dorso del postpecíolo finamente estriado, (iv) su mayor tamaño, y (v) la coloración más oscura. También se describe la reina de la especie *Lenomyrmex foveolatus*. Esta colección del noroeste de Ecuador amplía la distribución geográfica de *L. foveolatus* 400 kilómetros al sur de su registro previo en Colombia. Se presenta una clave taxonómica revisada para las obreras y reinas de todas las especies descritas de *Lenomyrmex*. Se discute la relación taxonómica de *L. hoelldobleri* con otras especies del género y su biología con base a la información limitada que está disponible actualmente. Finalmente, discutimos brevemente la ecología de la alimentación de las ranas venenosas dendrobátidas en el contexto de ser una valiosa fuente de especies de hormigas crípticas, nuevas y raramente recolectadas.

Keywords

Formicidae, Dendrobatidae, feeding ecology, myrmecophagy, cryptic species

Introduction

The subfamily Myrmicinae is the most diverse clade of ants with currently more than 6,600 species, which is roughly equivalent to half the number of all described ant species (Bolton 2016). Within the past two decades ten new myrmicine genera and many more species have been discovered and described from the New World, including the extant genera *Cryptomyrmex*, *Cyatta*, *Diaphoromyrma*, *Dolopomyrmex*, *Kalathomyrmex*, *Kempfidris*, *Lenomyrmex*, *Mycetagroicus*, *Patagonomyrmex*, and *Tropidomyrmex*, testifying to the enormous diversity of this ant subfamily (Sosa-Calvo et al. 2013, and references therein; Fernández et al. 2014, Johnson and Moreau 2016). The myrmicine ants likely originated some 100 Million years ago during the late Cretaceous and the species in this group dispersed to all major ecosystems around the world (Ward et al. 2015). In addition to their hyperdiversity, vast geographic distribution, and old age of the clade, myrmicine ants also occupy diverse ecological niches (Hölldobler and Wilson 1990). Generalist predators and scavengers are common in speciose genera, such as *Crematogaster*, *Monomorium*, *Myrmica*, *Pheidole*, *Solenopsis*, and *Tetramorium*. In addition, highly specialized feeding habits originated in multiple myrmicine clades during the Paleocene and potentially contributed to the species richness and ecological success of these lineages. Especially noteworthy are the intricate behaviors of the seed harvesting ants, the fungus-growing ants, and the highly predaceous dacetine ants, which were expertly reviewed in Hölldobler and Wilson's (1990) landmark monograph "The Ants".

Just prior to the turn of the millennium, Fernández and Palacio (1999) described the myrmicine genus *Lenomyrmex* from the Neotropical region. *Lenomyrmex* ants are rarely collected and seven species are currently known from this genus, including *Lenomyrmex hoelldobleri* sp. n., the species described here. The geographic distribution of *Lenomyrmex* extends from Costa Rica in the North to southwestern Ecuador in the South, and only *L. inusitatus* is found on the eastern slope of the Andes (Fernández and Palacio 1999, Fernández 2001, Fernández and Sendoya 2004, Longino 2006, Delsinne and Fernández 2012). So far, all *Lenomyrmex* species were found in moist tropical rainforests, associated with medium and high elevation between 500 and 1800 meters above sea level (Longino 2006, Delsinne and Fernández 2012). The slender, elongate, and highly conspicuous mandibles with minute peg-like denticles are a synapomorphy of all *Lenomyrmex* species, suggesting specialized predatory habits (Fernández and Palacio 1999). Unfortunately, the feeding behavior of these rather cryptic ants was never observed and the prey organisms *Lenomyrmex* feeds on are unknown. *Lenomyrmex* appears to be a close relative of *Daceton* trap-jaw ants, which are both part of a monophyletic group of specialized predators (Ward et al. 2015).

Here, we describe the new species *Lenomyrmex hoelldobleri* sp. n. from northwest Ecuador (Fig. 1), which was discovered in stomach content samples of the dendrobatid poison frog, *Oophaga sylvatica*. We also diagnose the gyne of *L. foveolatus* (Fig. 3), which also fell prey to *O. sylvatica*. This new record of *L. foveolatus* from northwest Ecuador expands the known geographic distribution range of this species from Colombia to Ecuador (Fig. 4). Many amphibians, including species of the aposematic poison frogs in the family Dendrobatidae, and non-avian reptiles are known to be specialized predators of ants (Weber 1938, Darst et al. 2005, Esteves et al. 2008, Sosa-Calvo 2015), and therefore they provide interesting sources of rarely collected and new arthropod species. Dendrobatid poison frogs sequester alkaloids that are found in their skin toxins from their diet (Daly et al. 2000, Saporito et al. 2004, 2007, Darst et al. 2005, McGugan et al. 2016), and therefore we briefly discuss the ecology of the specialized ant feeding behavior, or myrmecophagy, of dendrobatid frogs.

Materials and methods

Material examined. The examined ant and frog specimens have been deposited at the following institutions.

- CJ** Centro Jambatu de Investigación y Conservación de Anfibios, Fundación Otonga, Quito, Ecuador
- CRC** Christian Rabeling Collection, University of Rochester, Rochester, NY, U.S.A.
- DZUP** Coleção Entomológica Pe. Jesus Santiago Moure, Universidade Federal do Paraná, Curitiba, PR, Brazil

ICN	Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá D.C., Colombia
MCZC	Museum of Comparative Zoology, Harvard University, Cambridge, MA, U.S.A.
QCAZ	Museo de Zoología, Pontificia Universidad Católica del Ecuador, Quito, Ecuador
USNM	United States National Museum of Natural History, Washington, DC, U.S.A.

Morphological analysis. Specimens were examined and measured using a Leica M165 C stereomicroscope fitted with a stage micrometer. Measurements were recorded to the nearest 0.01 mm at 40x magnification. To generate composite images of the specimens we utilized a Leica DFC450 digital camera mounted on a Leica M205 C stereomicroscope. Composite images were assembled using the Leica Application Suite (Version 4.5) and the Helicon Focus (Version 6.2.2) software packages. Conventions for morphological terminology, measurements, and indices follow those utilized in recent taxonomic studies of Neotropical ants and frogs (Fernández and Palacio 1999, Brown et al. 2011, Delsinne and Fernández 2012, Rabeling et al. 2015). Measurements are given in millimeters. Measurements and indices are defined as follows:

EL	Eye length, in lateral view, the maximum diameter of the eye.
GL	Gaster length, in lateral view, from the anterior edge of the first tergum to the posterior edge of the last visible tergum.
HL	Head length, in full-face view, the maximum distance from the anterior margin of the clypeus to the posterior margin of the head, excluding the mandibles.
HW	Head width, in full-face view, the maximum width of the head excluding the compound eyes.
ML	Mandible length, in full-face view, the maximum distance from the anterior margin of clypeus to the distalmost margin of the mandibles.
PL	Petiole length, in lateral view, the axial distance from anteriormost margin of the ventral process to the posteriormost margin of petiole.
PPL	Postpetiole length, in lateral view, the maximum axial distance from the anteriormost to the posteriormost margin of the postpetiole.
PPW	Postpetiole width, in dorsal view, the maximum transverse distance across the disc of the postpetiole.
PW	Petiole width, in dorsal view, the maximum transverse distance across the node.
SL	Scape length, maximum length excluding the basal condyle.
SVL	Snout to vent length, in ventral view, from the anterior tip of the frog's head to the opening of the cloaca.
TL	Total length (ML + HL + WL + PL + PPL + GL).
WL	Weber's length, in lateral view, measured diagonally from the inflexion on the anterior edge of the pronotum to the posterior edge of the propodeal lobe.

CI	Cephalic index, $(HW/HL) \times 100$.
MI	Mandibular index, $(ML/HL) \times 100$.
OI	Ocular index, $(EL/HW) \times 100$.
SI	Scape index, $(SL/HL) \times 100$.

Results

Taxonomy

Lenomyrmex hoelldobleri sp. n.

<http://zoobank.org/AD681140-8B64-4835-A2B7-E9730BD2CA70>

Figure 1

Holotype worker. ECUADOR: Esmeraldas; 4 Km SW of Alto Tambo, next to Reserve Otokiki; elevation 676 meters above sea level; GPS coordinates: 0.912306, -78.583528; 09.vii.2013; from the stomach content of a male specimen (frog voucher number: CJ1689; SVL = 36.7 mm) of the Little Devil poison frog, *Oophaga sylvatica*; leg. L. A. O'Connell, E. E. Tapia, L. A. Coloma; unique ant specimen identifier: USNMMENT01124322; deposited in USNM.

Measurements of holotype. HL: 1.02; HW: 0.78; ML: 0.45; SL: 0.81; EL: 0.18; WL: 1.58; PL: 0.73; PW: 0.23; PPL: 0.46; PPW: 0.35; GL: 1.00; TL: 4.77; CI: 76; OI: 23; SI 79.

Description, holotype worker. Mandibles elongate, triangular with masticatory margin crenulated, 3 times longer than basal margin, sclerotized blunt peg-like denticles barely visible at 80x magnification (Fig. 1A). Clypeus without carinae, apical margin mostly convex and with a median angle; posterior margin convex, barely projects backward between frontal carinae. Frontal lobes inconspicuous, little expanded laterally, only partially covering antennal condyles. Antennal fossae large, deep, 1.5x longer than broad. Antennal scrobes absent. In full-face view, head with a broadly convex posterior cephalic margin; in full-face view, maximum width, just behind eyes, slightly narrowing posterad. Compound eyes large, protruding, with 15 facets along maximum diameter. Mesosomal profile with pronotum, mesonotum, and propodeum differentiated. Metanotal impression clearly marked (Fig. 1C). Propodeum armed with 2 long, acute spines, clearly longer than distance between their bases (Figs 1B, C). In lateral view, inferior lobes of propodeum triangular. Femora claviform. Meso- and metatibiae lacking spurs. Tarsal claws simple, elongated. In lateral view, petiole long, fusiform, pedunculate; petiolar node well-defined; antero-ventral subpetiolar process directed forward, compressed in anterior-posterior direction, giving appearance of a spine in lateral view; anterolateral edges of process continue dorsally toward sides of petiolar peduncle. In lateral view, postpetiole dome-like, lacking a ventral process.

Mandibles smooth, slightly shining (Fig. 1A). Head, mesosoma, dorsum of petiolar node and postpetiole costate. The costae longitudinal in the head frons, concentric

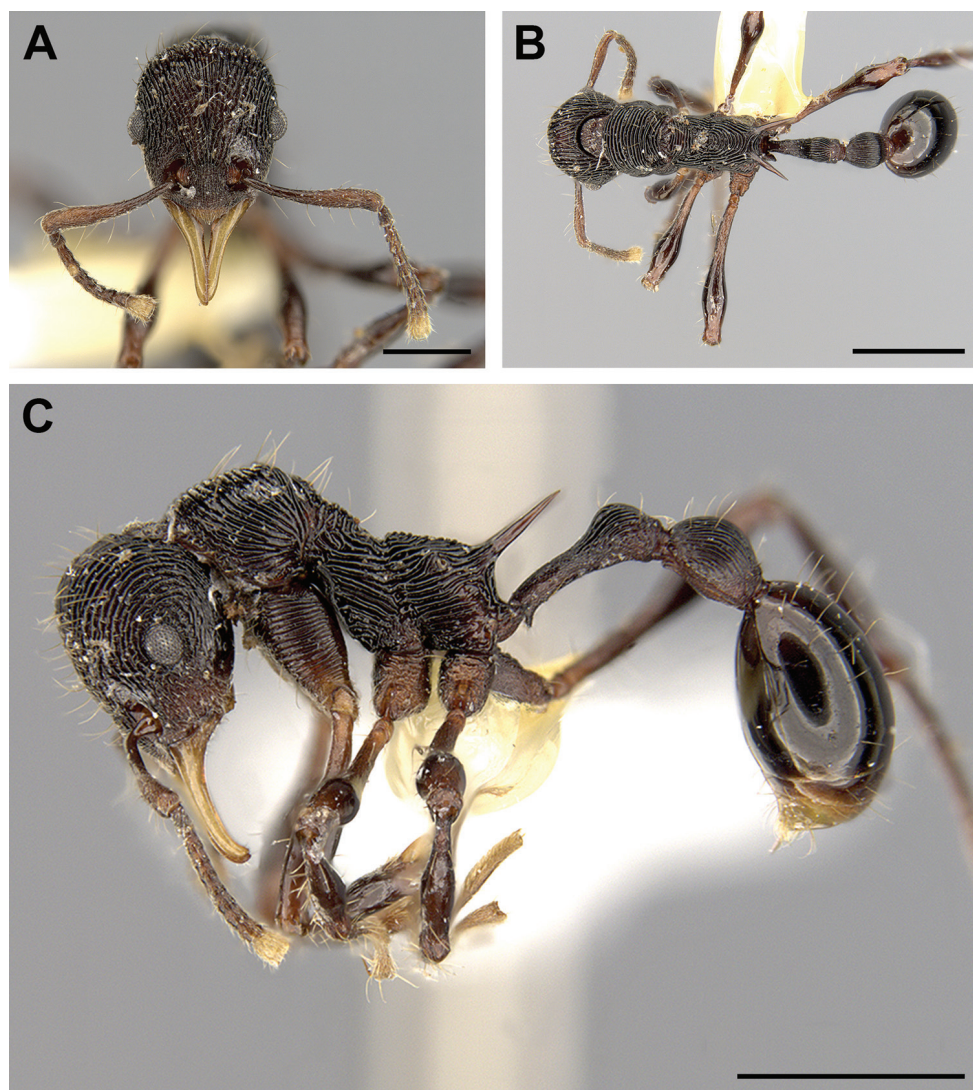


Figure 1. Worker of *Lenomyrmex hoelldobleri* in full-face (A), dorsal (B), and lateral (C) views. The depicted worker is the holotype with the unique specimen identifier USNMMENT01124322. Scale bars: 0.5 mm (A), 1 mm (B, C).

around eyes, predominantly transverse on pronotal dorsum, transverse on mesonotum, concentrically transverse on dorsum of propodeum (Fig. 1B), longitudinal on disc of petiole and postpetiole (Figs 1B, C). Lateral margins of mesosoma with longitudinal costae, coxae with transverse costae, discrete in meso- and meta-coxae. Petiolar peduncle with granulations. Postpetiole mostly shining, and dorsolaterally with fine longitudinal striae and granulations ventrolaterally. Gaster smooth, shining except for dense punctures on pygidium and hypopygium.

Clypeal apical margin with several short, erect hairs. Head frons, leading edge of antennal scape, pronotum, node of petiole, disc of postpetiole, and gaster with scattered erect hairs, most of them longer than maximum diameter of eye (Fig. 1A & B). Erect hairs on dorsum of petiole and legs as long as, or shorter than, maximum eye diameter. Hairs on antennal scape longer than maximum diameter of antennal scape. Funicular antennal segment with numerous short decumbent hairs. Otherwise body devoid of hairs. Body black; legs and coxae lighter; antennal club, mandibles, and gastric apex yellowish-brown.

Distribution and ecology. The single known specimen of *Lenomyrmex hoelldobleri* was recovered from a stomach content sample of the dendrobatid poison frog, *Oophaga sylvatica*. The habitat where the poison frog *Oophaga sylvatica* was collected was a secondary habitat with forest fragments and pastureland. The region encompasses remnant Evergreen Foothill Forests of the Western Cordillera (Ministerio del Ambiente del Ecuador 2012). This area is located in the Chocó Ecoregion, one of the most biologically diverse areas in the world with exceptionally high levels of endemism. The Chocó is considered one of the biodiversity hotspots for conservation purposes (Mittermeier et al. 1998, Myers et al. 2000) and one of the most threatened areas in the world (Brooks et al. 2002). The coastal northwest region of Ecuador, where the Alto Tambo area is found, is part of the wettest ecosystem known in Ecuador, with rainfalls ranging from 2000 up to 4000 mm annually (Ministerio del Ambiente del Ecuador 2012). Temperatures range from an annual average of 20 to 25° C (Ministerio del Ambiente del Ecuador 2012). The Foothill Forests are characterized by the dominance of tree species that can exceed 30 m in height. Trees are covered by orchids, bromeliads, ferns, and aroids. These forests have a dense herbaceous undergrowth layer dominated by Marantaceae, Araceae, and Polypodiopsida (Cerón et al. 1999). Two species of *Lenomyrmex* (*L. foveolatus*, *L. hoelldobleri*) occur in sympatry in the Alto Tambo area (Fig. 4).

Queen and male. Unknown.

Etymology. This species is named in honor of our colleague and friend Bert Hölldobler on the occasion of his 80th birthday. Because of Bert's passion for ants, his pioneering and high-caliber contributions to entomology and behavioral ecology, as well as his dedication to mentoring the next generation of myrmecologists, myrmecology has become its own discipline in entomology, and continues to attract enthusiastic students who share Bert's love for ants.

Comments. *Lenomyrmex hoelldobleri* can be distinguished from all other *Lenomyrmex* species by the following combination of character states: (i) petiolar node conspicuous, well-defined; (ii) a well-defined metanotal suture; (iii) conspicuous costae on its body; (iv) long erect hairs on the scape, and (v) size, being larger than all known species. *Lenomyrmex costatus* is morphologically most similar to *L. hoelldobleri* and both share the integumental sculpturing and the presence of long setae on the antennal scapes. However, *L. hoelldobleri* can be clearly distinguished from *L. costatus* by its well-defined petiolar node, the presence of the metanotal suture, its larger size, by having concentrically transverse striae on dorsum of propodeum (longitudinal in



Figure 2. Worker of *Lenomyrmex costatus* in full-face (A), dorsal (B), and lateral (C) views. The depicted worker is the holotype with the unique specimen identifier MCZ-ENT00036069. Scale bars: 0.5 mm (A), 1 mm (B, C).

L. costatus), and the distinctly darker coloration (compare Figs 1, 2). To differentially diagnose *L. hoelldobleri* and *L. costatus*, we examined the holotype of *L. costatus* (Fig. 2). The specimen is deposited at Museum of Comparative Zoology at Harvard University. The specimen information is as follows: Panama; Bocas del Toro; Fortuna to Chiriqui Grande rd.; elevation 1050 meters above sea level; GPS coordinates: 8°47'N, 82°12'W; 14.vii.1987; leg. D. M. Olson (DMO523); unique ant species identifier: MCZ-ENT00036069.

***Lenomyrmex foveolatus* Fernández & Palacio**

Figure 3

Gyne. ECUADOR: Esmeraldas; Reserve Otokiki-Alto Tambo; elevation 723 meters above sea level; GPS coordinates: 0.918533, -78.566800; 08.vii.2013; from the stomach content of a female specimen (frog voucher number: CJ1658, SVL = 36.7 mm) of the Little Devil frog, *Oophaga sylvatica*; leg. L. A. O'Connell, E. E. Tapia, L. A. Coloma; unique ant specimen identifier: USNMENT01127956; deposited in USNM.

Gyne measurements. HL: 0.91; HW: 0.83; ML: 0.49; SL: 0.75; EL: 0.23; WL: 1.47; PL: 0.78; PW: 0.25; PPL: 0.35; PPW: 0.29; GL: 1.41; TL: 5.40; CI: 91; MI: 55; OI: 0.29; SI 90 (n=1).

Description, dealate gyne. As in the worker description (Fernández and Palacio 1999: 13–14) but mesosoma with caste-specific morphology related to wing-bearing and with the following differences: in full-face view, mid portion of anterior margin of clypeus weakly concave, forming a pair of lateral angles; compound eyes larger than in worker, with 12 ommatidia in maximum diameter; three small but conspicuous ocelli present. Dorsum of pronotum, mesoscutum, axillae, and scutellum lustrous and weakly coriaceous; dorsolateral portion of pronotum with small and sparse foveae; in dorsal view, posterior lateral portions of pronotum concave. In dorsal view, mesoscutum somewhat triangular anteriorly; parapsidal lines short, conspicuous; scuto-scutellar sulcus well-developed; posterior margin of scutellum subquadrate, lacking tubercles. Dorsum and declivity of propodeum lustrous; posterior margin of propodeum angulate, lacking tubercles or spines (as in worker). Mesopleuron clearly divided to anepisternum and katepisternum by oblique mesopleural sulcus. Pilosity of body consisting of small, simple, appressed hairs.

Additional material examined. ECUADOR: Esmeraldas; Alto Tambo; elevation 788 meters above sea level; GPS coordinates: 0.907450, -78.540583; 05.vii.2013; from the stomach content of a male specimen (frog voucher number: CJ1770) of the Little Devil frog, *Oophaga sylvatica*; leg. L. A. O'Connell, E. E. Tapia, L. A. Coloma; [1w, CRC, USNMENT01127960]. Same as previous entry but, 200–300 m SW El Placer; elevation 551 meters above sea level; GPS coordinates: 0.901050, -78.618233; 07.vii.2013; from the stomach content of a male specimen (frog voucher number: CJ1632; SVL = 35.6 mm) of the Little Devil frog, *Oophaga sylvatica*; leg. L. A. O'Connell, E. E. Tapia, L. A. Coloma; [1w, QCAZ, USNMENT01127955]. Same as previous entry but, next to Reserva Otokiki (farm next to railway); elevation 676 meters above sea level; GPS coordinates: 0.912306, -78.583528; 09.vii.2013; from the stomach content of a male specimen (frog voucher number: CJ1690; SVL = 38.2 mm) of the Little Devil frog, *Oophaga sylvatica*; leg. L. A. O'Connell, E. E. Tapia, L. A. Coloma; [3w, DZUP, ICN, USNM; USNMENT01127957, USNMENT01127935, USNMENT01127958]. Same as previous entry but, from the stomach content of a female specimen (frog voucher number: CJ1691; SVL = 34.7 mm) of the Little Devil frog, *Oophaga sylvatica* [1w, QCAZ; USNMENT01127954]. Same as previous entry but, Lita; around bamboo forest; elevation 326 meters above sea level; GPS coordinates: 0.911944, -78.680833; 10.vii.2013; from

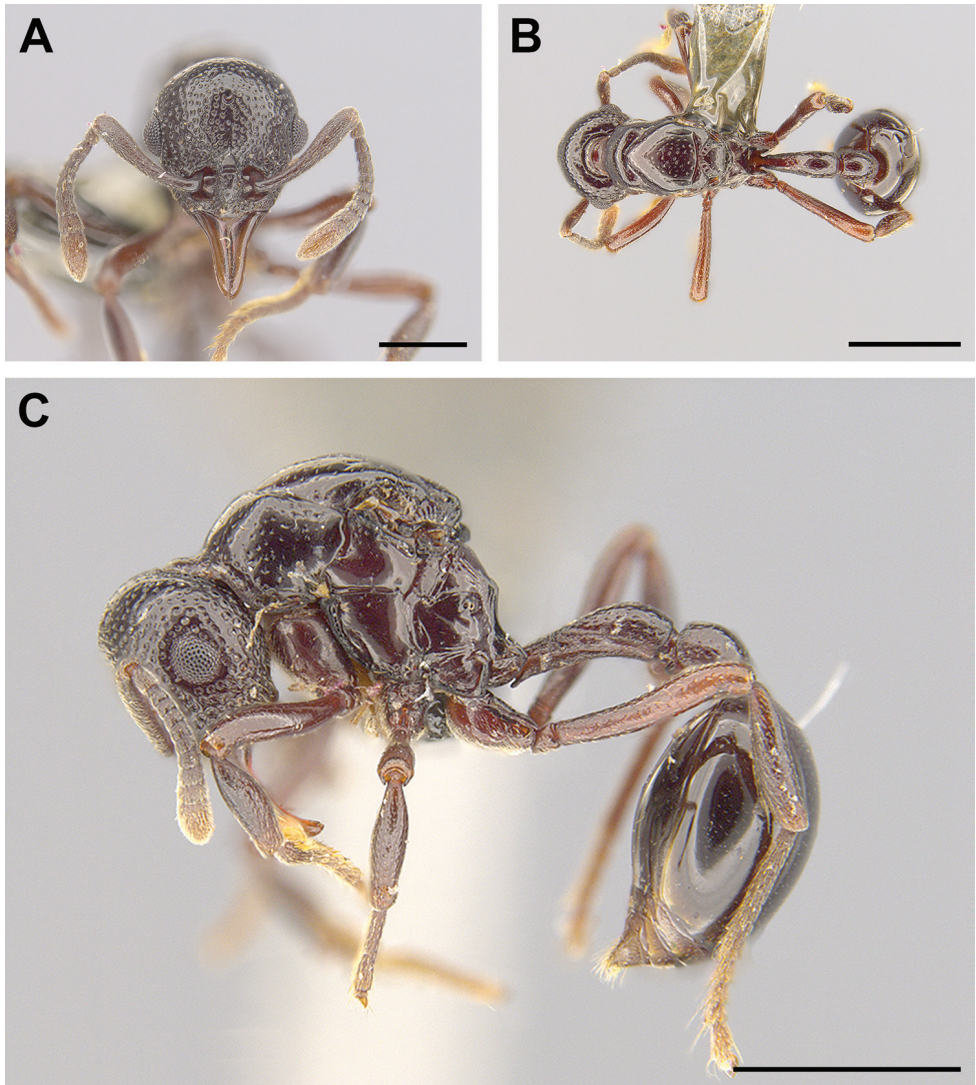


Figure 3. Dealate gyne of *Lenomyrmex foveolatus* in full-face (A), dorsal (B), and lateral (C) views. The depicted gyne has the unique specimen identifier USNMENT01127956. Scale bars: 0.5 mm (A), 1 mm (B, C).

the stomach content of a female specimen (frog voucher number: CJ1695; SVL = 32 mm) of the Little Devil frog, *Oophaga sylvatica*; leg. L. A. O'Connell, E. E. Tapia, L. A. Coloma; [1w, CRC, USNMENT01127936].

Worker measurements. HL: 0.81–0.90; HW: 0.73–0.83; ML: 0.42–0.47; SL: 0.61–0.73; EL: 0.17–0.20; WL: 1.06–1.42; PL: 0.65–0.73; PW: 0.21–0.23; PPL: 0.29–0.35; PPW: 0.25–0.28; GL: 0.98–1.34; TL: 4.31–5.19; CI: 90–94; MI: 51–57; OI: 0.25–0.28; SI 82–95 (n=7).

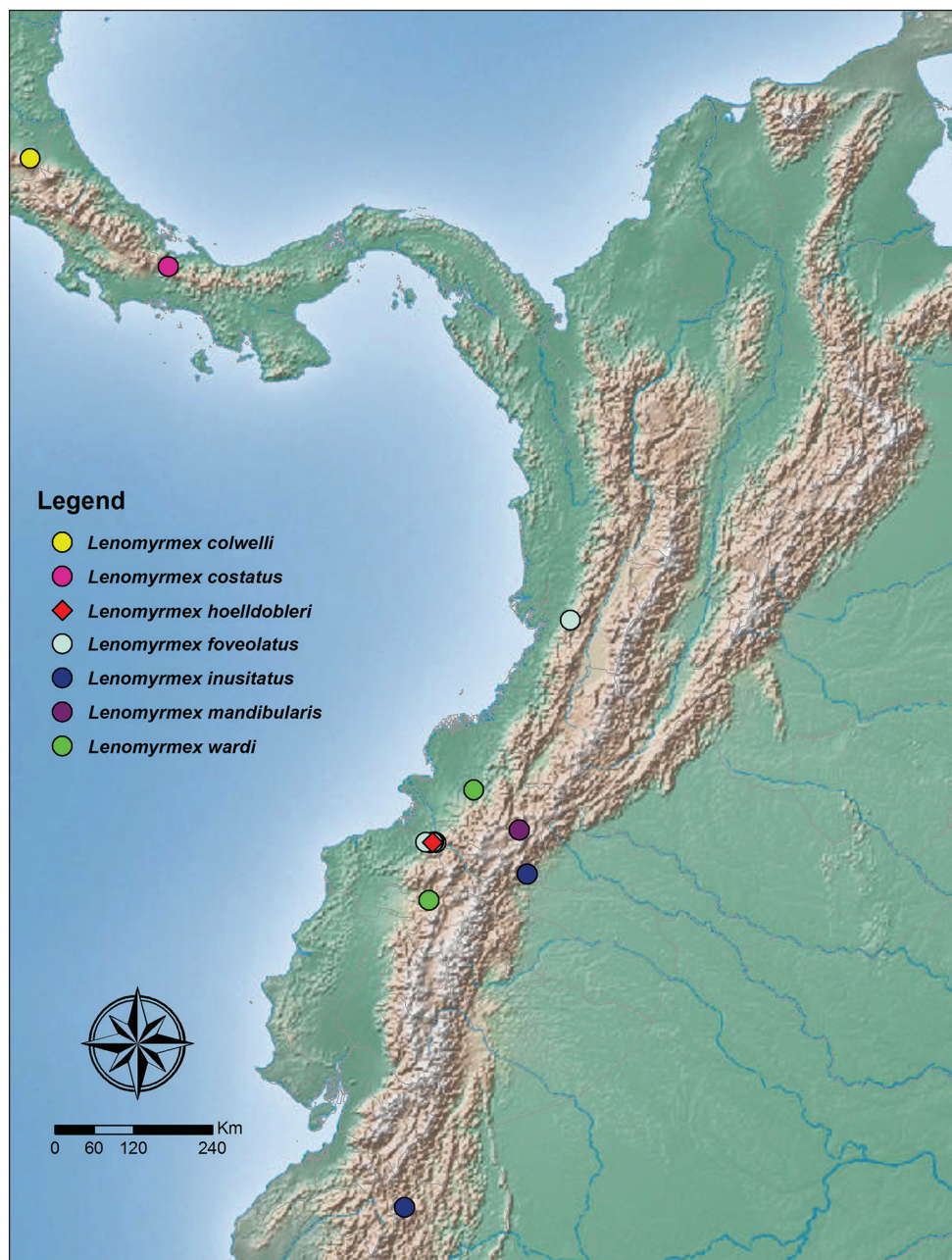


Figure 4. Geographic distribution of the genus *Lenomyrmex* in Central and South America.

Comments. Specimens from the Colombian type series could not be examined, but based on the Fernández and Palacio's (1999) description, the worker specimens collected from Ecuador closely resemble the specimens from Colombia. The main

differences between the specimens belonging to these two populations are: (i) the fovea on dorsum of head are scattered in the Colombian specimens and more densely clustered in the Ecuadorian individuals; (ii) the specimens from Ecuador have rounded propodeal lobes differing from the acute propodeal lobes observed in the type series from Colombia; (iii) in the specimens from Ecuador the metapleural gland bulla is striate, and striae seem absent from bulla of the Colombian specimens.

Distribution and ecology. Previously only known from the type locality in western Colombia, Departamento del Valle, Darién, middle Río Calima basin. The current record near Alto Tambo extends the species geographic range 400 km south of the type locality (Fig. 4). General habitat data is the same as in the *Lenomyrmex hoelldobleri* account, except that the frog was collected in a banana plantation.

Key to the workers of *Lenomyrmex* (modified from Delsinne and Fernández 2012)

- 1 Mesosoma predominantly smooth and shiny, without erect hairs 2
- Mesosoma with conspicuous sculpture and at least one pair of erect hairs... 3
- 2(1) Propodeum without spines; head only foveolate (SW Colombia)... *L. foveolatus*
- Propodeum with a pair of acute and well-defined spines; head foveolate, with median longitudinal striae (Cordillera Oriental of the Andes in S Colombia and S Ecuador) *L. inusitatus*
- 3(1) Dorsum of head and petiole with longitudinal conspicuous costae; erect hairs of antennal scape as long as or longer than maximum diameter of scape 4
- Dorsum of head densely rugo-reticulate; sculpture of the petiole variable, rugulate to rugo-reticulate or longitudinally striate but never costate; erect hairs of antennal scape not longer than maximum diameter of the scape 5
- 4(3) Node of petiole inconspicuous and ill-defined; dorsum of propodeum with longitudinal striae; in dorsal view, disc of postpetiole weakly sculptured; body ferruginous yellow (W Panama) *L. costatus*
- Node of petiole conspicuous, well-defined; dorsum of propodeum with transverse striae; in dorsal view, disc of postpetiole finely striate; body black (W Ecuador) *L. hoelldobleri*
- 5(3) Length of propodeal spines approximately equal to distance between their bases; mesopleuron with some irregular longitudinal striae, but mostly smooth and shiny; metapleuron with irregular longitudinal striae; HL > 0.80 mm; mesosoma with only two suberect hairs on the pronotum (SW Colombia) *L. mandibularis*
- Length of propodeal spines variable, either shorter or longer than distance between their bases; metapleuron and subsequent portion of mesopleuron with fine transverse rugulae or rugo-reticulate, without smooth areas; HL < 0.80 mm; mesosoma with numerous erect to suberect hairs 6
- 6(5) Propodeal spines shorter than distance between their bases; eyes with six or seven facets in maximum diameter; petiolar node protruding over the pe-

- duncle and well defined; postpetiolar dorsum with longitudinal striae (NW Ecuador, SW Colombia).....***L. wardi***
- Propodeal spines longer than distance between their bases; eyes with about nine facets in maximum diameter; petiolar node undifferentiated from the peduncle; postpetiolar dorsum smooth and polished (Costa Rica)***L. colwelli***

Key to the known queens of *Lenomyrmex* (modified from Delsinne and Fernández 2012)

- 1 Head foveolate; median longitudinal striae may be present. Body lacking erect hairs **2**
- Head densely rugo-reticulate. Body with erect hairs..... **3**
- 2(1) Propodeal spines present. Mesosoma shiny with sparse punctures on pronotum, mesopleuron, metapleuron, and propodeum. Scutellum and axillae foveolate, mesoscutum foveolate-striate ***L. inusitatus***
- Propodeal spines absent. Mesosoma predominantly smooth and shiny, lacking punctures in mesopleuron, metapleuron, and propodeum. Pronotum with a few foveae on lateral portions. Scutellum and axillae smooth. Mesoscutum smooth and shining ***L. foveolatus***
- 3(1) Propodeal spines approximately equal in length to distance between their bases; integument predominantly shiny; HL > 0.80.....***L. mandibularis***
- Propodeal spines notably shorter than distance between their bases; integument predominantly opaque; HL <0.80 ***L. wardi***

Discussion

All seven species of the myrmicine ant genus *Lenomyrmex* are characterized by their elongate, highly modified mandibles, which are indicative of specialized predatory habits (Fernández and Palacio 1999, Fernández 2001, Longino 2006, Delsinne and Fernández 2012). Interestingly, *Lenomyrmex* ants combine morphological characters typical of highly specialized predators with plesiotypic characters, such as the flexible suture between pronotum and mesonotum, which is atypical for myrmicine ants, but characteristic of early ant lineages with a predatory lifestyle in low-light environments (Bolton 1990, Rabeling et al. 2008, Yamane et al. 2008). This combination of plesiomorphic and derived morphological characters made it difficult to place the genus *Lenomyrmex* within the myrmicine phylogeny and its phylogenetic relationship to other members of the subfamily remained uncertain at first (Fernández and Palacio 1999). A recent molecular phylogenetic reconstruction of the subfamily Myrmicinae inferred *Lenomyrmex* as a close relative of the genus *Daceton* (Ward et al. 2015), which are predatory, arboreal ants (Wilson 1962, Azorsa and Sosa-Calvo 2008). Interestingly, the *Daceton*-species group is the sister group of the fungus-growing ants. Unfortu-

nately, the sister-group relationship of the predatory trap-jaw ants and fungus-growing ants does not provide new insights into the much-debated evolutionary origins of the unique and highly derived fungus-growing behavior (Hölldobler and Wilson 1990, 2011, Mueller et al. 2001, Rabeling et al. 2006, Mehdiabadi and Schultz 2010). The current phylogenetic hypothesis suggests that either ant fungiculture evolved from a predatory ancestral state or, alternatively, the fungicultural and the predatory behaviors evolved along independent evolutionary trajectories from a common ancestor with generalist feeding habits. The discovery of a “missing link” would mark a real advance in our understanding about the evolutionary trajectories towards highly derived behaviors.

Lenomyrmex ants are rare in museum collections and the majority of the specimens have been collected sporadically in leaf-litter samples (Fernández and Palacio 1999, Fernández 2001, Longino 2006, Delsinne and Fernández 2012). So far only colonies of *L. mandibularis* have been collected manually because this species constructs nests in stems of a *Palicourea* species in the plant family Rubiaceae and in rotten logs (Fernández and Palacio 1999). In addition to systematic leaf litter sampling and hand collecting, the examination of stomach contents of leaf-litter foraging amphibians is a valuable source of cryptic and rarely collected ant species (Weber 1938, Delsinne and Fernández 2012, Sosa-Calvo 2015). Many species of amphibians and non-avian reptiles specialize on ant feeding and some species are predominantly myrmecophagous (Solé et al. 2002, Darst et al. 2005, Esteves et al. 2008). In the Neotropical poison frog family Dendrobatidae, myrmecophagy evolved at least twice, possibly three times independently (Santos et al. 2003, Darst et al. 2005), and the frogs sequester the skin alkaloids mostly from their ant and mite diet (McGugan et al. 2016). In addition to ants and mites, other arthropods, such as beetles and millipedes, are considered alkaloid sources for poison frogs (Dumbacher et al. 2004, Saporito et al. 2003, 2004, 2007).

To study the feeding ecology of the Little Devil poison frog, *Oophaga sylvatica*, the stomach contents of more than 300 individuals from different populations in Ecuador have been examined recently (McGugan et al. 2016, O'Connell, Sosa-Calvo et al., unpublished data). The majority of the frogs' diet consisted of ants, constituting between 40 and 86 % of diet volume in different frog populations. Of the more than 3000 examined prey items, 44 different ant genera could be identified, representing nine different subfamilies (Sosa-Calvo, O'Connell et al., unpublished data). The majority of the eaten ant genera belong to the subfamily Myrmicinae, including the rarely collected genus *Lenomyrmex*, with a total of nine specimens belonging to two species, *L. hoelldobleri* (the holotype worker) and *L. foveolatus* (seven workers and one gyne). Other cryptic and rarely collected ant genera include *Leptanilloides*, *Stigmatomma*, and *Cerapachys*, among others. To sample stomach contents of amphibians and other vertebrates solely for nutritional studies, it is not necessary to kill the animals. Stomach flushing methods have been developed and successfully applied in numerous studies, which avoids killing individuals of the study species (Solé et al. 2005). To conclude, the study of vertebrate stomach contents is not only a way of studying the trophic ecology of vertebrates themselves, but also an interesting source of cryptic and new arthropod species, including ants.

Acknowledgements

We gratefully acknowledge the Ministerio de Ambiente de Ecuador for permission to collect frog specimens in Ecuador (001-13 IC-FAU-DNB/MA) and export them to the United States (CITES 32V/S). We thank Stefan Cover (MCZ) for providing access to the holotype of *Lenomyrmex costatus*. Rodrigo Feitosa and one anonymous reviewer improved the manuscript with helpful comments. Lina Pedraza assisted in taking morphometric measurements of the ants. CR and JSC are supported by the National Science Foundation (DEB-1456964). LAO is supported by a Bauer Fellowship from Harvard University, a For Women in Science Fellowship from the L'Oreal Foundation, and is grateful to Lola Guarderas and Elicio E. Tapia for providing assistance during field research in Ecuador. LAC acknowledges support from Wikiri and the Saint Louis Zoo.

References

- Azorsa F, Sosa-Calvo J (2008) Description of a remarkable new species of ant in the genus *Daceton* Perty (Formicidae: Dacetini) from South America. *Zootaxa* 1749: 27–38.
- Bolton B (1990) The higher classification of the ant subfamily Leptanillinae (Hymenoptera: Formicidae). *Systematic Entomology* 15: 267–282. doi: 10.1111/j.1365-3113.1990.tb00063.x
- Bolton B (2016) An online catalog of the ants of the world. Available from <http://antcat.org> [accessed 20 June, 2016]
- Brooks TM, Mittermeier RA, Mittermeier CG, da Fonseca GA, Rylands AB, Konstant WR, Flick P, Pilgrim J, Oldfield S, Magin G, Hilton-Taylor C (2002) Habitat loss and extinction in the hotspots of biodiversity. *Conservation Biology* 16: 909–923. doi: 10.1046/j.1523-1739.2002.00530.x
- Brown JL, Twomey E, Amezcuita A, Souza MB, Caldwell JP, Lötters S, May R, Melo-Sampaio PR, Mejía-Vargas D, Perez-Peña P, Pepper M, Poelman EH, Sanchez-Rodriguez M, Summers K (2011) A taxonomic revision of the Neotropical poison frog genus *Ranitomeya* (Amphibia: Dendrobatidae). *Zootaxa* 3083: 1–20.
- Cerón C, Palacios W, Valencia R, Sierra R (1999) Las formaciones naturales de la Costa del Ecuador. In: Sierra R (Ed.) *Propuesta preliminar de un sistema de clasificación de vegetación para el Ecuador continental*. Proyecto INEFAN/GERF-BIRF y Ecociencia, Quito.
- Daly JW, Garraffo HM, Poonam J, Spande TF, Snelling RR, Jaramillo C, Rand AS (2000) Arthropod-frog connection: decahydroquinoline and pyrrolizidine alkaloids common to microsympatric myrmicine ants and dendrobatid frogs. *Journal of Chemical Ecology* 26: 73–85. doi: 10.1023/A:1005437427326
- Darst CR, Menéndez-Guerrero PA, Coloma LA, Cannatella DC (2005) Evolution of dietary specialization and chemical defense in poison frogs (Dendrobatidae): a comparative analysis. *The American Naturalist* 165: 56–69. doi: 10.1086/426599
- Delsinne T, Fernández F (2012) First record of *Lenomyrmex inusitatus* (Formicidae: Myrmicinae) in Ecuador and description of the queen. *Psyche* 2012: 145743. doi: 10.1155/2012/145743

- Dumbacher JP, Wako A, Derrickson SR, Samuelson A, Spande TF, Daly JW (2004) Melyrid beetles (Choresine): a putative source for the batrachotoxin alkaloids found in poison-dart frogs and toxic passerine birds. *Proceedings of the National Academy of Science USA* 101: 15857–15860. doi: 10.1073/pnas.0407197101
- Esteves EA, Brandão CRF, Viegas K (2008) Subterranean ants (Hymenoptera, Formicidae) as prey of fossorial reptiles (Reptilia, Squamata: Amphisbaenidae) in Central Brazil. *Papéis Avulsos de Zoologia, Museu de Zoologia da Universidade de São Paulo* 48: 329–334. doi: 10.1590/S0031-10492008002800001
- Fernández F, Palacio EE (1999) *Lenomyrmex*, an enigmatic new ant genus from the Neotropical Region (Hymenoptera: Formicidae: Myrmicinae). *Systematic Entomology* 24: 7–16. doi: 10.1046/j.1365-3113.1999.00063.x
- Fernández F (2001) Hormigas de Colombia. IX: Nueva especie de *Lenomyrmex* (Formicidae: Myrmicinae). *Revista Colombiana de Entomología* 27: 201–204
- Fernández F, Sendoya S (2004) Synonymic list of Neotropical ants (Hymenoptera: Formicidae). *Biota Colombiana* 5: 3–105
- Fernández F, Feitosa RM, Lattke J (2014) *Kempfidris*, a new genus of myrmicine ants from the Neotropical region (Hymenoptera: Formicidae). *European Journal of Taxonomy* 85: 1–10. doi: 10.5852/ejt.2014.85
- Hölldobler B, Wilson EO (1990) *The Ants*. Harvard University Press, Harvard, 732 pp.
- Hölldobler B, Wilson EO (2011) *The leafcutter ants—civilization by instinct*. W. W. Norton & Company, New York, 157 pp.
- Johnson RA, Moreau CS (2016) A new ant genus from southern Argentina and southern Chile, *Patagonomyrmex* (Hymenoptera: Formicidae). *Zootaxa* 4139: 1–31. doi: 10.11646/zootaxa.4139.1.1
- Longino JT (2006) New species and nomenclatural changes for the Costa Rican ant fauna (Hymenoptera: Formicidae). *Myrmecologische Nachrichten* 8: 131–143
- McGugan JR, Byrd GD, Roland AB, Caty SN, Kabir N, Tapia EE, Trauger SA, Coloma LA, O'Connell LA (2016) Ant and mite diversity drives toxin variation in the Little Devil Poison frog. *Journal of Chemical Ecology* 42: 537–551. doi: 10.1007/s10886-016-0715-x
- Mehdiabadi NJ, Schultz TR (2010) Natural history and phylogeny of the fungus-farming ants (Hymenoptera: Formicidae: Myrmicinae: Attini). *Myrmecological News* 13: 37–55
- Ministerio del Ambiente del Ecuador (2012) *Sistema de clasificación de los ecosistemas del Ecuador continental*. Subsecretaría de Patrimonio Natural. Quito
- Mittermeier RA, Myers N, Thomsen J, da Fonseca GA, Olivieri S (1998) Biodiversity hotspots and major tropical wilderness areas: Approaches to setting conservation priorities. *Conservation Biology* 12: 516–520. doi: 10.1046/j.1523-1739.1998.012003516.x
- Mueller UG, Schultz TR, Currie C, Adams R, Malloch D (2001) The origin of the attine ant-fungus mutualism. *Quarterly Review of Biology* 76: 169–197. doi: 10.1086/393867
- Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GA, Kent J (2000) Biodiversity hotspots for conservation priorities. *Nature* 403: 853–858. doi: 10.1038/35002501
- Rabeling C, Verhaagh M, Mueller UG (2006) Behavioral ecology and natural history of *Blepharidatta brasiliensis* (Formicidae, Blepharidattini). *Insectes Sociaux* 53: 300–306. doi: 10.1007/s00040-006-0872-y

- Rabeling C, Brown JM, Verhaagh M (2008) Newly discovered sister lineage sheds light on early ant evolution. *Proceedings of the National Academy of Sciences USA* 105: 14913–14917. doi: 10.1073/pnas.0806187105
- Rabeling C, Schultz TR, Bacci Jr M, Bollazzi M (2015) *Acromyrmex charruanus*: a new inquiline social parasite species of leaf-cutting ants. *Insectes Sociaux* 62: 335–349. doi: 10.1007/s00040-015-0406-6
- Solé M, Ketterl J, Di-Bernardo M, Kwet A (2002) Ants and termites are the diet of the microhylid frog *Elachistocleis ovalis* (Schneider, 1799) at an *Auracaria* forest in Rio Grande do Sul. *Herpetological Bulletin* 79: 14–17.
- Solé M, Beckmann O, Pelz B, Kwet A, Engels W (2005) Stomach-flushing for diet analysis in anurans: an improved protocol evaluated in a case study in *Araucaria* forests, southern Brazil. *Studies on Neotropical Fauna and Environment* 40: 23–28. doi: 10.1080/01650520400025704
- Santos JC, Coloma LA, Cannatella DC (2003) Multiple, recurring origins of aposematism and diet specialization in poison frogs. *Proceedings of the National Academy of Science USA* 100: 12792–12797. doi: 10.1073/pnas.2133521100
- Saporito RA, Donnelly MA, Hoffman RL, Garraffo HM, Daly JW (2003) A siphonotid millipede (*Rhinotus*) as the source of spiropyrrolizidine oximes of dendrobatid frogs. *Journal of Chemical Ecology* 29: 2781–2786. doi: 10.1023/B:JOEC.0000008065.28364.a0
- Saporito RA, Garraffo HM, Donnelly MA, Edwards AL, Longino JT, Daly JW (2004) Formicine ants: an arthropod source for the pumiliotoxin alkaloids of dendrobatid poison frogs. *Proceedings of the National Academy of Science USA* 101: 8045–8050. doi: 10.1073/pnas.0402365101
- Saporito RA, Donnelly MA, Norton RA, Garraffo HM, Spande TF, Daly JW (2007) Oribatid mites as a major dietary source for alkaloids in poison frogs. *Proceedings of the National Academy of Sciences USA* 104: 8885–8890. doi: 10.1073/pnas.0702851104
- Sosa-Calvo J, Schultz TR, Brandão CR, Klingenberg C, Feitosa RM, Rabeling C, Bacci M Jr., Lopes CT, Vasconcelos HL (2013) *Cyatta abscondita*: taxonomy, evolution, and natural history of a new fungus-farming ant genus from Brazil. *PLoS ONE* 8(11): e80498. doi: 10.1371/journal.pone.0080498
- Sosa-Calvo J (2015) Systematics of the cryptic fungus-farming ant genus *Myrmicocrypta* Fr. Smith, with the description of a new genus and species of fungus-farming ants (Hymenoptera: Myrmicinae). Ph.D. Dissertation, University of Maryland.
- Ward PS, Brady SG, Fisher BL, Schultz TR (2015) The evolution of myrmicine ants: phylogeny and biogeography of a hyperdiverse ant clade (Hymenoptera: Formicidae). *Systematic Entomology* 40: 61–81. doi: 10.1111/syen.12090
- Weber NA (1938) The food of the giant toad, *Bufo marinus* (L.), in Trinidad and British Guiana with special reference to the ants. *Annals of the Entomological Society of America* 31: 499–503. doi: 10.1093/aesa/31.4.499
- Wilson EO (1962) Behavior of *Daceton armigerum* (Latreille), with a classification of self-grooming movements in ants. *Bulletin of the Museum of Comparative Zoology* 127: 401–422.
- Yamane S, Bui TV, Eguchi K (2008) *Opamyрма hungvuong*, a new genus and species of ant related to *Apomyрма* (Hymenoptera: Formicidae: Amblyoponinae). *Zootaxa* 1767: 55–63.

New genera of Australian stiletto flies (Diptera, Therevidae)

Michael E. Irwin¹, Shaun L. Winterton²

1 Illinois Natural History Survey, Champaign, Illinois USA **2** California State Collection of Arthropods, California Department of Food & Agriculture, Sacramento, California, USA

Corresponding author: Shaun L. Winterton (wintertonshaun@gmail.com)

Academic editor: P. Stoev | Received 5 February 2016 | Accepted 11 September 2016 | Published 19 September 2016

<http://zoobank.org/3B4D7966-762D-4D7D-ACCC-1A31F51FBD73>

Citation: Irwin ME, Winterton SL (2016) New genera of Australian stiletto flies (Diptera, Therevidae). ZooKeys 618: 97–128. doi: 10.3897/zookeys.618.8059

Abstract

Two new stiletto fly genera of Agapophytinae (Diptera: Therevidae) are described from Australia. *Sidarena* gen. n. comprises six new species (*Sidarena aurantia* sp. n., *S. flavipalpa* sp. n., *S. geraldton* sp. n., *S. hortorum* sp. n., *S. macfarlandi* sp. n., and *S. yallingup* sp. n.) and is largely endemic to Western Australia. *Zelothrix* gen. n. is described based on two species; *Z. warrumbungles* sp. n. is a locally abundant species in Eastern Australia, while *Z. yeatesi* sp. n. is restricted to southwestern Western Australia. These sister genera are likely closely related to *Taenogerella* Winterton & Irwin and *Actenomeros* Winterton & Irwin.

Keywords

Stiletto fly, Therevidae, Therevoid clade, Asiloidea, Australia

Introduction

The stiletto fly (Diptera: Therevidae) fauna of Australasia is the most species-rich biogeographical region, comprising over 400 described species in 26 genera. Two of the four subfamilies of therevidae are present in Australasia, Agapophytinae (209 species in 23 genera) and Therevinae (166 spp. in 3 gen.) (Winterton 2009, 2011; Winterton et al. 2016); Xestomyzinae and Phycusinae (previously Phycinae) (Gaimari et al. 2013; ICZN 2015) are entirely absent from the region. All agapophytine genera and all but one therevine genus (i.e., *Irwiniella* Lyneborg, 1976) are endemic to Australasia.

Numerous publications describing new subfamilies, genera and species of Australian therevids have been published over the last 10 years (e.g., Ferguson et al. 2013, 2014; Winterton 2007a–d, 2009, 2011a, b; Winterton and Ferguson 2012; Winterton and Lambkin 2012; Lambkin and Turco 2013) including overarching phylogenies of the family by Lambkin et al. (2009) and Winterton et al. (2016), yet new genera and species continue to be discovered and described. Herein we describe two new genera of agapophytine therevids from Australia, *Sidarena* gen. n. (Figs 1–2) and *Zelothrix* gen. n. (Fig. 3). Both genera were recovered as sister groups (both identified as ‘undescribed genus S’) in the recent paper on therevid phylogeny by Winterton et al. (2016) and appear closely related to genera such as *Taenogerella* Winterton & Irwin and *Actenomeros* Winterton & Irwin. The new genera are diagnosed and keys to species are presented for each.

Materials and methods

Adult morphological terminology follows Cumming and Wood (2009) with genitalic morphology as modified by Winterton et al. (1999a,b) and Winterton (2006). Genitalia were macerated in 10% KOH to remove soft tissue, then rinsed in distilled water and dilute glacial acetic acid, and dissected in 80% ethanol. Genitalia preparations were placed in glycerine in a genitalia vial mounted on the pin beneath the specimen.

Specimen images were taken at different focal points using a digital camera and subsequently combined into a serial montage image using Helicon Focus software. All new nomenclatural acts are to be registered in ZooBank (Pyle and Michel 2008). Types are deposited in the following institutions and collections: Australian National Insect Collection (Canberra) (ANIC), Western Australian Museum (Perth) (WAM), California Academy of Sciences (San Francisco) (CAS), California State Collection of Arthropods (Sacramento) (CSCA), Canadian National Insect Collection (Ottawa) (CNC), Queensland Museum (Brisbane) (QM). Numbers quoted with individual specimens as MEI000000 are unique identifiers in the therevid database MANDALA and are attached to each specimen as a yellow or white label (Kampmeier and Irwin 2009). Material examined lists were exported from MANDALA. Abbreviations in text: notopleural setae (np); supra alar setae (sa); postalar setae (pa); dorsocentral setae (dc); scutellar setae (sc).

Taxonomy

Sidarena gen. n.

<http://zoobank.org/CCC28E9C-EB5D-4776-A269-770CFDE5FBF0>

Figs 1–2, 5–20, 28B

Type species. *Sidarena macfarlandi* sp. n., designated here.

Diagnosis. Both sexes with eyes widely dichoptic; multiple poorly defined rows of postocular macrosetae present dorsally in both sexes; antennal scape lacking macrosetae along medial surface, scape shorter than head length; flagellum conical, tapering to a terminal arista; parafacial setae absent; one pair of scutellar macrosetae, most other scutal macrosetae variable in number; velutum patches absent on femora and sparsely



Figure 1. Adult male *Sidarena hortorum* sp. n.; Talbot Road Nature Reserve, Stratton, Perth, Western Australia. (Photo credit: Fred and Jean Hort).



Figure 2. *Sidarena hortorum* sp. n. mating pair; Bullsbrook Nature Reserve, Bullsbrook, Western Australia. (Photo credit: Fred and Jean Hort).



Figure 3. Adult male *Zelothrix yeatesi* sp. n.; Warrumbungle National Park, New South Wales. (Photo credit: Shaun L. Winterton).

present ventrally on gonocoxites; single anteroventral seta present apically on hind femur; wing slightly to dark infusate with maculae, cell m_3 open to wing margin; abdomen narrow elongate; male abdomen typically with silver velutum; male genitalia with articulating inner gonocoxal process well developed; outer gonocoxal process well developed and rounded; gonocoxal apodeme short and rounded; gonostylus narrow apically; gonocoxites lacking medial atrium; aedeagus with distiphallus broad apically, not directed ventrally at apex; epandrium shape quadrangular; ventral apodeme of parameral sheath forked. Female tergite 8 with broad anteromedial process; three spermathecae, ducts joining to spermathecal sac duct; spermathecal sac present (Fig. 28B).

Included species. *Sidarena aurantia* sp. n., *S. flavipalpa* sp. n., *S. geraldton* sp. n., *S. hortorum* sp. n., *S. macfarlandi* sp. n., and *S. yallingup* sp. n.

Comments. *Sidarena* gen. n. is mostly endemic to Western Australia and is distinctive in general appearance, with male eyes widely dichoptic, numerous bristles on the occiput and often with grey metallic pubescent stripe medially on the scutum (often adjoining broad matte black-brown pubescent stripes laterally). The presence of these characters alone differentiates this genus from all other genera in the subfamily. Similar genera to *Sidarena* gen. n. include *Squamopygia* Kröber, *Ectinorhynchus* Macquart and *Zelothrix* gen. n. The new genus can be quickly differentiated from *Squamopygia* by the much shorter scape (narrowly elongate cylindrical in *Squamopygia*), and the wing not distinctly banded (two black bands in *Squamopygia*). *Sidarena* gen. n. is separable from *Ectinorhynchus* by the absence of a medial atrium in the male gonocoxites (present in *Ectinorhynchus*) and separable from *Zelothrix* gen. n. by a single scutellar macroseta (two macrosetae in *Zelothrix* gen. n.) and three spermathecae (two in *Zelothrix* gen. n.). The male genitalia are remarkably uniform throughout the genus. A single species is herein described from Queensland while the remaining species are from Western Australia.

Etymology. The genus name is derived from the Greek *Sideros*, meaning iron, referring to the broad metallic-grey stripe typically present on the thorax; and *arena*, referring to its habit of landing in sandy patches. Gender is feminine.

Key to species of *Sidarena* gen. n.

- 1 Abdomen predominantly bright orange to dark yellow (Figs 5, 14) **2**
- Abdomen predominantly dark brown, often with yellow laterally (e.g., Figs 10, 12)..... **3**
- 2 Abdominal tergite 1 uniformly orange; femora with extensive black suffusion, hind femur mostly brown to black; pubescence on lower half of pleuron and coxae sparse (Fig. 5) ***Sidarena aurantia* sp. n.**
- Abdominal tergite 1 dark anteriorly; femora mostly orange with brown black suffusion evident only on hind femur; pubescence on lower half of pleuron and coxae relatively dense (Figs 14–15)..... ***Sidarena macfarlandi* sp. n.**
- 3 Femora dark yellow (Eastern Australia) (Figs 7–8) ***Sidarena flavipalpa* sp. n.**
- Femora brown to dark brown (Figs 10, 12, 17) (Western Australia) **4**
- 4 Wing distinctly mottled; six to eight dorsocentral macrosetae (Fig. 17)
..... ***Sidarena yallingup* sp. n.**



Figure 4. Warrumbungle National Park (New South Wales, Australia), creek bed in dry sclerophyll forest. Habitat of *Zelothrix warrumbungle* sp. n. where large numbers of individuals may be present during the summer months (Photo credit: Shaun L. Winterton).

- Wing with relatively few faint markings; fewer than six large dorsocentral macrosetae (Fig. 10).....**5**
- 5 Scape largely greyish pubescent; abdomen base colour mostly blackish-brown; smaller species with wing relatively narrow (body length = 5.5 mm) (Fig. 10).....***Sidarena geraldton* sp. n.**
- Scape largely cream-yellow pubescent (sometimes brown on lateral surface); abdomen base colour only brown dorsally, yellow laterally; larger species; wing relatively broad (body length = 8–9 mm) (Figs 12–13)***Sidarena hortorum* sp. n.**

***Sidarena aurantia* sp. n.**
<http://zoobank.org/89BA1398-15FC-4E48-AC36-0107EC85556B>
Figs 5–6

Type material. Holotype male, AUSTRALIA: Western Australia: 158 km S Newman, 9 km N Kumarina Roadhouse, Malaise in wide sandy wash, 21/23.V.2003, M.E. Irwin F.D. Parker, 638 m 24°37.8'S, 117°36.8'E (GPS) (ANIC).

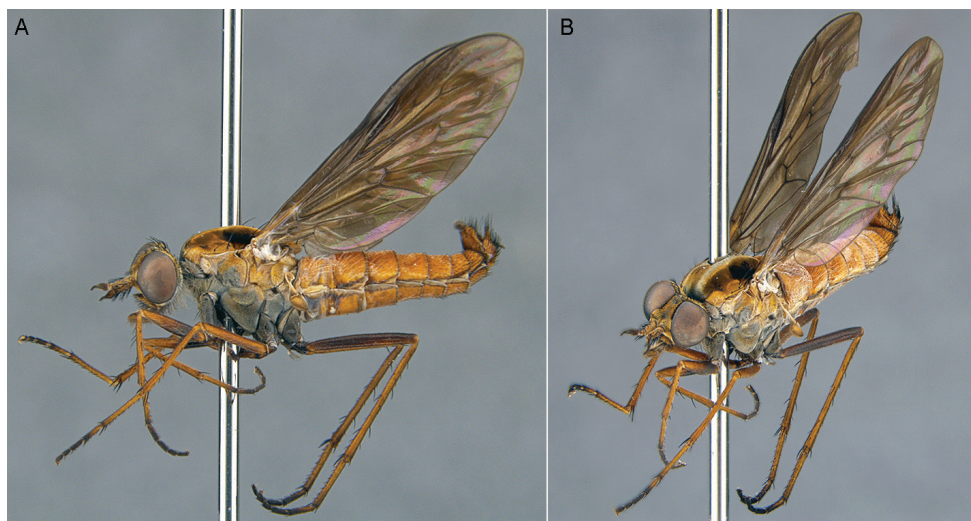


Figure 5. *Sidarena aurantia* sp. n.: **A** Male lateral view **B** same, oblique view.

Paratypes. AUSTRALIA: Western Australia: 13 males, same data as holotype (ANIC, CSCA); male, same data as holotype, 18/21.V.2003 (CSCA); male, 74 km S Newman on Great Northern Highway, Malaise in wash with drying pools, 6/18.V.2003, M.E. Irwin F.D. Parker, 631 m 23°56.0'S, 119°46.0'E (GPS) (ANIC).

Diagnosis. Medium-sized fly with distinct yellow-orange colouration on body; pleuron base colour yellow-orange dorsally, blackish suffusion ventrally and on coxae; abdomen yellow-orange, sternites 1–3 with black suffusion laterally; postocular macrosetae relatively short; femora orange with black suffusion (variable); wing uniformly dark infuscate.

Description. *Body length.* 8.0 mm (male). *Head.* (Fig. 5) Yellowish-grey pubescent; frons flat, brownish-orange pubescent, admixed with scattered black setae dorsolaterally and immediately above antennal socket, narrow dark pubescence present along eye margin; occiput convex with two rows of dark postocular setae, dark setae extending ventrally onto gena and admixed with finer white setae; antennal scape length less than pedicel and flagellum combined, orange-yellow pubescent with numerous robust black setae laterally; pedicel brownish-orange pubescent with numerous black setae; flagellum with extensive brown pubescence; mouthparts yellowish with dark pile. *Thorax.* Base colour dark yellow-orange on scutum and dorsally on pleuron; scutum overlain with pubescence as broad brown lateral stripes and broad metallic-grey stripe medially (some individuals with brownish suffusion anteromedially); very fine, sparse setal pile on scutum; postpronotal lobe orange pubescent; pleuron dark-yellow orange dorsally, darker suffusion ventrally and anteriorly; pleuron with fine white pile on anepisternum and katatergite; chaetotaxy: notopleural setae (np), 4; supra alar setae (sa), 2; postalar setae (pa), 1; dorsocentral setae (dc), 3; scutellar setae (sc), 1; wings dark infuscate, most cells slightly paler centrally; venation dark; coxae black; femora dark yellow-

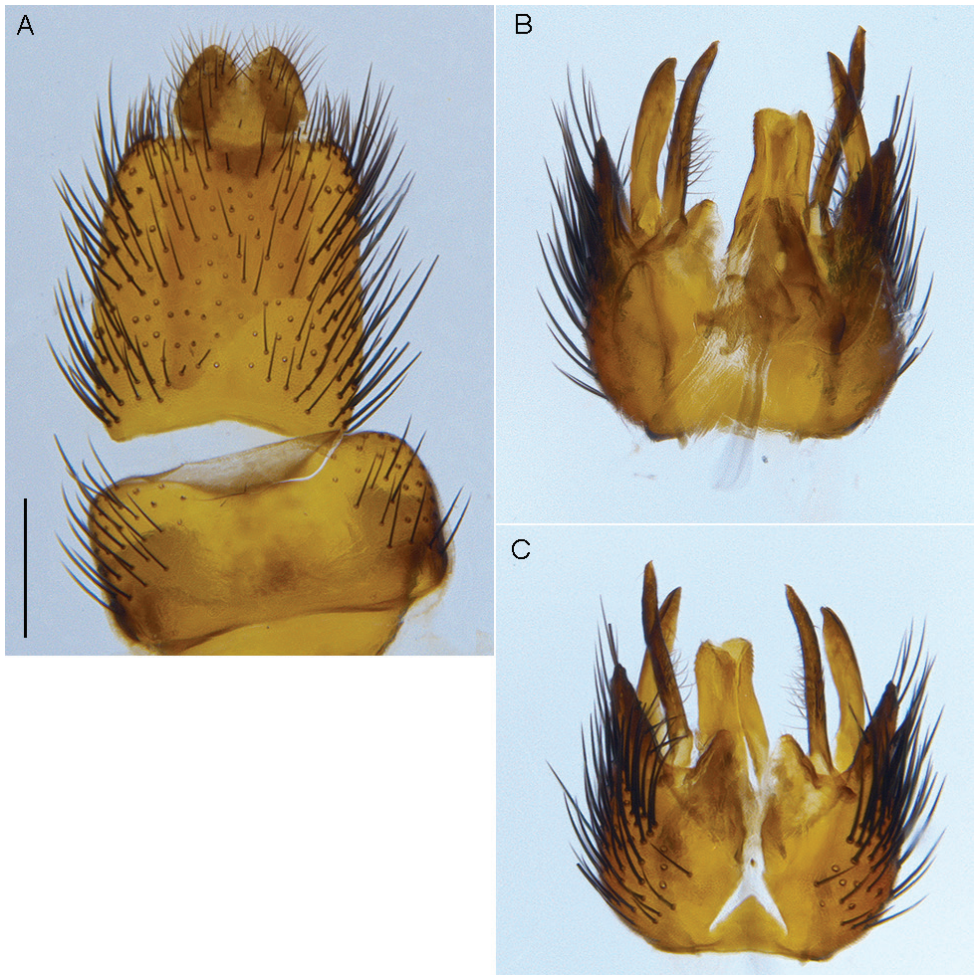


Figure 6. *Sidarena aurantia* sp. n., male genitalia: **A** Epandrium and tergite 8, dorsal view **B** Gonocoxites and aedeagus, dorsal view (epandrium removed) **C** same, ventral view. Scale line: 0.2 mm.

orange with variable extent of black suffusion, more pronounced on hind femur; tibiae and tarsi dark yellow-orange, distal tarsomeres blackish. *Abdomen*. Mostly dark yellow-orange, dark suffusion laterally on anterior sternites; silver velutum on tergites 1–5; sparse setal pile on all segments, short and dark dorsally, elongate and white laterally on anterior segments, setal pile denser and more robust posteriorly. *Male Terminalia*. (Fig. 6) Dark yellow-orange; epandrium numerous dark robust setal laterally, setal pile shorter medially; tergite 8 quadrangular with dark setae laterally; gonocoxite rounded with outer process well developed and rounded, extensive robust setal pile present; hypandrium triangular and fused with gonocoxites laterally; velutum pile very sparse ventrally on gonocoxites (barely evident in some cases); ventral lobe triangular; gonocoxite halves approximating medially, lacking medial atrium; inner gonocoxal process

articulating dorsally on gonocoxite, curved medially and with few setae apically; gonostylus narrow and slightly curved medially, numerous setae midway along medial surface; aedeagus with dorsal apodeme of parameral sheath 'T'-shaped, subequal in length to ventral apodeme; distiphallus broad distally with small spines apically.

Comments. *Sidarena aurantia* sp. n. is known only from a series of males collected in Malaise traps in northern Western Australia. The yellow-orange abdomen is highly distinctive for this species, and it is very similar to *S. macfarlandi* sp. n. There are subtle differences between the two species, which at this stage, based on the material examined, we recognise as separate species. The abdomen of *S. aurantia* sp. n. is more uniformly orange (darker on anterior tergites in *S. macfarlandi* sp. n.), while the lower half of the pleuron, coxae and hind femora are darker in *S. aurantia* sp. n. The female is unknown for this species.

Etymology. The specific epithet is a Latin adjective in the nominative feminine singular, meaning orange-coloured, referring to the body colouration.

***Sidarena flavipalpa* sp. n.**

<http://zoobank.org/6BBF2B14-6CB7-4127-965F-96D074D17C1D>

Figs 7–9

Type material. **Holotype** male, AUSTRALIA: Queensland: Beaudesert, 14.vii.1953, K.R.N., in cop. (CSCA) (MEI028781).

Paratype. AUSTRALIA: Queensland: female, same data and mounted with holotype (CSCA) (MEI028782).

Diagnosis. Medium sized fly with light brownish-grey coloured thorax; abdomen base colour dark brown; scutum brown with light grey dorsocentral stripes; occiput overlain with tan-grey pubescence; wing slightly infusate, darker along wing veins.

Description. *Body length.* 6.5 mm (male), 7.0 mm (female). *Head.* Light brown-tannish grey pubescent; frons darker along eye margin (as a spot in female), admixed with sparse dark setae, especially above antennal socket; occiput convex with dark postocular setae not arranged in rows in either sex, setae of similar length in both sexes, dark setae extending onto gena where they are admixed with paler setae; antennal scape longer than pedicel and flagellum combined, distinctly thicker; scape yellow with brownish suffusion laterally, numerous robust dark setae laterally; pedicel brownish with dark setae; flagellum brown, conical, darker apically; mouthparts yellow with white setae. *Thorax.* Scutum with extensive dark brown matte pubescence, orange pubescence laterally and on postpronotal lobe, distinct light grey dorsocentral stripes along entire scutum length; scutal pile very fine and sparse; scutellum yellow with sparse grey pubescence; pleuron tan-grey pubescent with fine white setae confined to anepisternum and katatergite. Coxae yellow, overlain with grey pubescence; legs entirely yellow with black macrosetae; chaetotaxy: np, 4; sa, 2; pa, 1; dc, 4; sc, 1; wing hyaline with brownish tint. *Abdomen.* Tergites dark brown to black with yellow laterally, sternites yellow; all segments with sparse short setae, lateral setae white in male,



Figure 7. *Sidarena flavipalpa* sp. n.: **A** Male, anterior view **B** same, dorsal view.



Figure 8. *Sidarena flavipalpa* sp. n.: **A** Female, anterior view **B** same, dorsal view.

especially on anterior segments. *Male terminalia.* (Fig. 9) Epandrium longer than wide, with medium length black setae over entire surface; hypoproct rounded apically; gonocoxite ovate, outer gonocoxal process elongate and rounded, reaching half the distance to the tip of the inner gonocoxal process; inner gonocoxal process slightly spatulate at

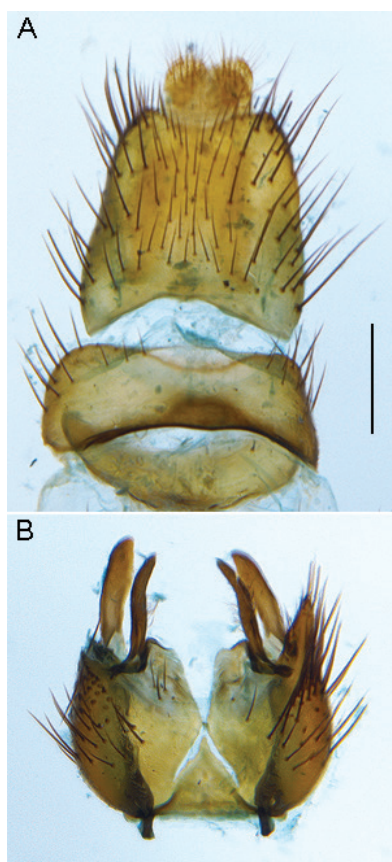


Figure 9. *Sidarena flavipalpa* sp. n.: **A** Epandrium and tergite 8, dorsal view **B** Gonocoxites, ventral view (epandrium and aedeagus removed). Scale line: 0.2 mm.

apex, extending to tip of gonostylus; gonostylus with a brush of light brown setae along inner surface; gonocoxite with elongate dark brown setae on lateral surface, denser over area ventrad of outer gonocoxal process; aedeagus typical of other species in the genus. *Female terminalia.* Short white setae dorsally on 4/5th of tergite 8; tergite 8 longer than broad; sternite 8 setose surface thinly sclerotized, ovoid in shape, narrower posteriorly; six acanthophorite A1 setae strong, 11 longer, thinner A2 setae directed ventrally.

Comments. *Sidarena flavipalpa* sp. n. is the only species of the genus found in eastern Australia. The species is only known from a pair collected in copula, and mounted together on the same pin; the holotype is the male, while the female is the paratype. This species is also distinctive by the scutal pubescent pattern, which is different from other species in the genus.

Etymology. The specific epithet is a noun in apposition derived from combining the Latin adjective *flavus*, meaning yellow, and noun *palpus*, meaning feeler, referring to the colour of the palpi.

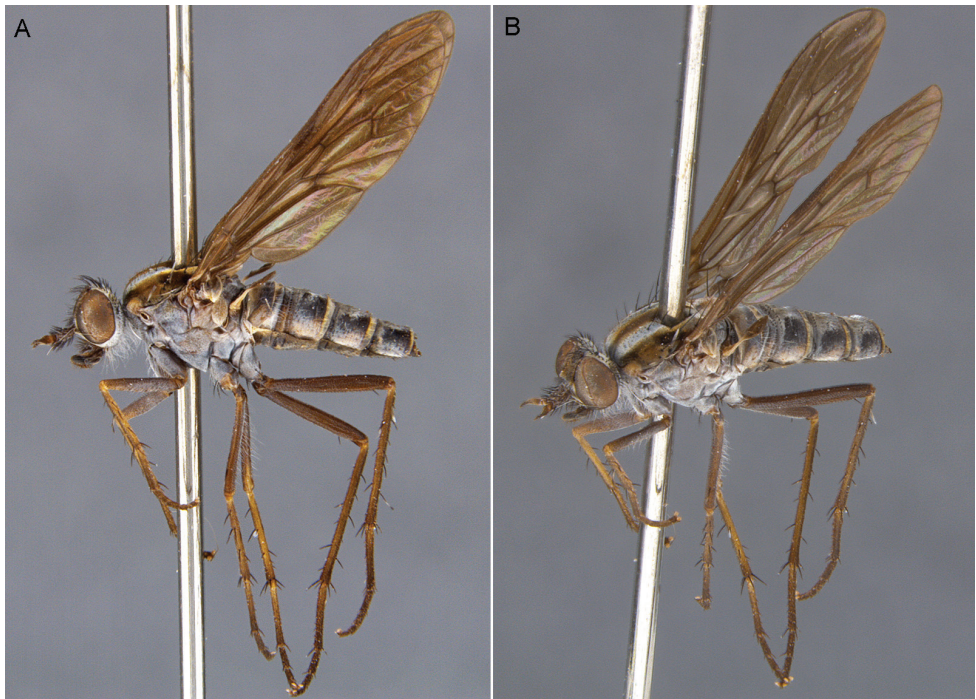


Figure 10. *Sidarena geraldton* sp. n.: **A** Male lateral view **B** same, oblique view.

***Sidarena geraldton* sp. n.**

<http://zoobank.org/B7AD7460-2384-4D24-B9C3-583D771E6219>

Figs 10–11

Type material. **Holotype** male, AUSTRALIA: Western Australia: Geraldton, 7.vii.1972, N. McFarland (at light) (ANIC) (MEI028795).

Diagnosis. Relatively small and slender species; scape grey pubescent, relatively narrow; pleuron uniform grey pubescent; abdomen dark brown-black; scutum with broad metallic grey stripe with medial brown suffusion; wing with faint infuscation, darker anteriorly and along veins; legs brown.

Description. *Body length.* 5.5 mm (male). *Head.* Occiput silver-grey pubescent with two rows of elongate postocular setae; frons flat, dark brown pubescent admixed with elongate black setae, especially just above antennal socket; pubescence also as black line along eye margin and silver spot lateral to antennal socket; face silver pubescent; gena silver-white pubescent with fine, white, elongate setae; antenna as long as head length, scape similar width to pedicel and flagellum and as long as both combined, brown with grey pubescence with extensive black setae on outer surface; flagellum brown pubescent, tapered to dark arista; mouthparts dark brown with black setae. *Thorax.* Dark base colour overlain with extensive grey pubescence; scutum overlain with pubescence as broad brown lateral stripes and broad metallic-grey stripe medially with brown suffusion along axis; scutellum yellowish with grey pubescence; pleuron mostly grey pubescent, lacking

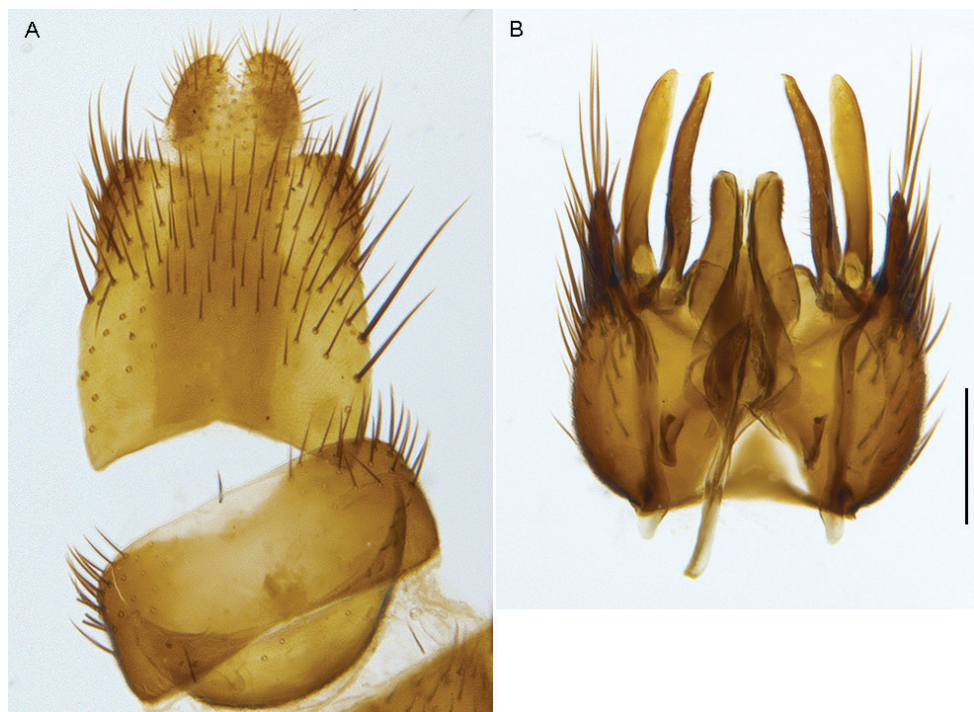


Figure 11. *Sidarena geraldton* sp. n., male genitalia: **A** Epandrium and tergite 8, dorsal view **B** Gonocoxites and aedeagus, dorsal view (epandrium removed). Scale line: 0.2 mm.

setae except anepisternum and katatergite which have scattered short, white setae; coxae dark grey pubescent; legs uniformly brown with black setae; chaetotaxy: np, 4; sa, 1; pa, 1; dc, 3; sc 1; wing uniformly tinted infusate, venation dark. *Abdomen.* Dark brown-black with cream-yellow areas laterally and on tergite 1, extensive silver velutum on at least tergites 1–4 in male, velutum not unidirectional, but with triangular pattern depending on angle viewed; thin setae present on all segments, black medially and posteriorly, white laterally and anteriorly. *Male terminalia.* (Fig. 11) Epandrium longer than wide, setae more robust laterally; cerci distinctly separate, ovoid; tergite 8 quadrangular with short robust setae laterally; gonocoxite wider than long; outer gonocoxal process elongate, pointed; inner gonocoxal process elongate, its apex protruding posteriorly to apex of gonostylus but more thinly sclerotized and slightly spatulate apically; gonostylus slender with scattered black setae anteriorly at about 1/3 distance from base to apex; ventral lobe broad and rounded; hypandrium connected to gonocoxite along anterior edge; aedeagus shape similar to other species in genus.

Comments. *Sidarena geraldton* sp. n. is a western species known only from the male holotype collected from Geraldton, Western Australia. This is a relatively diminutive species with dark legs, abdomen and narrow wings.

Etymology. The specific epithet is the unaltered place name of the type locality for this species; a noun in apposition.

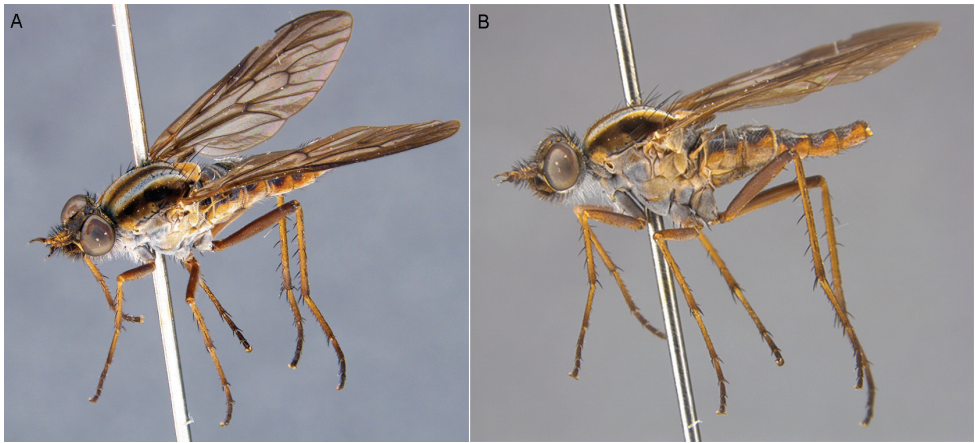


Figure 12. *Sidarena hortorum* sp. n.: **A** Male oblique view **B** same, lateral view (terminalia removed).

***Sidarena hortorum* sp. n.**

<http://zoobank.org/B49F0340-28A9-413E-8A31-B250AE827F94>

Figs 1–2, 12–13

Type material. **Holotype** male, AUSTRALIA: Western Australia: 37 km W Binnu, [-28.033, 114.667], 9.VII.1972, hand netted, N. McFarland. (ANIC) (MEI028783).

Paratypes. Two males, female, same data as holotype (ANIC, CSCA) (MEI028784, 028785, 028794); female, Cooralya H.S., [-24.45, 114.067], 10.IX.1971, hand netted, K. T. Richards. (WAM) (MEI028780); male, Gin Gin, 8 mile peg, [-31.35, 115.9], 17.VIII.1964, hand netted, P. Lawrence; 8 mile peg. (WAM) (MEI028779).

Diagnosis. Medium sized flies; thorax yellowish dorsally on pleuron, darker ventrally, scutum with broad grey strip and narrow medial brown suffusion; abdomen dark brown, yellow laterally; wing hyaline, faintly infuscate anteriorly and along veins; legs yellowish with dark suffusion; male postocular setae variable in length but often elongate.

Description. *Body length.* 8.0 mm (male), 9.0 mm (female). *Head.* (Figs 12, 13A) Yellowish-grey pubescent (male), silver-grey pubescent (female); frons flat, dark brownish pubescent, admixed with scattered black setae dorsolaterally and immediately above antennal socket, narrow dark pubescence present along eye margin; occiput convex with two poorly defined rows of dark postocular setae, dark setae extending ventrally onto gena and admixed with finer white setae; parafacial with yellow-silver pubescence; antennal scape length less than pedicel and flagellum combined, orange-silver pubescent, darker laterally, with numerous robust black setae laterally; pedicel brownish-orange pubescent with numerous black setae; flagellum with extensive dark brown pubescence; mouthparts yellowish with dark pile. *Thorax.* Base colour dark yellow-orange on scutum and posterodorsally on pleuron; scutum overlain with pubescence as broad brown lateral stripes and broad metallic-grey stripe medially (dark brownish suffusion along axis distinct in male); fine, sparse setal pile on scutum; post-

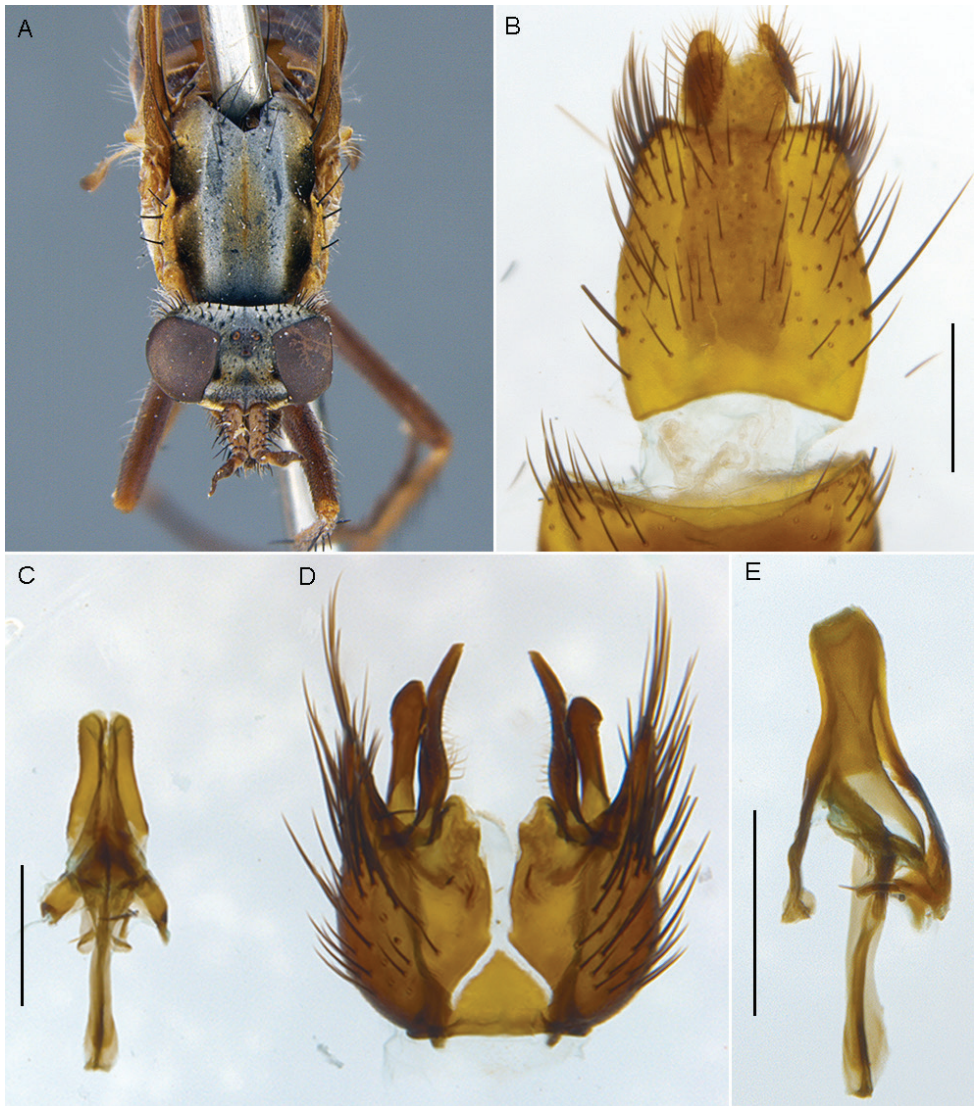


Figure 13. *Sidarena hortorum* sp. n.: **A** Female, head and thorax, dorsal view; male genitalia **B** Epandrium and tergite 8, dorsal view **C** Aedeagus, dorsal view **D** Gonocoxites, ventral view (epandrium and aedeagus removed) **E** Aedeagus, lateral view. Scale line: 0.2 mm.

pronotal lobe orange pubescent; scutellum yellow; pleuron dark-yellow orange posterodorsally, darker suffusion ventrally and anteriorly, covered with dense grey pubescence; pleuron with fine white pile on anepisternum and katatergite; chaetotaxy: np, 3; sa, 2; pa, 1; dc, 4; sc, 1; wing hyaline with dark tinge, especially anteriorly and along wing veins; venation dark; coxae dark with grey pubescence; legs dark yellow, femora with distinct brown suffusion; distal tarsomeres black. *Abdomen.* Dark yellow, extensive black-brown area medially on all tergites; silver velutum on tergites 1–5 in

male. *Male terminalia*. (Fig. 13B–E) Epandrium longer than wide, with brown marking medially and robust dark setae, longer laterally; cercus darker than epandrium; gonocoxites with outer gonocoxal process heavily sclerotized, pointed apically; inner gonocoxal process with few setae, spatulate apically; gonostylus narrow with setae mid-way along medial surface; ventral lobe bluntly rounded; aedeagus typical for genus. Female terminalia typical for genus.

Comments. *Sidarena hortorum* sp. n. is a western species closely related to *S. aurantia* sp. n. and *S. macfarlandi* sp. n. based on body colouration and scutal pattern.

Etymology. This species is a patronym named in honour of Fred and Jean Hort, field naturalists and photographers who enthusiastically document the flora and fauna of Western Australia.

***Sidarena macfarlandi* sp. n.**

<http://zoobank.org/2E3A2F57-634D-4DD0-871D-564B73BC3935>

Figs 14–16, 28B

Type material. Holotype male, AUSTRALIA: Western Australia, Moresby Range, 12.9 km NE Geraldton, Mills Park, [−28.660, 114.661], 1.viii.1973, hand netted, N. McFarland. (MEI028790) (ANIC).

Paratypes. AUSTRALIA: Western Australia: 2 males, female, same data as holotype (MEI028791, 028255 [male in copula], 028256 [female in copula]) (ANIC); males, same data as holotype (MEI028787); 6 males, 2 females, Moresby Range, Howatharra Rd., [−28.54, 114.667], 1.viii.1974, black light (UV), N. McFarland. (ANIC, CSCA) (MEI028788, 028789, 129016, 028257, 028792, 028793, 129014, 028254); female, Greenough, [−28.95, 114.733], 29.viii.1978, hand netted, R. P. McMillan. (WAM) (WAM872094).

Diagnosis. Abdomen distinctly orange; wing slightly mottled; legs dark yellow with brown suffusion on hind femur; pleuron dark yellow dorsally; abdominal tergite 1 with dark brown markings.

Description. *Body length.* 7.0 mm (male), 8.0 mm (female). *Head.* (Fig 14–15) Yellowish-grey pubescent; frons flat, brownish-orange pubescent, admixed with scattered black setae dorsolaterally and immediately above antennal socket, narrow dark pubescence present along eye margin; occiput convex with dark postocular setae not arranged in rows, dark setae extending ventrally onto gena and admixed with finer white setae; face yellow-grey pubescent; antennal scape length less than pedicel and flagellum combined, orange-yellow pubescent with numerous robust black setae laterally; pedicel brownish-orange pubescent with numerous black setae; flagellum with extensive brown pubescence; mouthparts yellowish with dark pile. *Thorax.* Base colour dark yellow-orange on scutum and dorsally on pleuron; scutum overlain with pubescence as broad brown lateral stripes and broad metallic-grey stripe medially (with light yellow-brownish suffusion medially and narrow dark stripe along axis); very fine, sparse setal pile on scutum; postpronotal lobe orange pubescent; pleuron dark-yellow

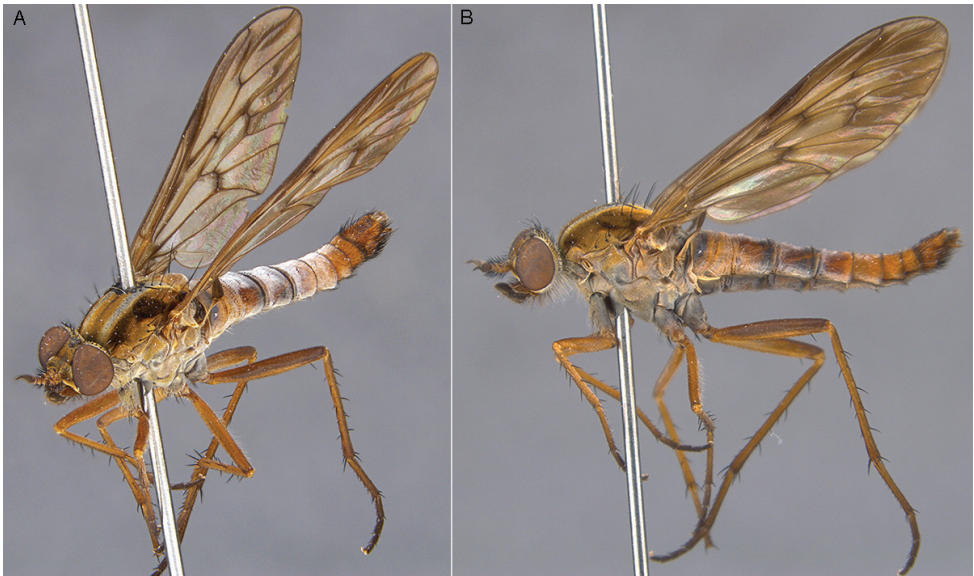


Figure 14. *Sidarena macfarlandi* sp. n.: **A** Male oblique view **B** same, lateral view.



Figure 15. *Sidarena macfarlandi* sp. n.: **A** Female anterior view **B** same, lateral view.

orange dorsally, darker suffusion ventrally, pleuron with fine white pile on anepisternum and katatergite; chaetotaxy: np, 3–4; sa, 2; pa, 1; dc, 3; sc, 1; wings infusate, darker along veins, more distinctive and extensive around crossveins to give mottled appearance; venation dark; coxae dark, overlain with grey pubescence; femora dark yellow-orange with dark suffusion more pronounced on hind femur; tibiae and tarsi dark yellow-orange, distal tarsomeres blackish. *Abdomen.* Mostly dark yellow-orange,

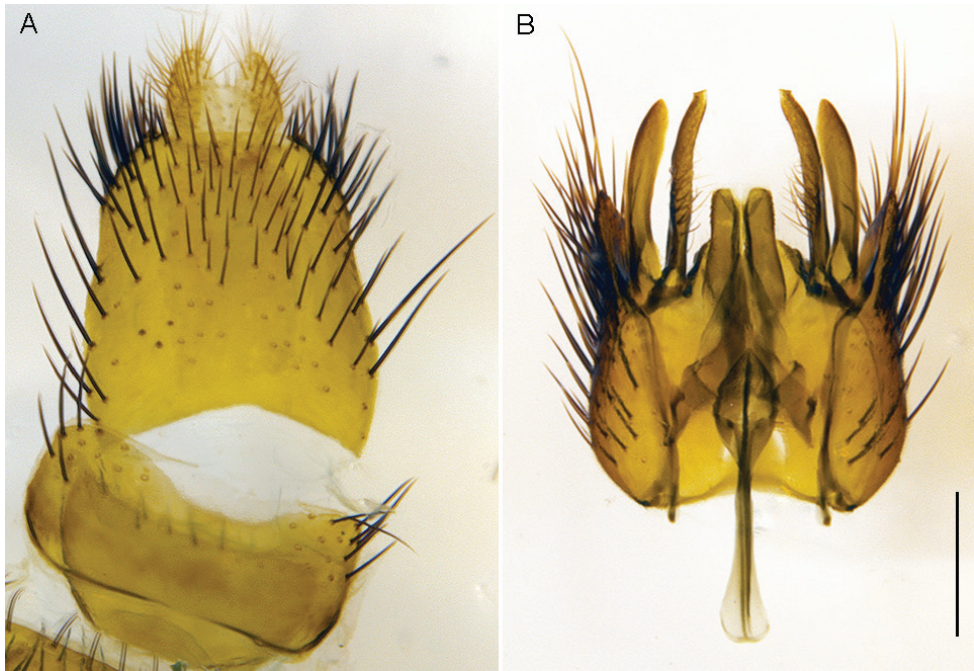


Figure 16. *Sidarena macfarlandi* sp. n., male genitalia: **A** Epandrium and tergite 8, dorsal view **B** Gonocoxites and aedeagus, dorsal view (epandrium removed). Scale line: 0.2 mm.

dark suffusion laterally on segments 1–3 and sometimes segment 4; silver velutum on tergites 1–6; sparse setal pile on all segments, short and dark dorsally, elongate and white laterally on anterior segments, setal pile darker and more robust posteriorly. *Male terminalia* (Fig. 16). Epandrium slightly longer than wide with extensive robust dark setae; cercus with pale setae; tergite 8 slightly emarginate posteriorly; gonocoxite and aedeagus typical of species in the genus. Female terminalia typical for the genus.

Comments. *Sidarena macfarlandi* sp. n. is similar in appearance to *S. aurantia* sp. n. based on body colouration, especially the abdomen (see comments above).

Etymology. This species is named after the collector, Noel McFarland, of this and other species of *Sidarena* in Western Australia.

***Sidarena yallingup* sp. n.**

<http://zoobank.org/1FD0C2F9-7A52-4E00-A5B3-0A3A64378F34>

Figs 17–20

Type material. **Holotype** male, AUSTRALIA: Western Australia, 37 km N Augusta, [–34.333, 115.167], 1.x.1975, hand netted, K. A. Spencer. (WAM872079) (WAM).

Paratypes. AUSTRALIA: Western Australia: male, 2 females, Leeuwin Naturaliste National Park, Yallingup portion, 14.ix.1983, hand netted, E. I. Schlinger, M. E.



Figure 17. *Sidarena yallingup* sp. n.: Male (upper) and female (lower), oblique view (terminalia removed).



Figure 18. *Sidarena yallingup* sp. n.: Male (upper) and female (lower), lateral view (terminalia removed).

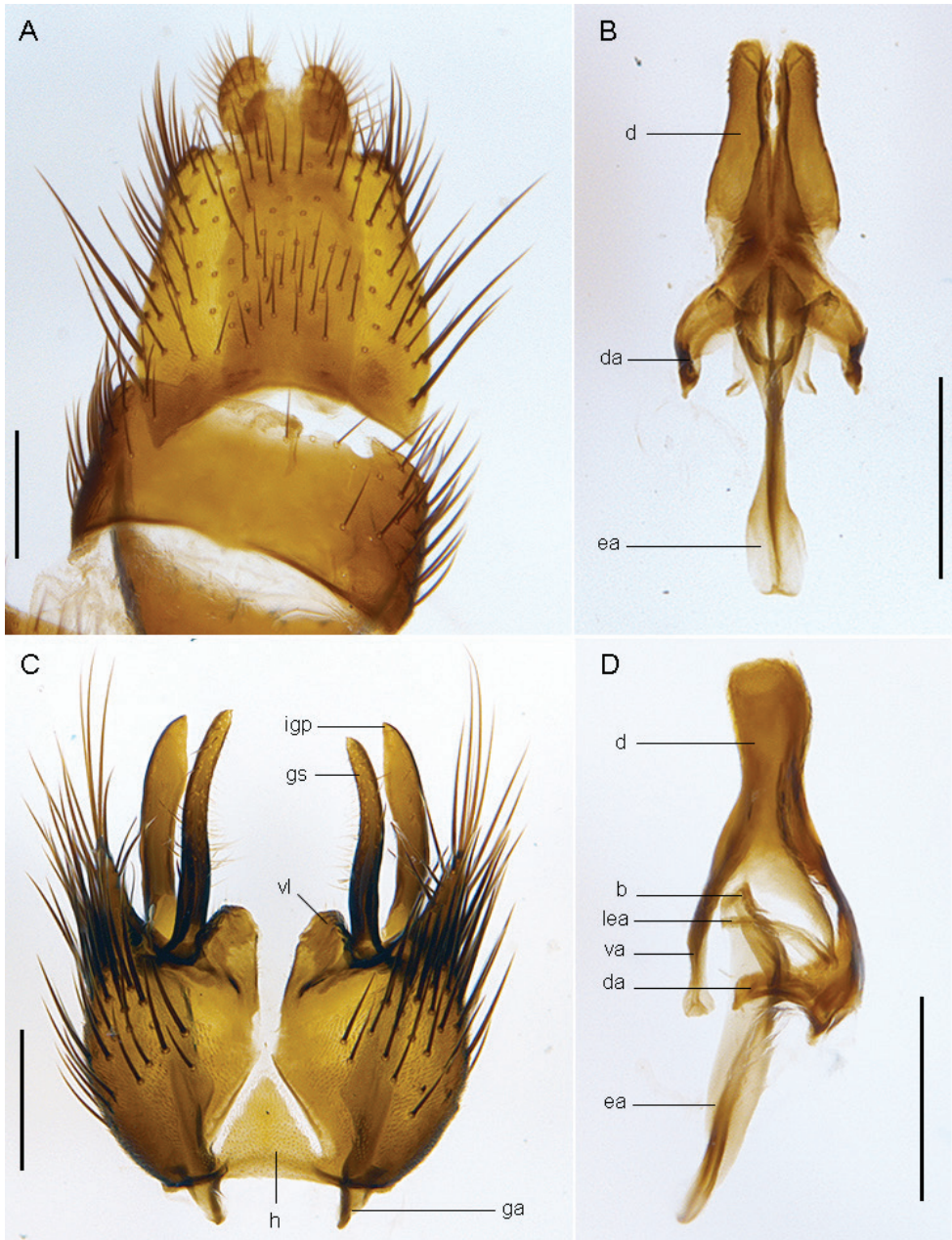


Figure 19. *Sidarena yallingup* sp. n.: Male genitalia: **A** Epandrium and tergite 8, dorsal view **B** Aedeagus, dorsal view **C** Gonocoxites, ventral view (epandrium and aedeagus removed) **D** Aedeagus, lateral view. Scale line: 0.2 mm. Abbreviations: *b*, basiphallus; *d*, distiphallus; *da*, dorsal apodeme of parameral sheath; *ea*, ejaculatory apodeme; *ga*, gonocoxal apodeme; *gs*, gonostylus; *h*, hypandrium; *igp*, inner gonocoxal process; *lea*, lateral ejaculatory apodeme; *va*, ventral apodeme of parameral sheath; *vl*, ventral lobe. Scale line = 0.2 mm.

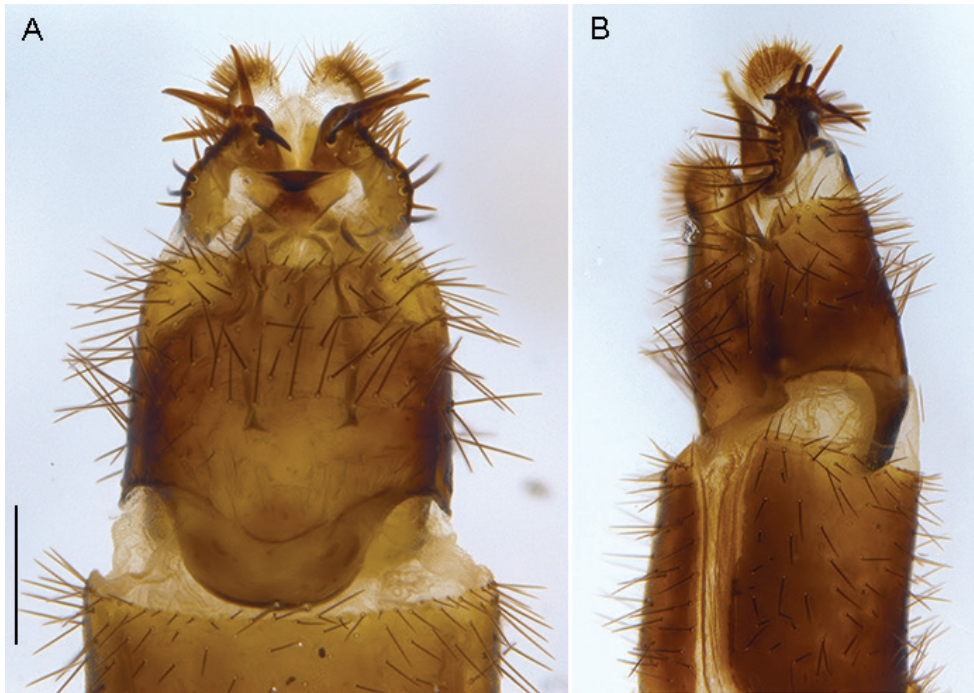


Figure 20. *Sidarena yallingup* sp. n.: Female genitalia: **A** dorsal view **B** lateral view. Scale line: 0.2 mm.

Irwin; limestone caves and Acacia-Eucalyptus forest, cycads (CSCA) (MEI028771, 028772, 028773); 2 females, Hamelin Bay, [-34.2, 115.017], 1.x.1975, hand netted, K. A. Spencer. (WAM872077, 872078) (WAM); female, Hamelin Bay, [-34.2, 115.017], 2m, 26.ix.1962, hand netted, E. S. Ross, D. Q. Cavnaro (CASC) (MEI028774).

Diagnosis. Wing dark mottled infusate; scutal macrosetae elongate, legs dark brown; abdomen dark brown; genal pile uniformly dark.

Description. *Body length.* 7.5 mm (male), 8.5 mm (female). *Head* (Fig 17–18). Grey-silver (male) or yellow-silver (female) pubescent; male occiput convex with silver-grey and matte black pubescence (depending on angle viewed); postocular setae not arranged in rows, setae variable in length, some elongate; frons flat with elongate black, scattered setae, at midpoint of the frons is a small dark patch of dark brown pubescence set against eye margin; gena with dark setae; face golden (female), silver (male) pubescent, without setae; antennae light yellowish-brown; palpus brown with dark setae; antenna light yellow-orange, scape wider than pedicel and flagellum, with dark setae on outer lateral surface, longer than pedicel and flagellum combined; flagellum orange, tapered to a dark brown arista. *Thorax.* Scutum with distinct matte black (gold when viewed laterally) stripes laterally, broad medial stripe greyish in male, yellowish-grey in female, narrow dark brown suffused stripe along axis in male; postpronotal lobe orange; scutal macrosetae elongate, black, sparse thin scutal pile other-

wise; pleuron dark yellow, darker ventrally and on coxae, with sparse covering of grey pubescence; sparse elongate, thin black setae on anepisternum and katatergite; scutellum pale yellow-orange with grey pubescence; chaetotaxy: np, 3; sa, 2; pa, 1; dc, 6–7; sc 2; wing dark mottled infusate; coxae dark brown with grey pubescence; femora dark brown; rest of legs light brown. *Abdomen*. Dark brown, dorsally black with silver velutum on tergites 2–5 (more extensive in male), velutum pattern not unidirectional and silver pattern changes depending on angle viewed; anterolaterally on tergite 1 is a small patch of orange. *Male* (Fig. 19) and *female* (Fig. 20) *terminalia*. Similar to other members of the genus.

Comments. The mottled wing of *S. yallingup* sp. n. is highly distinctive and easily identifies it among the other species with the metallic grey stripe on the scutum. There is more sexual dimorphism in this than in other species. This species appears to be closely related to *S. geraldton* sp. n. based on overall body colouration and wing pattern.

Etymology. The specific epithet is the unaltered place name Yallingup (which is an Aboriginal word meaning 'Place of Love') for a location in southwestern Western Australia where this species was collected; a noun in apposition.

Zelothrix gen. n.

<http://zoobank.org/AF6D8499-EF68-4415-A8B4-4F9DAE431A6A>

Figs 3, 21–28

Type species. *Zelothrix warrumbungles* sp. n., designated here.

Diagnosis. Male eyes contiguous dorsally; male occiput concave with a single row of postocular macrosetae present dorsally in male; antennal scape with macrosetae along medial surface; scape narrow and only slightly elongate; two pair of scutellar macrosetae; parafacial without setal pile; velutum patches absent on femora and sparsely present ventrally on gonocoxites; single anteroventral seta present apically on hind femur; wing cell m_3 open; male genitalia with inner gonocoxal process well developed; gonostylus narrow apically; gonocoxites with medial atrium lacking; aedeagus with distiphallus broad apically; ventral apodeme of parameral sheath as broad plate, not forked; epandrium quadrangular. Female tergite 8 with narrow process anteromedially; two spermathecae, ducts joining to spermathecal sac duct; spermathecal sac present (Fig. 28A), female abdominal segment 8 with elongate posteriorly directed setae (Fig. 27).

Included species. *Z. warrumbungles* sp. n. and *Z. yeatesi* sp. n.

Comments. *Zelothrix* gen. n. is a distinctive genus with a disparate distribution. Similar genera include *Squamopygia* Kröber, *Taenogerella* Winterton & Irwin and *Sidarena* gen. n. This new genus can be differentiated from *Squamopygia* and *Sidarena* gen. n. by the presence of two scutellar macrosetae and a medial atrium in the male gonocoxites. The wing is extensively patterned in *Zelothrix* gen. n. but not banded as in *Squamopygia*. *Zelothrix* gen. n. is separable from *Taenogerella* by the latter having a downward directed distiphallus in the male genitalia and three spermathecae (two

in *Zelothrix* gen. n.). A significant departure from the female genitalic complement of three spermathecae typically found in Agapophytinae, is that *Zelothrix* gen. n. only has two spermathecae, a condition found in Therevinae. No other genus of Agapophytinae has two spermathecae, although the distantly related agapophytine genus *Bonjeania* Winterton & Skevington has only a single spermatheca (Winterton et al. 2000).

Zelothrix warrumbungles sp. n. is a locally highly abundant species found mainly in Warrumbungle National Park (New South Wales) (Fig. 4), while *Z. yeatesi* sp. n. is a rarely collected species endemic to Porongurup National Park (Western Australia). The two species are very similar in appearance.

Etymology. This name is derived from the Greek, *Zelos*– emulation, and *thrix*– hair, for the setal pile on the female abdomen. Gender is feminine.

Key to species of *Zelothrix* gen. n.:

- 1 Forefemur dark brown; male frons predominantly silver pubescent immediately above antennal socket; antenna greater than 1.5× head length (Figs 3, 25–27) (Eastern Australia) ***Z. warrumbungles* sp. n.**
- Forefemur dark yellow; male frons black and silver pubescent immediately above antennal socket; antenna less than 1.5× head length (Figs 21–24) (Western Australia) ***Z. yeatesi* sp. n.**

Zelothrix warrumbungles sp. n.

<http://zoobank.org/778C5F21-B145-4EB6-B6A6-899D650F0CB3>

Figs 3, 21–23

Type material. Holotype male, AUSTRALIA: New South Wales: Warrumbungle National Park, 1.7 km N Camp Blackman, Buckleys Creek, [-31.25, 149.002], 480m, 30.x.-14.xi.1997, malaise trap, S. Winterton, J. Skevington. (ANIC) (MEI153269).

Paratypes. AUSTRALIA: New South Wales: 22 males, 10 females, same data as holotype, (ANIC) (MEI140101, MEI140126, MEI140128, MEI140131, MEI140138, MEI140141, MEI140143, MEI140150, MEI140153, MEI140159, MEI140375-95, MEI153269). Queensland: female, Stanthorpe, [-28.667, 151.917], 10.i.1924, hand netted, F. M. Hull. (CNC) (MEI027295); female, near Stanthorpe, Mount Marlay, [-28.667, 151.933], 1.x.1987, hand netted, D. K. Yeates. (QM) (MEI033880). Victoria: 24.2 km NNE Orbost, [-37.75, 148.5], 5.xi.1969, hand netted, I. F. B. Common. (ANIC) (MEI028778).

Diagnosis. Forefemur dark brown; male frons predominantly silver pubescent immediately above antennal socket; antenna greater than 1.5x head length.

Description. *Body length.* 7.5 mm (male), 8.5 mm (female). *Head.* (Figs 21–22) Silver-grey pubescent; ocellar tubercle black, raised (prominent in male); frons flat, with only a few black setae above the antennal socket, silver and black pubescent in male, matte black, silver and gold patterned in female; occiput silver-gold pubescent,



Figure 21. *Zelothrix warrumbungles* sp. n.: **A** Male lateral view **B** same, oblique view.



Figure 22. *Zelothrix warrumbungles* sp. n.: **A** Female lateral view **B** same, oblique view.

concave with a single row of black postocular setae dorsally in male, two rows in female; gena silver pubescent admixed with pale setae; parafacial silver in male, silver and matte black in female; palpus narrow, pointed apically, with brown with black setae; Antennal scape elongate and cylindrical, orange, with erect black setae on all surfaces, slightly thinner than flagellum and length equalling length of combined pedicel and flagellum; flagellum elongate and cylindrical, brown pubescent with distinct angled arista at apex. *Thorax*. Scutum and scutellum gold-silver pubescent, scutum with three distinct dark brown stripes, medial stripe extending onto scutellum; chaetotaxy: np, 4; sa, 2; pa, 1; dc, 3; sc, 2; pleuron base colour black, overlain with dense greenish-silver pubescence extending onto coxae; thin white hairs on anepisternum and katatergite; femora bright yellow, forefemur mostly with dark

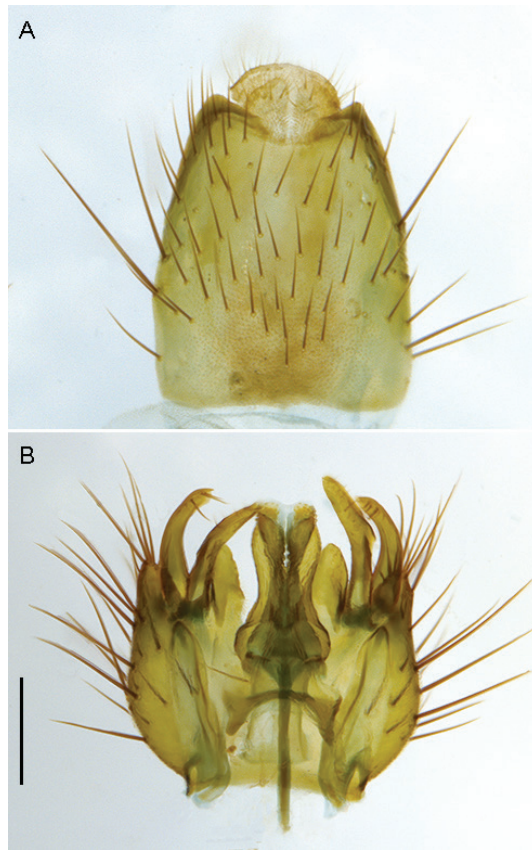


Figure 23. *Zelothrix warrumbungles* sp. n.: Male genitalia: **A** Epandrium, dorsal view **B** Gonocoxites and aedeagus, dorsal view (epandrium removed). Scale line: 0.2 mm.

brown to black suffusion; tibiae and tarsomeres 1 and 2 dark yellow, brown apically; remaining tarsomeres dark brown; wing distinctly infuscate with extensive mottled pattern; venation dark. *Abdomen.* Slender, elongate, bright yellow with small dark brown area anteromedially on tergites in male, tergites more extensively dark brown in female and overlain with sparse grey pubescence; sparse thin elongate setae on all segments, mostly pale, but darker dorsomedially and on terminalia. *Male Terminalia.* (Fig. 23) Epandrium longer than wide, slightly tapered posteriorly, sclerotised posterolaterally, setae sparse, more elongate laterally; cercus relatively small; gonocoxites rounded with short round outer gonocoxal process; hypandrium small and fused to gonocoxites anteriorly; gonocoxal apodemes small and rounded; setae on gonocoxites sparse, elongate and erect, with sparse velutum ventrally on gonocoxite; inner gonocoxal process and gonostylus narrow and curved medially; ventral lobe elongate and rounded apically; dorsal apodeme of parameral sheath ‘T’-shaped; ventral lobe broad, not forked and projecting beyond dorsal apodeme; distiphallus broad, irregularly shaped dorsally, small spines apically; lateral ejaculatory apodemes

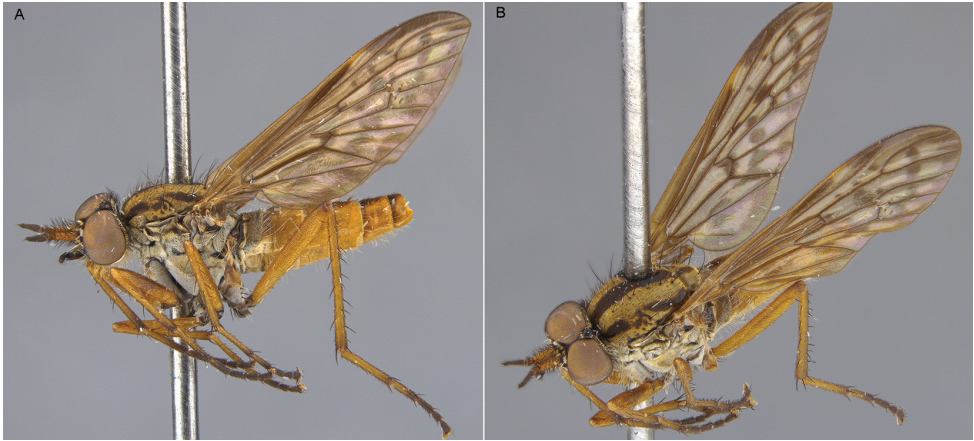


Figure 24. *Zeloithrix yeatesi* sp. n.: **A** Male lateral view **B** same, oblique view (terminalia removed).



Figure 25. *Zeloithrix yeatesi* sp. n.: **A** Female oblique view **B** same, lateral view (terminalia removed).

narrow and angled posteriorly, basiphallus small. *Female terminalia*. Similar to the other species in this genus.

Comments. *Zeloithrix warrumbungles* sp. n. is a distinctive, elegant and abundant species in the type locality during the late summer months, and in some years may be the most commonly encountered species of stiletto fly during this time.

Etymology. The specific epithet is the unaltered place name Warrumbungles (which is an Aboriginal name for this mountain range, meaning “crooked mountains”) referring to the mountain range where this species was collected; a noun in apposition.

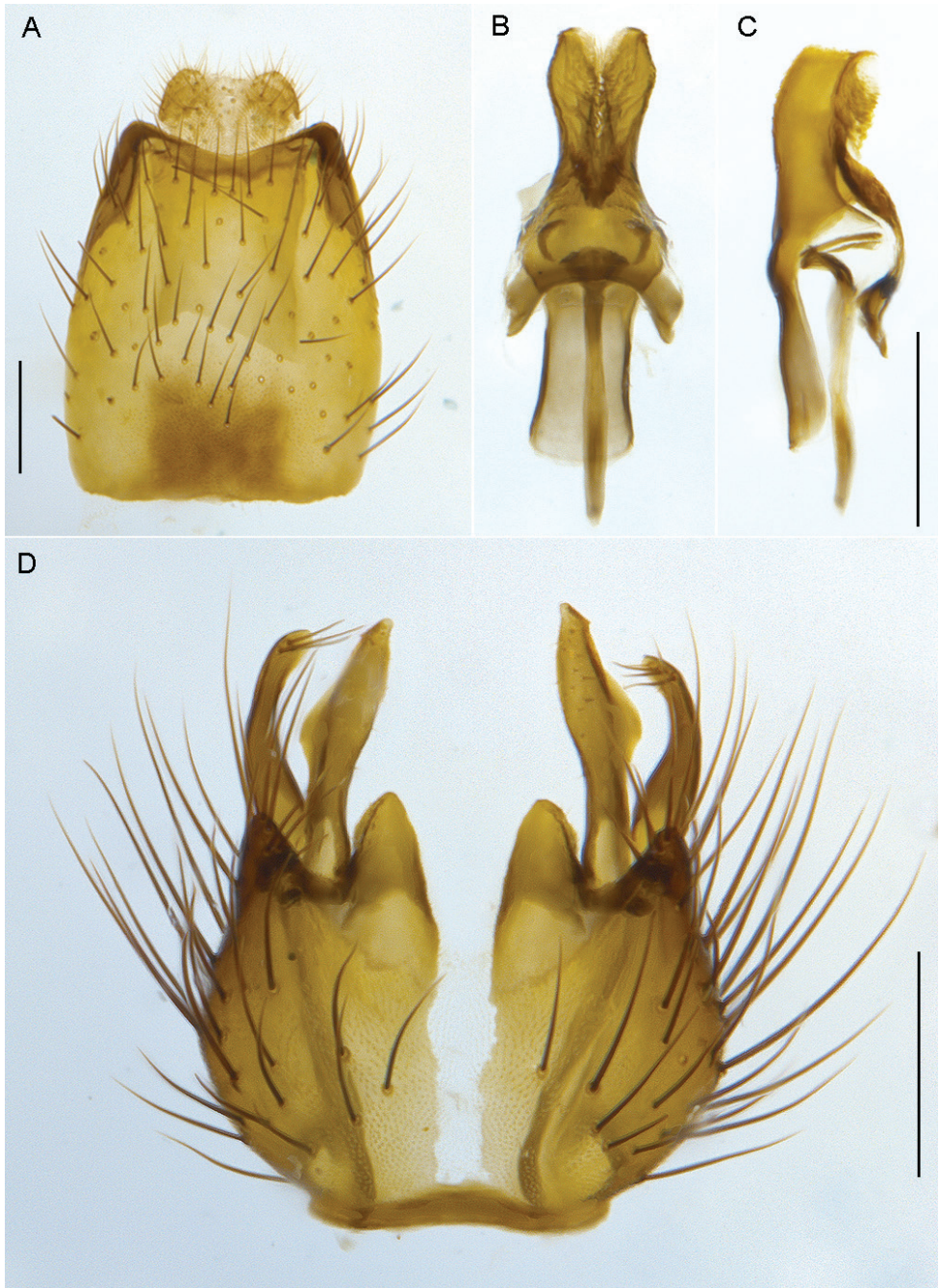


Figure 26. *Zelothrix yeatesi* sp. n.: Male genitalia: **A** Epandrium, dorsal view **B** Aedeagus, dorsal view **C** Same, lateral view **D** Gonocoxites, ventral view (epandrium and aedeagus removed). Scale line: 0.2 mm.

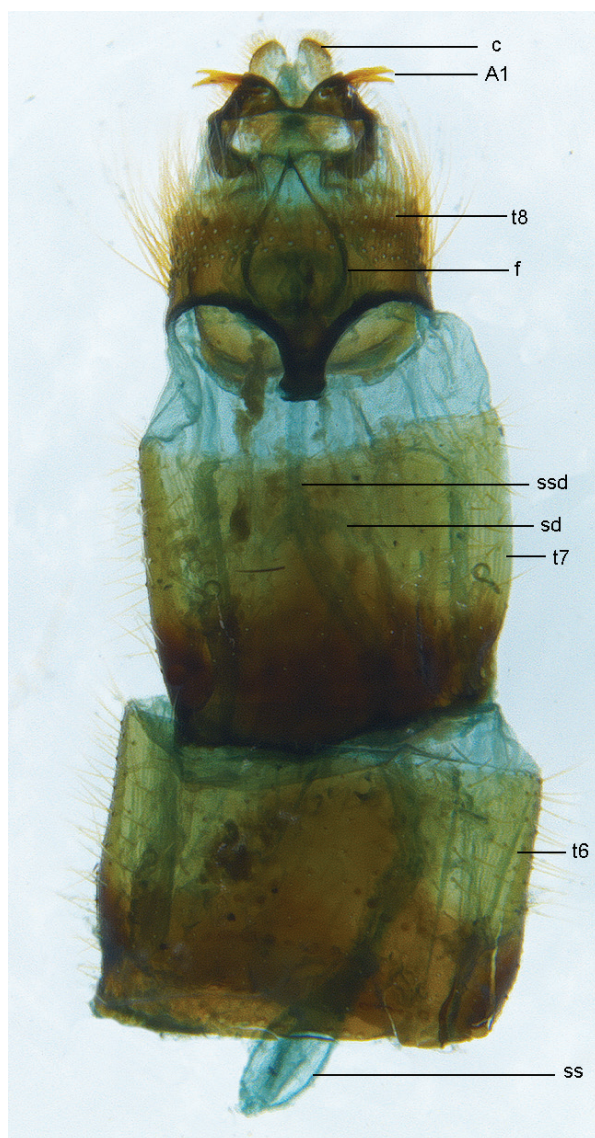


Figure 27. *Zelothrix yeatesi* sp. n.: Female genitalia, dorsal view. Abbreviations: c, cercus; A1 acanthophorite spines A1; t6–t8; tergites 6–8; sd, spermathecal duct; ss, spermathecal sac; ssd, spermathecal sac duct.

***Zelothrix yeatesi* sp. n.**

<http://zoobank.org/38B927CB-50F3-44F0-85FA-FEB0B0F25A1C>

Figs 24–28

Type material. **Holotype** male, AUSTRALIA: Western Australia, Porongurup National Park, [Porongurup Range], Yate Flats, [-34.667, 117.85], 11.xi.1987, malaise trap, M. E. Irwin, E. I. Schlinger (ANIC) (MEI028776).

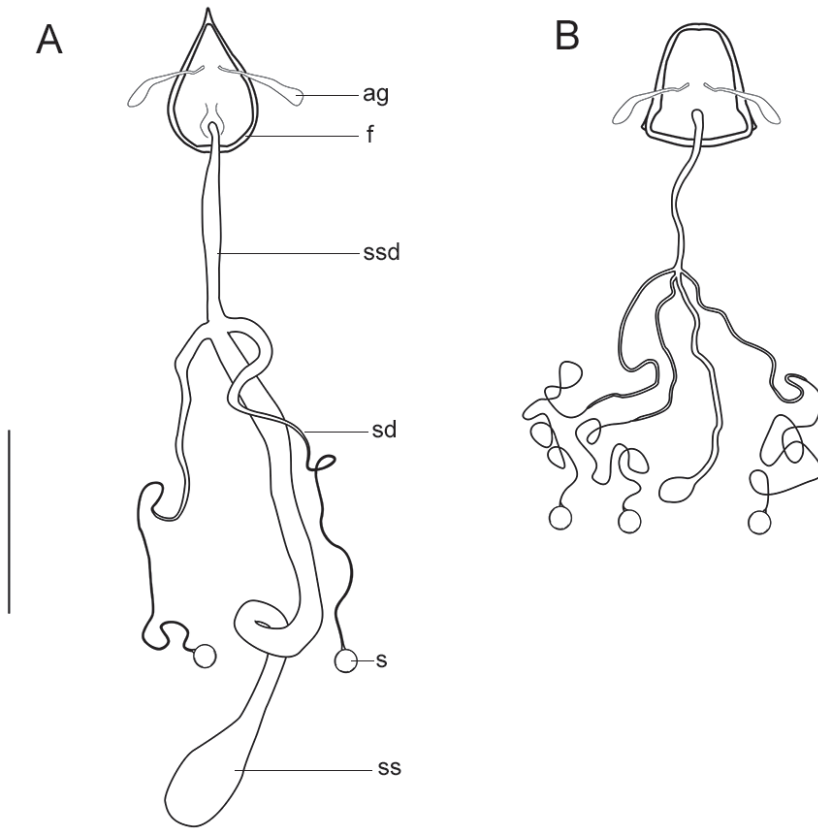


Figure 28. Distal reproductive complex. **A** *Zelothrrix yeatesi* sp. n. **B** *Sidarena macfarlandi* sp. n. Scale line: 0.2 mm. Abbreviations: *ac*, accessory gland; *f*, furca; *s*, spermatheca; *sd*, spermathecal duct; *ss*, spermathecal sac; *ssd*, spermathecal sac duct.

Paratypes. AUSTRALIA: Western Australia: female, same data as holotype (ANIC) (MEI028776); male, Porongurup National Park, [Porongurup Range], Jarra-Karri Forest, Mira Flores Hut, [-34.667, 117.85], 11.xi.1987, hand netted, M. E. Irwin, E. I. Schlinger. (CSCA) (MEI028775).

Diagnosis. Forefemur dark yellow; male frons black and silver pubescent immediately above antennal socket; antenna less than 1.5x head length.

Description. *Body length.* 6.0 mm (male), 6.5 mm (female). (Figs 24–25) Similar to *Z. warrumbungles* sp. n. except as follows: Antenna shorter, scape slightly wider; frons of male with matte black pubescence more extensive above antennae socket; scutal chaetotaxy: np, 4; sa, 1–2; pa, 1; dc, 4; sc, 2; foreleg dark yellow; male abdominal tergites with more extensive dark markings medially. *Male* (Fig. 26) and *female* (Fig. 27, 28A) *terminalia*. Very similar to the other species in this genus. Female tergite 8 with anterior process relatively narrow; broad band of elongate setae directed posteriorly on both tergite 8 and sternite 8; furca broadly tear-drop shaped

Comments. *Zelothrix yeatesi* sp. n. is very similar in body colour and wing patterning to *Z. warrumbungles* sp. n., but is much less commonly collected. The shape and vestiture of the frons and antennal shape differentiate this species.

Etymology. This distinctive species is a patronym named in honour of our colleague, friend and oft mentor, Dr David K. Yeates.

Acknowledgements

Funding for this study was provided originally by the National Science Foundation (NSF) (DEB-0614213, DEB-9521925, and DEB-9977958), the Schlinger Foundation and the Australian Biological Resources Study (ABRS-209-48). Statements and viewpoints expressed herein do not necessarily reflect the views of NSF or ABRS. Thank you to Fred and Jean Hort for permission to use their photographs of *Sidarena* during their excellent studies and photography of Western Australian insects.

References

- Cumming JM, Wood DM (2009) Adult morphology and Terminology. In: Brown BV, Borkent A, Cumming JM, Wood DM, Woodley NE, Zumbado MA (Eds) Manual of Central American Diptera. National Research Council of Canada Monograph 1: 9–50.
- Ferguson DJ, Irwin ME, Yeates DK (2013) New species of *Anabarhynchus* Macquart (Diptera: Therevidae) from arid and monsoon tropical Australia. *Zootaxa* 3680: 55–95. doi: 10.11646/zootaxa.3680.1.6
- Ferguson DJ, Lambkin CL, Yeates DK (2014) Eight new species of Australian stiletto flies in the genus *Anabarhynchus* Macquart (Diptera: Therevidae) from South East Queensland. *Zootaxa* 3802: 553–582. doi: 10.11646/zootaxa.3802.4.7
- Gaimari SD, Hauser M, Fricke R (2013) Case 3605 PHYCINAE Lyneborg, 1976 (Insecta: Diptera: Therevidae): proposed emendation of spelling to PHYCUSINAE to remove homonymy with PHYCINAE Swainson, 1838 (Osteichthyes: Gadiformes: Phycidae); and Phycis Walbaum [ex Artedi], 1792 (Osteichthyes: Gadiformes: Phycidae): proposed conservation of usage by designation of *Blennius phycis* Linnaeus, 1766 as the type species. *Bulletin of Zoological Nomenclature* 70(1): 22–29.
- International Commission on Zoological Nomenclature (2015) Opinion 2372 (Case 3605): PHYCINAE Lyneborg, 1976 (Insecta, Diptera, THEREVIDAE): spelling emended to PHYCUSINAE and Phycis Walbaum, 1792 (Osteichthyes []): usage conserved by designation of *Blennius phycis* Linnaeus, 1766 as the type species. *Bulletin of Zoological Nomenclature* 72(4): 324–326.
- Kampmeier GE, Irwin ME (2009) Meeting the interrelated challenges of tracking specimen, nomenclature, and literature data in Mandala. In: Pape T, Meier R, Bickel D (Eds) *Diptera Diversity: Status, Challenges and Tools*. Brill Academic Publishers, Leiden, 407–438. doi: 10.1163/ej.9789004148970.1-459.65
- Lambkin CL, Trueman JW, Yeates DK, Holston KC, Webb DW, Hauser M, Metz MA, Hill HN, Skevington JH, Yang L, Irwin ME, Wiegmann BM (2009) Supertrees and the Tree of

- Life: Generating a metaphylogeny for a diverse invertebrate family (Insecta: Diptera: Therevidae) using constraint trees and the parsimony ratchet to overcome low taxon overlap. *Invertebrate Systematics* 23: 171–191. doi: 10.1071/IS08035
- Lambkin CL, Turco F (2013) *Collessiama* (Diptera: Therevidae: Agapophytinae: *Taenogera* genus-group) a new genus from eastern Australia, with a key to the Australian genera of Therevidae. *Zootaxa* 3680: 96–117. doi: 10.11646/zootaxa.3680.1.7
- Pyle RL, Michel E (2008) Zoobank: Developing and nomenclatural tool for unifying 250 years of biological information. *Zootaxa* 1950: 39–50.
- Winterton SL (2006) New species of *Eupsilocephala* Kröber from Australia (Diptera: Therevidae). *Zootaxa* 1372: 17–25.
- Winterton SL (2007a) Revision of the Australian stiletto fly genus *Acatopygia* Kröber (Diptera: Therevidae: Agapophytinae). *Zootaxa* 1405: 51–62.
- Winterton SL (2007b) New species of *Laxotela* Winterton & Irwin from Australia (Diptera: Therevidae: Agapophytinae). *Zootaxa* 1407: 43–55.
- Winterton SL (2007c) New species of *Nanexila* Winterton & Irwin and *Taenogera* Kröber from Australia (Diptera: Therevidae). *Zootaxa* 1413: 55–64.
- Winterton SL (2007d) New species of *Acraspisoides* Hill & Winterton and *Bonjeania* Irwin & Lyneborg (Diptera: Therevidae: Agapophytinae), with the description of a new genus. *Zootaxa* 1438: 1–25.
- Winterton SL (2009) Revision of the stiletto fly genus *Neodialineura* Mann (Diptera: Therevidae: Agapophytinae): an empirical example of cybertaxonomy. *Zootaxa* 2157: 1–33.
- Winterton SL (2011a) Review of the stiletto fly genus *Actenomerus* Winterton & Irwin (Diptera, Therevidae, Agapophytinae). *ZooKeys* 120: 55–63. doi: 10.3897/zookeys.120.1615
- Winterton SL (2011b) Revision of the stiletto fly genera *Acupalpa* Kröber and *Pipinnipons* Winterton (Diptera, Therevidae, Agapophytinae) using cybertaxonomic methods, with a key to Australasian genera. *ZooKeys* 95: 29–79. doi: 10.3897/zookeys.95.1461
- Winterton SL, Ferguson D (2012) New species of *Vomerina* Winterton (Diptera: Therevidae: Agapophytinae) from Australia. *ZooKeys* 218: 65–75. doi: 10.3897/zookeys.218.2380
- Winterton SL, Hardy NB, Gaimari SD, Hauser M, Hill HN, Holston KC, Irwin ME, Lambkin CL, Metz MA, Turco F, Webb D, Yang L, Yeates DK, Wiegmann BM (2016) The phylogeny of stiletto flies (Diptera: Therevidae). *Systematic Entomology* 41: 144–161. doi: 10.1111/syen.12147
- Winterton SL, Irwin ME, Yeates DK (1999a) Systematics of *Nanexila* Winterton & Irwin, gen. n. (Diptera: Therevidae) from Australia. *Invertebrate Taxonomy* 13: 237–308. doi: 10.1071/IT97029
- Winterton SL, Irwin ME, Yeates DK (1999b) Phylogenetic revision of the *Taenogera* Kröber genus-group (Diptera: Therevidae), with descriptions of two new genera. *Australian Journal of Entomology* 38: 274–290. doi: 10.1046/j.1440-6055.1999.00126.x
- Winterton SL, Skevington JH, Irwin ME, Yeates DK (2000) Phylogenetic revision of *Bonjeania* Irwin & Lyneborg (Diptera: Therevidae). *Systematic Entomology* 25: 295–324. doi: 10.1046/j.1365-3113.2000.00107.x
- Winterton SL, Lambkin CL (2012) New Australian stiletto flies: revision of *Manestella* Metz and description of *Medomega* gen. n. (Diptera: Therevidae: Agapophytinae). *ZooKeys* 240: 1–119. doi: 10.3897/zookeys.240.2967

A note on captive breeding and reproductive parameters of the Chinese pangolin, *Manis pentadactyla* Linnaeus, 1758

Fuhua Zhang¹, Shibao Wu¹, Cuiyun Zou¹, Qiaoyun Wang¹,
Shaoshan Li¹, Ruyong Sun¹

¹ School of Life Science, South China Normal University, Guangzhou 510631, P. R. China

Corresponding author: Shibao Wu (wushibao@163.com)

Academic editor: P. Stoev | Received 22 June 2016 | Accepted 29 August 2016 | Published 19 September 2016

<http://zoobank.org/3EC354B3-C669-4C96-A82C-4015ED231DCD>

Citation: Zhang F, Wu S, Zou C, Wang Q, Li S, Sun R (2016) A note on captive breeding and reproductive parameters of the Chinese pangolin, *Manis pentadactyla* Linnaeus, 1758. ZooKeys 618: 129–144. doi: 10.3897/zookeys.618.8886

Abstract

The Chinese pangolin (*Manis pentadactyla* Linnaeus, 1758) is a critically endangered species, and documents on its captive breeding and reproductive parameters are scarce. MP8, kept in the Pangolin Research Base for Artificial Rescue and Conservation Breeding of South China Normal University (the PRB-SC-NU), gave birth to a male offspring (MP86) on 19 October 2011. The baby pangolin was well developed, with a weight of 120 g and a total length of 23.2 cm. The gestation length of MP8 was estimated to be from 182 to 225d. Reproductive parameters of the Chinese pangolin are discussed based on collected data about this species. The Chinese pangolin has an obvious reproductive seasonality and its gestation length is typically six to seven months. In this observation, estrus and mating principally occurred in a one-year period from February to July. Parturition principally took place from September to February of the next year. Chinese pangolins usually give birth to one offspring at a time ($n = 27$). Sex ratio at birth was 0.71:1 (♀:♂, $n = 12$). Average weight for the reproducible females was 3.57 ± 1.38 kg (2.14–6.8 kg, $n = 15$). We estimated that Chinese pangolins could reach sexual maturity before they were one year old.

Keywords

Age of sexual maturity, birth record, breeding season, gestation period, parturition, Pholidota

Introduction

The Chinese pangolin (*Manis pentadactyla* Linnaeus, 1758) belonging to the order Pholidota of Mammalia is one of eight extant species of pangolins around the world (Gaubert and Antunes 2005, Wu et al. 2004a), and mainly distributes in the southern area of the Yangtze River, China (Wu et al. 2005). Due to its high value for medicine and food, a high proportion of the population has been illegally hunted and traded. Moreover, its habitats has also been heavily destroyed, thus the population has declined sharply in recent years (Wu et al. 2004b). In 2014, the Chinese pangolin was assessed as critically endangered by IUCN (Challender et al. 2014). Keeping critically endangered species in artificial facilities is *ex-situ* conservation – a temporary measure for urgently saving these species from wild. Chinese pangolins were first maintained in captivity in 1877; and afterwards, at least twenty-four zoos and five universities and institutions tried to keep them. In these practices, however, most of the captive pangolin individuals died within one year (Chao et al. 1993, Cheng et al. 2000, Chin et al. 2011, Clark et al. 2008, Gu et al. 1983, Heath and Vanderlip 1988, Hoyt 1987, Masui 1967, Shi and Wang 1985, Wang 2000, Wilson 1994, Wu 1998, Yang et al. 2001, 2007). Even fewer reproductive records about the female Chinese pangolins' rutting, mating, pregnancy rate, and births in captivity have been reported. So far, only four cases, three in Taipei Zoo and one at the Research Institute of Forestry of Gaoan County in Jiangxi Province, China, have been made available for study (Chin et al. 2011, Shi and Wang 1985, Yang et al. 2007).

Reproductive parameters, such as age of sexual maturity, breeding season, gestation period, litter size, and sex ratio at birth are basic data for the scientific management of wildlife populations and the prediction of future trends. They are also fundamental for making plans for the captive breeding of pangolins. However, reproductive parameters of the Chinese pangolin are fragmentary, and presented in few studies; data have been mainly based on talking with hunters, dissecting dead pregnant pangolins, and noting rescued pregnant pangolins that gave birth in captivity. Additionally, most of these parameters have been presented as a range, which is not accurate (Chao et al. 1993, Cheng et al. 2000, Heath and Vanderlip 1988, Liu and Xu 1981, Luo et al. 1993, Masui 1967, Ogilvie and Bridgwater 1967, Wang 1990, Wu 1998, Zhu-Ge and Huang 1989). Data from direct observations in captivity are unusually scarce (Chin et al. 2011, Shi and Wang 1985, Yang et al. 2001, 2007). Length of the gestation period of the Chinese pangolin in the Taipei Zoo has varied considerably. Yang et al. (2007) suggested the gestation period was less than 169d, however, Chin et al. (2011) thought that it might be from 318 to 372d. Further research on reproductive parameters of the Chinese pangolin is needed, with more direct observations taken.

In June, 2010, the Pangolin Research Base for Artificial Rescue and Conservation Breeding of South China Normal University (the PRB-SCNU) was built in the village of Sima, in the town of Changping, in the city of Dongguan. There, studies on rescu-

ing and keeping pangolins in captivity were conducted. From then until now, one Chinese pangolin and eight Sunda pangolins (*Manis javanica* Desmarest, 1822) have conceived and given birth to offspring in captivity (Zhang et al. 2015). The information on captive breeding of the Chinese pangolin reported in this paper is intended to enrich the reproductive knowledge and the direct observations of the reproductive parameters of this species. By combining data in this note with existing reproductive data about the Chinese pangolin, the reproductive parameters of this species was discussed in the present paper, findings in this study will then provide guidance for selecting individuals to be mated, determining the season for mating, predicting the parturition time, and creating breeding plans. Data are intended to provide benefits to the management of captive Chinese pangolins, and provide further information for enhancing management practices and predicting population trends of the wild population.

Materials and methods

Subjects

Subjects of this study were two wild-born Chinese pangolins marked as MP1 (♂) and MP8 (♀) who were sent to the PRB-SCNU on 24 June and 16 July 2010, respectively. When they arrived, their weights were measured (2.5 kg for MP1 and 3.3 kg for MP8). They were individually housed. Pangolins received treatment for all apparent parasites and disease, adjusted to domestic feeding habits, and adapted to the captive environment.

Housing

Housing details for the two Chinese pangolins were the same as housing the Sunda pangolin at PRB-SCNU that have been described by Zhang et al. (2015).

Housing together and mating

The female MP8 and the male MP1 were housed together during the period from 8 March to 20 April 2011. After that, MP8 was housed individually until she gave birth to a baby pangolin. After their separation, we observed the breasts of MP8 to be enlarged, with a little secretion on her papilla. Accordingly, we suspected that MP8 was pregnant, which was proved true several months later by a baby pangolin birth. Undoubtedly, MP8 mated with MP1 during the period in which they were housed together. Unfortunately, we missed observing their mating behavior so we cannot determine the exact mating date.

Results

Gestation length and number of offspring

The keeper found a dead pangolin baby (MP86) whose umbilical cord (with a length of 9.2 cm) was still connected to the placenta. The baby was buried in the sandy soil substrates of the nest and under a brick. Examining MP8, we observed breast swelling, indicating her breasts were full of milk. In addition, her vulvae displayed redness with some red viscous secretion. She was sensitive to sound, and in response to noise, became quite alert. When we got close to her, she made a sound of “fu-fu-” and curled herself tightly. Undoubtedly, she was showing strong epimeletic behavior after parturition. When the keeper checked her nest at about 0 o'clock on 19 October, he did not observe MP8 showing any sign of approaching parturition. So MP86 was likely born between 0:00 and 8:00 am of 19 October. Considering MP8 and MP1 were housed together between 8 March and 20 April, the gestation length of MP8 was estimated to be from 182 to 225d. The number of offspring was one.

When the keeper checked the nest of MP8 on the morning of 19 October at approximately 8:00 am, he also found that her bowls for food and water had changed position and had fallen over. Approximately 30 g of artificial food remained in her food bowl. The sandy soil substrate of her enclosure was freshly turned, so we suspected that around the time of delivery MP8 showed several abnormal behaviors with dysphoric emotion.

Changes of morphology and weight for MP8

During the pregnancy, MP8 did not show any significant morphological changes except for enlarged breasts and concurrent weight gain. Before the pangolins were housed together, her two breasts were only small bumps with a height less than 0.3 cm, with a diameter at the base of the breast of 0.5 cm. Her nipples were not obvious either. After being housed together, the female's breasts were first observed to be swollen on 24 April 2011; the nipples began to distend outward with some waxy secretion on their surfaces. Her breasts and nipples enlarged gradually, with the breasts becoming significantly fuller and more upright. At the same time, secretions also became thicker. Before her parturition, her breasts had a height of 1.5 cm with a diameter at the base of the breast of 1.1 cm (Fig. 1). Swollen breasts and waxy secretions have also been noted in other reports (Heath and Vanderlip 1988, Yang et al. 2001, 2007). During the gestation period, MP8's daily intake for food increased from 50 g to 70 g and her weight increased from 5.0 to 6.55 kg – an increase of 31% (Fig. 2).



Figure 1. Breast and waxy secretion covering the nipple surface of the female pangolin MP8 for the parturition the day before (by Fuhua Zhang, 18 Oct 2011). **a** breast **b** nipple **c** waxy secretion covering the nipple surface.

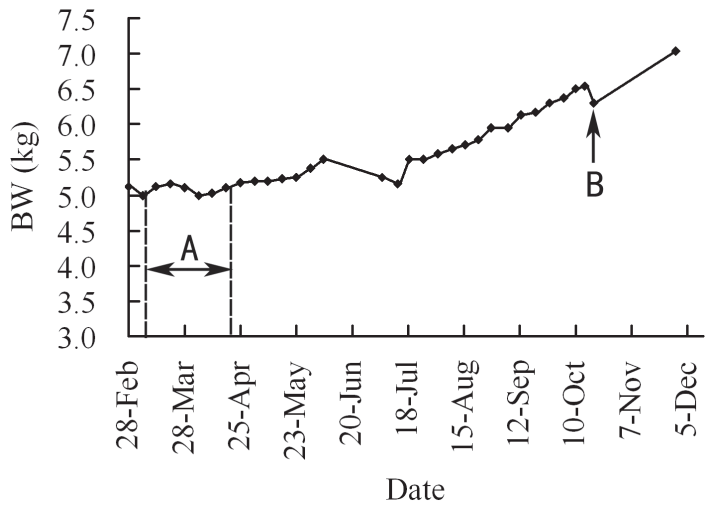


Figure 2. Body weight change of the female pangolin MP8 during the gestation period (from 8 Mar to 19 Oct 2011). **A** duration for MP8 housing with MP1 together **B** date of MP8 giving birth.

Morphological features of MP86

After clearing the sand on its surface, the skin of the head and abdomen of MP86 were found to be broken. It was a male pangolin with its head, limbs, claws, and tail well developed.

Its extraoral tongue extended 2.6 cm. Overlapping scales covered its body, with most closely tied to its surface. Scales tied to the surface were soft and not cuticularized; free parts of the scales were narrow, with a length of about 1 to 2 mm. The scales were grey, and at the base much darker; free parts were milky white, transparent, and membranous (Fig. 3). Delicate and milky white hair was found among some scales.



Figure 3. The newborn Chinese pangolin baby MP86 (by Fuhua Zhang, 19 Oct 2011).

Table 1. Measurements of the morphological indexes for the baby MP86.

Items	Outcome
Body mass	120 g
Length of head and body	15.6 cm
Tail length	7.6 cm
Total length	23.2 cm
Head length	4.6 cm
Length of the middle claw of fore limb	1.7 cm
Length of the middle claw of hind limb	0.8 cm
Ear length	0.6 cm
Number of rows of scales around middle of the body	15
Number of rows of ridge scales on one side of the body	4
Number of the scales on the edge of one side of the tail	16
Exposed tongue length	2.6 cm

Its claws were bent and sharp. Their ends were wrapped in soft ivory skin membrane. This membrane structure may be related to preventing the baby’s claws from scratching the dam’s vagina during its transit through the birth canal. Its abdomen was naked, without any scales. Morphological index data are presented in Table 1. According to the degree of MP86’s development and Table 1, we concluded it had been mature and reached full term. MP86 was the offspring of natural childbirth.

Discussion

Data from a total of twenty Chinese pangolin births were collected, with five from captive breeding (Table 2). MP86, reported in this paper, was the fifth Chinese pangolin baby in the world to be bred in captivity (Table 2). The Chinese pangolin remains critically endangered, and it is difficult to get samples in the wild. Data from captive breeding records and reproductive parameters of the species in this study are valuable, as information collected enriches our understanding of the reproductive biology and ecology of this critically endangered species.

Weight change of the pregnant pangolins and survival of the newborns

During pregnancy, the weight of MP8 increased by 31% (1.55 kg) (Fig. 2). It has been suggested that weight increase in pregnant pangolins before delivery would improve the survival rate of newborn infants (Bagatto et al. 2000, Dehnhard et al. 2006, Heath 1987, Heath and Hammel 1986). Chin et al. (2011) reported that the weight of two young pregnant Chinese pangolins increased by 63.89% (from 3.6 kg to 6.05 kg) and 134.0% (from 2.14 kg to 4.78 kg), respectively, before they gave birth to babies. At birth, these babies weighed 80 g and 110 g, respectively, and this modest increase in weight indicates good nutritional status of the mothers, and is helpful for the development of fetuses and births of healthy cubs. Weight gain also contributes to meeting the nutritional need of postpartum lactation so the fetus can survive more easily, unlike a decrease in weight, which would restrict the survival of the cubs or even cause stillbirth. A female Chinese pangolin whose weight decreased by 950 g (from 2.8 kg to 1.85 kg) gave birth to a cub with a weight of 52 g, who died within a few minutes after its birth (Chin et al. 2011).

Development and health of the newborn pangolins

Twenty birth records of Chinese pangolins have been counted in this paper (see Table 2). Descriptions of the babies were similar. The average weight of baby pangolins was 103.92 ± 37.40 g (52–180 g, $n = 12$); Total length was 21.86 ± 2.98 cm (18.5–26.5 cm, $n = 7$). The weight of newborn baby pangolins has been suggested as an assessment criterion for the successful reproduction of Chinese pangolins, as cubs with higher weights usually survive easily (Chin et al. 2011). Among the thirteen viable cubs in Table 2, seven had known weights (No. 2, No. 8, No. 10, No. 12, No. 13, No. 14, No. 16) and were relatively big, with weights over 80 g (the largest was 180 g). Four were stillborn without any vital signs. Three of these had known weights (from 52 to 75 g). They were relatively small and weakly developed. To further describe the degree of development in the newborn Chinese pangolins, we tried to use DI, an index of fatter and thinner, which represents a ratio of the fetus' weight to its total length. The

Table 2. The parturition records and estimated gestation period of Chinese pangolins.

No.	ID	Mothers					Litter size	New born babies			Source
		Arrival Data (BW, g)	Mating Date (BW, g)	Mating sites	Giving birth date (BW, g)	Giving birth sites		Gender	BW (g)	TL (cm)	
1	3#	24 Mar 1984 (3850)	1–30 Jun 1984 (?)	Captive	3 Jan 1985 (?)	Gaoan Forestry Institute	1	?	165	?	Shi and Wang 1985
2	1#	4 Nov 1984 (2350)	Bef 4 Nov 1984 (?)	Wild	5 Feb 1985 (3000)	University of California	1	F	92	21	Heath and Vanderlip 1988
3	‡	6 Nov 1995† (3855)	Bef 6 Nov 1995 (?)	Wild	15 Feb 1996 (?)	Xiashan Rare Animals Farm	1	M	75	18.5	Wu 1998
4	‡	?	?	Wild	12 Feb 1993 (?)	Taipei Zoo	1	?	?	?	Yang et al. 2001
5	‡	21 Dec 1991† (4000)	Bef 21 Dec 1991 (?)	Wild	26 Feb 1992 (?)	Taiwan Forestry Institute	1	M	70	19.2	Chao et al. 1993
6	‡	?	?	Wild	?	Rochester Zoo	1	?	?	?	Ogilvie and Bridgewater 1967
7	B	7 Nov 2005 (2800)	Bef 7 Nov 2005 (?)	Wild	20 Sep 2006 (1850)	Taipei Zoo	1	F	52	?	Chin et al. 2011
8	‡	?	?	Wild	23 Sep 2015	Hunter's home	1	F	110	24.5	Interview hunter
9	‡	?	?	Wild	?	Wild	1	?	?	?	Liu and Xu 1981
10	C	2 Oct 2006 (2140)	Bef 2 Oct 2006 (?)	Wild	9 Oct 2007 (4780)	Taipei Zoo	1	M	80	?	Chin et al. 2011
11	MP8	16 Jul 2010 (3300)	8 Mar – 20 Apr 2011 (5000)	Captive	19 Oct 2011 (6300)	PRB-SCNU	1	M	120	23.2	this study
12	‡	?	?	Wild	1 Nov 2010 (?)	PRB-SCNU	1	F	180	26.5	PRB-SCNU
13	A	9 Jul 2005 (2280)	23–27 Dec 2005 (3600)	Captive	9 Nov 2006 (6050)	Taipei Zoo	1	F	110	?	Chin et al. 2011
14	3#	4 Nov 1984 (2950)	Bef 4 Nov 1984 (?)	Wild	14 Nov 1984 (3000)	University of California	1	M	93	20	Heath and Vanderlip 1988
15	‡	29 Mar 1998 (3000)	15 Jun - ? 1998 (3100)	Captive	28 Nov 1998 (?)	Taipei Zoo	1	?	?	?	Yang et al. 2001
16	‡	?	?	Wild	?	Wild	1	?	100	?	Liu and Xu 1981
17	‡	12 May 1996 (?)	?	Captive	11 Dec 1997 (?)	Taipei Zoo	1	M	?	?	Yang et al. 2001
18	‡	9 Dec 1965 (?)	Bef 9 Dec 1965 (?)	Wild	25 Dec 1965 (?)	Ueno Zoo	1	M	?	?	Masui 1967
19	‡	?	?	Wild	?	Wild	1	?	?	?	Interview hunter
20	‡	?	?	?	1986 or 1987 (?)	Taipei Zoo	1	?	?	?	Hoyt 1987

† = wild-caught date; ? = Unknown; ‡ = No ID Number; Bef = Before; BW = Body weight; TL = Total length.

DIs of the four surviving baby pangolins (Table 2: No. 2, No. 8, No. 12, No. 14) were 4.38, 4.49, 4.65, and 6.79, all of which exceeded 4.2. The DIs of the two dead animals (Table 2: No. 3, No. 5) were 3.65 and 4.05, so below 4.2. We suggest a weight of 80 g and a DI of 4.2 could be used as the scale to describe the degree of development in newborn Chinese pangolins. Of course, more samples are needed to confirm this idea.

Gestation length

The gestation length of the Chinese pangolin was calculated based on direct observations and published data (Table 2). It seemed that gestation length is not very clear and more data is needed, especially for those collected in captivity. In the present study, the gestation period was from 182 to 225d, in agreement with findings reported by Shi and Wang (1985) (187–216d). Yang et al. (2007) reported a gestation length of less than 169d, and Wang (1990) argued for a period of about eight months (240 d). However, Chin et al. (2011) also reported the gestation length of three Chinese pangolins (A, B, and C) to be 317–321d, > 318 d, and >372 d, respectively (Table 2), all of which were over 300d, the results have large differences from that of the others. The gestation lengths of the Indian pangolin (*Manis crassicaudata* Gray, 1827), Sunda pangolin, and Cape pangolin (*Manis temminckii* Smuts, 1832) were about 165d, 180d, and 139d, respectively (Panda et al. 2010, Zhang et al. 2015, Van Ee 1966), all of which were less than 300d. Wang et al. (1984) reported the gestation length of ninety-seven species of mammals which belonging to thirteen orders. Only a few large-sized species had gestation periods of over 300d, for example, Asian elephants (*Elephas maximus*), Asian rhinoceros (*Rhinoceros unicornis*), and bactrian camel (*Camelus bactrianus*). Small animals tend to have shorter gestation lengths. Perhaps Chin et al. (2011) overestimated the gestation of the three Chinese pangolins (A, B, C).

The gestation period is usually stable as it is genetically controlled (Liu and Zheng 1997). But the gestation period of a few mammals may show larger fluctuations, such as that of the giant panda, whose gestation lengths have been noted as 140.3 ± 20.5 d (from 89 to 186d) (Zhang and Wei 2006). Environmental conditions, health and nutritional status of the mother, delayed implantation, and reproductive hormone levels may each affect animals' pregnancy lengths (Silk 1986, Yang 2010). The three Chinese pangolins reported by Chin et al. (2011) were kept in very narrow space (1.2×1.2×0.8 m). Thus, the relatively long gestation length of the three Chinese pangolins (A, B, and C) may be related to the disturbance of hormones caused by environmental stress.

Mating and parturition season

It is usually suggested that the Chinese pangolin has a specific mating and parturition season. Mating behavior has been mainly observed to occur in summer and seldom occurs in April and May or early autumn; births have mainly taken place in winter

(Heath 1992a, Liu and Xu 1981, Luo et al. 1993, Wang 1990, Wu 1998, Zhu-Ge and Huang 1989). However, according to the descriptions of thirty-one interviewed hunters, Chao et al. (1993) stated the Chinese pangolins mainly give birth to infants in the spring, between March and May, with the parturition season also later.

In this study, a total of three female Chinese pangolins' mating times were collected (No. 1, No. 11, and No. 13 in Table 2). Mating for No. 11 and No. 1 occurred between March and May and in June, respectively. Additional mating times for two pregnant pangolins (No. 2 and No. 3) were also estimated according to the times of their death and the degrees of development of embryos in the uterus. Their mating seemed to occur between February and March and between June and July, respectively (Table 3). These few instances appear to show the mating season of the Chinese pangolins mainly occurred between February and July.

Among the twenty birth records of Chinese pangolins gathered in this study, nineteen of their birth months were known, usually occurring from October to February of the next year, i.e., in autumn and winter (eighteen cases, accounting for a percentage of 94.7%) with a few occurring in August (Table 2). This finding suggests the breeding season of Chinese pangolin is similar to that of the Cape pangolin, but different from the Sunda and Indian pangolins. Regarding the Cape pangolin, mating occurred from late summer to early autumn (between March and May in the southern hemisphere), with the birthing season in winter (from June to September in the southern hemisphere) (Heath 1992b); for Sunda and Indian pangolins, births have also been observed throughout the year (Heath 1995, Mohapatra and Panda 2014, Zhang et al. 2015). Given that direct observations of the mating and parturition for the Chinese pangolin were few, more direct observations and documentation are necessary.

Litter size and sex ratio at birth

It is commonly suggested that the Chinese pangolin give birth to one offspring at a time. In the current study, MP8 was observed to give birth to a single offspring, coinciding with other breeding records collected ($n = 19$) in the current study. This is in line with findings from our dissection of seven pregnant Chinese pangolins, where only one fetus was found in each uterus (Table 3). This agrees with birth records for other pangolin species – the Sunda pangolin, Indian pangolin, Cape pangolin, and tree pangolin – where usually a single young was produced (Hoyt 1987, Israel et al. 1987, Menzies 1967, Mohapatra and Panda 2014, Van Ee 1966, Zhang et al. 2015). However, for the Chinese pangolin and Indian pangolin, it is also stated that two baby pangolins can be born at the same time (Liu and Xu 1981, Wang 1990, Prater 2005). In August 2015, we visited China's Xishuangbanna Natural Reserve, located in Yunnan Province, and interviewed several staff. Interviewees Li Xiaokun (on the staff of this nature reserve) and Lv Xinghua (from Ninger county, Yunnan province) told us that in 1979, they dug out an adult female pangolin and four similar-sized babies (approximately 1.0 kg) in a burrow with a depth of 1 meter in the Wulu River Forest

Table 3. Records of dissection and estimated mating time of the dead pregnant Chinese pangolins.

No.	Mother ID	Arrival date	Estimated mating time	Mating site	Died date	Litter size	Embryo/fetus mass (g)	Fetus gender	Source
1	†	?	?	Wild	? Jan 1982	1	43.0	?	Wang 1990
2	FS3	4 Mar 2011	Feb or Mar 2011	Wild	10 Mar 2011	1	6.5	?	PRB-SCNU
3	MP7	16 Jul 2010	Jun or Jul 2010	Wild	22 Jul 2010	1	1.4	?	PRB-SCNU
4	†	?	?	Wild	? Sep 1978	1	75.0	?	Liu and Xu 1981
5	†	?	?	Wild	? Oct 1979	1	80.0	?	Liu and Xu 1981
6	†	10 Nov 2013	?	Wild	15 Nov 2013	1	46.8	?	PRB-SCNU
7	96004	26 Oct 1996	?	Wild	15 Nov 1996	1	108.0	F	Cheng et al. 2000

† = No ID Number; ? = Unknown

Farm in Mengwang Town, Jinghong City, Yunnan Province. This may indicate that the Chinese pangolin could give birth to four babies at a time.

Sex ratio at birth of the Chinese pangolin has not been reported in other literature. A total of twelve newborn cubs whose gender were recorded in this paper (Table 2), including five females and seven males, suggests a sex ratio of 0.71:1 (♀:♂, n = 12). Female individuals were fewer than males, but this might be ascribed to the small sample size. Natural selection may force parents to regulate the sex ratio of their offspring according to parental ability to invest (Trivers and Willard 1973). Dams in good health have higher levels of investment and tend to give birth to more male cubs. A higher percentage of males mean females have more opportunities to select an excellent mate. This benefits the health of a population and prevents their decline and extinction. It must be kept in mind, however, that a greater number of males will consume more resources, thereby affecting the development of the population (Lumley et al. 2015).

Weight of the pregnant and puerperal pangolins and age at sexual maturity

The age of sexual maturity for the Chinese pangolin remains unclear. Weights of fifteen sexually mature female Chinese pangolins were recorded in the present study (Table 4), however, it is unclear whether those pangolins were primiparous. Their average weight was 3.57 ± 1.39 kg, with a range of 2.14–6.8 kg (n = 15). Eight females, whose weights were between 2–3 kg, gave birth to offspring, accounting for 53.3% of the total. The

Table 4. Weight of the collected female Chinese pangolins which have the ability to reproduce in this paper.

No.	ID of the female	Body weight (kg)	Source	Note
1	C	2.14	Chin et al. 2011	pregnant
2	†	2.25	Liu and Xu 1981	pregnant
3	P1	2.35	Heath and Hammel 1986	pregnant
4	MP7	2.42	PRB-SCNU	pregnant
5	†	2.5	Liu and Xu 1981	lactation
6	B	2.80	Chin et al. 2011	pregnant
7	P3	2.95	Heath and Hammel 1986	pregnant
8	†	3.0	Yang et al. 2001	lactation
9	A	3.6	Chin et al. 2011	When mating
10	†	3.855	Wu 1998	pregnant
11	†	4.0	Chao et al. 1993	pregnant
12	FS3	4.1	PRB-SCNU	pregnant
13	MP8	5.17	PRB-SCNU	When mating
14	†	5.6	Interview hunter	lactation
15	†	6.8	Yang et al. 2001	pregnant

† = No ID number.

weight of the smallest mother pangolin was only 2.14 kg. It has been reported that the six-month-old baby Chinese pangolin could attain a weight of 1.2–2.0 kg (Liu and Xu 1981, Yang et al. 2001), or more (2.7 kg) (Masui, 1967). This indicates that the female Chinese pangolin could reproduce when she was approximately six months old. Chin et al. (2011) argue that the Chinese pangolin could breed before the age of 1–1.5 year, which is in agreement with our conclusion. Sunda pangolins with average weights of 3.49 ± 0.90 kg (1.75–5.54 kg, $n = 24$) were confirmed to be pregnant, and reached sexual maturity at six to seven months old (Zhang et al. 2015).

Conclusions

- 1) There is an obvious breeding season for the Chinese pangolin: estrus and mating principally occurred from February to July in a one-year period, and parturition principally took place from September to February of the next year. The gestation length is typically six to seven months.
- 2) Female Chinese pangolins may reach sexual maturity before one year old, even as early as six months old, or when their body weights reach over two kilograms.
- 3) During the pregnancy, the Chinese pangolin does not show significant morphological changes except for its breast and body weight.
- 4) The Chinese pangolin usually gives birth to one offspring at a time. The body weight of all the surviving newborn babies was more than 80 g.

Acknowledgements

We would like to thank all those who work at the Pangolin Research Base for Artificial Rescue and Conservation Breeding of South China Normal University (the PRB-SCNU). We would further like to acknowledge and thank the Wildlife Rescue Center of Guangdong Province for providing the animals for this study. This research was supported by the Natural Science Foundation of Guangdong Province (No. S2013010013356), the Special Fund for Significant Science and Technology in Social Development Field of Guangdong Province (No. 2011A030100012), and the National Natural Science Foundation of China (31572286). We are also grateful to Prof. Martha Schirn (U.S.) and Dr. Zaiwang Zhang (Binzhou University) for reading through and checking the manuscript to correct the grammatical and semantic errors.

References

- Bagatto B, Crossley II DA, Burggren WW (2000) Physiological variability in neonatal armadillo quadruplets: within-and between-litter differences. *Journal of Experimental Biology* 203(11): 1733–1740.

- Challender D, Baillie J, Ades G, Kaspal P, Chan B, Khatiwada A, Chin S, KC R, Nash H, Hsieh H (2014) *Manis pentadactyla*. The IUCN Red List of Threatened Species. Version 2015.1. www.iucnredlist.org [Downloaded on 04 June 201]
- Chao JT, Chen YM, Yeh WC, Fang KY (1993) Notes on a newborn Formosan pangolin, *Manis pentadactyla pentadactyla*. Taiwan Museum 46(1): 43–46.
- Cheng DY, Li QS, Yu ZB, Zhang Q, Li ZC (2000) Discussion on birth time of pangolins. Resource Development and Market 16(5): 287.
- Chin SC, Lien CY, Chan YT, Chen CL, Yang YC, Yeh LS (2011) Monitoring the gestation period of rescued Formosan pangolin (*Manis pentadactyla*) with progesterone radioimmunoassay. Zoo Biology 30: 1–11.
- Clark L, Thai NV, Phuong TQ (2008) A long way from home: the health status of Asian pangolins confiscated from the illegal wildlife trade in Viet Nam. In: Pantel S, Chin SY (Eds) Proceeding of the Workshop on Trade and Conservation of Pangolins Native to South and Southeast Asia. Singapore Zoo, Singapore, 111–118.
- Dehnhard M, Hildebrandt TB, Knauf T, Jewgenow K, Kolter L, Göritz F (2006) Comparative endocrine investigations in three bear species based on urinary steroid metabolites and volatiles. Theriogenology 66: 1755–1761. doi: 10.1016/j.theriogenology.2006.01.012
- Gaubert P, Antunes A (2005) Assessing the taxonomic status of the Palawan pangolin *Manis culionensis* (Pholidota) using discrete morphological characters. Journal of Mammalogy 86(6): 1068–1074. doi: 10.1644/1545-1542(2005)86[1068:ATTSOT]2.0.CO;2
- Gu WY, Tao SZ, Liu P (1983) Primary exploration on rearing technique of pangolin. Chinese Journal of Zoology 18(3): 26–28.
- Heath ME, Hammel HT (1986) Body temperature and rate of O₂ consumption in Chinese pangolins. American Journal of Physiology 250: 377–382.
- Heath ME (1987) Twenty-four-hour variations in activity, core temperature, metabolic rate, and respiratory quotient in captive Chinese pangolins. Zoo Biology 6: 1–10. doi: 10.1002/zoo.1430060102
- Heath ME, Vanderlip SL (1988) Biology, husbandry, and veterinary care of captive Chinese Pangolins (*Manis pentadactyla*). Zoo Biology 7: 293–312. doi: 10.1002/zoo.1430070402
- Heath ME (1992a) *Manis pentadactyla*. Mammalian species (414): 1–6. doi: 10.2307/3504143
- Heath ME (1992b) *Manis temminckii*. Mammalian species (415): 1–5. doi: 10.2307/3504220
- Heath ME (1995) *Manis crassicaudata*. Mammalian species (513): 1–4. doi: 10.2307/3504173
- Hoyt R (1987) Pangolins: past, present, and future. AAZPA National Conference Proceedings, 107–134.
- Israel S, Sinclair T, Grewal B, Hoefler HJ (1987) Indian wildlife. APA Productions (HK) Ltd., Hong Kong.
- Liu LY, Zheng GM (1997) General Zoology (3rd edition). Higher Education Press, Beijing, 533 pp.
- Liu ZH, Xu LH (1981) Pangolin's habits and its resource protection. Chinese Journal of Zoology 16: 40–41.
- Lumley AJ, Michalczyk L, Kitson JJN, Spurgin LG, Morrison CA, Godwin JL, Dickinson ME, Martin OY, Emerson BC, Chapman T, Gage MJG (2015) Sexual selection protects against extinction. Nature 522: 470–473. doi: 10.1038/nature14419

- Luo R, Chen Y, Wei K (1993) The mammalian fauna of Guizhou. Guizhou Science and Technology Publishing House, Guiyang, 169–172.
- Masui M (1967) Birth of Chinese pangolin (*Manis pentadactyla*) at Ueno Zoo, Tokyo. International Zoo Yearbook 7(1): 114–116. doi: 10.1111/j.1748-1090.1967.tb00340.x
- Menzies JI (1967) A preliminary note on the birth and development of a Small-scaled tree pangolin *Manis tricuspis* at Ife University Zoo. International Zoo Yearbook 7(1): 114. doi: 10.1111/j.1748-1090.1967.tb00339.x
- Mohapatra RK, Panda S (2014) Husbandry, Behaviour and Conservation Breeding of India Pangolin. Folia Zoologica 63(2): 73–80.
- Ogilvie PW, Bridgwater DD (1967) Notes on the breeding of an Indian pangolin *Manis crassicaudata* at Oklahoma Zoo. International Zoo Yearbook 7(1): 116–118. doi: 10.1111/j.1748-1090.1967.tb00341.x
- Panda S, Mishra S, Mohapatra SN (2010) Nandankanan faunal diversity. Nandankanan Biological Park, Forest and Environment Department, Government of Odisha, 28.
- Prater HS (2005) The book of India animals. Oxford University Press, New Delhi, 301–303.
- Shi YQ, Wang YG (1985) The preliminary study on captive breeding pangolins. Forest Science and Technology (10): 28–29.
- Silk JB (1986) Eating for two: Behavioral and environmental correlates of gestation length among free-ranging baboons (*Papio cynocephalus*). International Journal of Primatology 7(6): 583–602. doi: 10.1007/BF02736663
- Trivers RL, Willard DE (1973) Natural selection of parental ability to vary the sex ratio of offspring. Science 179: 90–92. doi: 10.1126/science.179.4068.90
- Van Ee CA (1966) A note on breeding the cape pangolin *Manis temminckii* at Bloemfontein Zoo. International Zoo Yearbook 6: 163–164. doi: 10.1111/j.1748-1090.1966.tb01734.x
- Wang CY (2000) Behavior observations of the Chinese pangolin under artificial rearing. Journal of Economic Animal 4(3): 41–44.
- Wang M, Yuan J, An G (1984) The gestation length of wild mammals. Chinese Journal of Wildlife 6: 55–58.
- Wang QS (1990) The mammal fauna of Anhui. Anhui Publishing House of Science and Technology, Hefei, 106–109.
- Wilson AE (1994) Husbandry of pangolins *Manis* spp. International Zoo Yearbook 33: 248–251. doi: 10.1111/j.1748-1090.1994.tb03578.x
- Wu S (1998) Notes on a newborn Chinese pangolin (*Manis pentadactyla aurita*). J Qinghai Normal University (Natural Science) (1): 40–42.
- Wu SB, Liu NF, Ma GZ, Tang M, Chen H, Xu ZR (2004a) A current situation of ecology study on pangolins. Chinese Journal of Zoology 39(2): 46–52.
- Wu S, Liu N, Zhang Y, Ma G (2004b) Assessment of threatened status of Chinese pangolin (*Manis pentadactyla*). Chinese Journal of Applied and Environmental Biology 10(4): 456–461.
- Wu SB, Ma GZ, Liao QX, Lu KH (2005) Studies of conservation biology on Chinese Pangolin (*Manis pentadactyla*). Chinese Forest Press, Beijing, 3–4.
- Yang CW, Chen SM, Chang CY, Lin MF, Block E, Lorentsen R, Chin JSC, Dierenfeld ES (2007) History and dietary husbandry of pangolins in captivity. Zoo Biology 26: 223–230. doi: 10.1002/zoo.20134

- Yang CW, Guo JC, Li ZW, Yuan XW, Cai YL, Fan ZY (2001) The study of Chinese pangolin in Taiwan. The research report of Taipei Zoo in 2001, Taipei.
- Yang L (2010) Animal reproduction (2nd edition). China Agriculture Press, Beijing, 195–198.
- Zhang F, Wu S, Yang L, Zhang L, Sun R, Li S (2015) Reproductive of parameters of the Sunda pangolin, *Manis javanica*. Folia Zoologica 64(2): 129–135.
- Zhang Z, Wei F (2006) Giant panda ex-situ conservation theory and practice. Science Press, Beijing, 267–272.
- Zhu-Ge Y, Huang MH (1989) Fauna of Zhejiang (Mammalia). Zhejiang Science and Technology Press, Hangzhou, 60–61.

On natural history collections, digitized and not: a response to Ferro and Flick

Derek S. Sikes¹, Kyle Copas², Tim Hirsch², John T. Longino³, Dmitry Schigel²

1 University of Alaska Museum, 907 Yukon Drive, Fairbanks, AK 99775-6960, USA **2** GBIF Secretariat, Universitetsparken 15, DK-2100 Copenhagen Ø, Denmark **3** Department of Biology, University of Utah, Salt Lake City, UT 84112, USA

Corresponding author: Derek S. Sikes (dssikes@alaska.edu)

Academic editor: J. Miller | Received 23 July 2016 | Accepted 13 September 2016 | Published 26 September 2016

<http://zoobank.org/691C98E8-B2F9-4C87-BD4B-9B81B48C7B09>

Citation: Sikes DS, Copas K, Hirsch T, Longino JT, Schigel D (2016) On natural history collections, digitized and not: a response to Ferro and Flick. ZooKeys 618: 145–158. doi: 10.3897/zookeys.618.9986

Response to: Ferro ML, Flick AJ (2015) “Collection Bias” and the importance of natural history collections in species habitat modeling: A case study using *Thoracophorus costalis* Erichson (Coleoptera: Staphylinidae: Osoriinae), with a critique of GBIF.org. The Coleopterists Bulletin, 69(3):415–425. doi: 10.1649/0010-065X-69.3.415

Abstract

Ferro and Flick (2015) describe their efforts to estimate the distribution for a species of rove beetle via the study of specimens from entomological collections, and compare these results to digitally accessible open data. Their study provides an informed and accurate case study that contrasts targeted data capture with generalized public repositories of digital specimen data. However, we feel the conclusions on how global biodiversity data aggregation and publication work require clarification and correction of common misconceptions that we believe will interest those concerned with the future of natural history collections and taxonomy.

Keywords

Natural History collections, Museums, digitization, GBIF, georeferencing, data sharing

Summary of the original statements

Ferro and Flick (2015) used a classical approach to gather distribution data for a species of rove beetle, *Thoracophorus costalis*. They borrowed specimens from 38 collections, recorded specimen data, and analyzed them with niche modeling software. They were able to show that, on average, data from at least 15 separate collections were sufficient to construct a satisfactory model. They then used data currently published through the Global Biodiversity Information Facility (GBIF) network. GBIF provided an incomplete and biased set of records that, used alone, produced poor results in species distribution modeling. Therefore, the authors argue, while online sources of data like GBIF.org may have some value, their use makes it too easy to produce low quality research. They also suggest that GBIF.org should provide more frequent and prominent notices highlighting that data may be of insufficient quality.

Our response

We thank Ferro and Flick (2015) for raising a number of important issues regarding specimen digitization and data aggregators like GBIF.org. We take this opportunity to highlight some issues which we hope our community can work together towards resolving and add a counterpoint to Ferro and Flick's (2015) critique of GBIF.

Taxonomy and digitization a zero-sum game?

Ferro and Flick raise the concern that funding for digitization efforts is siphoning funds away from the maintenance of natural history collections (NHCs). We argue that the distinction between funding NHCs and the production of GBIF-mediated data is artificial – specimen records from NHCs are the foundation of the entomological data accessible through GBIF.org, with U.S. institutions alone providing 7.5 million geo-referenced insect occurrence records citing a specimen as the 'basis of record', including 3.5 million records relating to insect specimens collected from U.S. lands (GBIF.org (2016-09-14) GBIF Occurrence Download <http://doi.org/10.15468/dl.5txrti> and GBIF.org (2016-09-14) GBIF Occurrence Download <http://doi.org/10.15468/dl.1kayda>). There need be no 'choice' between maintaining good regional specimen collections and the digitization and publication of data through online aggregated databases. Increasingly in the U.S. (Kaiser 2015), and the world, sharing of digitized data and published results is expected for government funded research. This is becoming such a standard that open sharing of digitized specimen data significantly increases the probability of obtaining funding for natural history collections. The choice is, therefore, becoming one of either funding NHCs and digitizing NHC data, or doing neither. The Berlin Declaration on Open Access to Knowledge in the Sciences and Humanities of 2003 (2003), promoting open access to scientific data, has been signed by

302 worldwide scientific organizations. The National Science Foundation's Advancing the Digitization of Biodiversity Collections (ADBC) initiative is in its fifth year of implementation and has resulted in a massive mobilization of NHC data. A new five-year national initiative in the U.S., the Biodiversity Collections Network (BCoN), funded by the National Science Foundation, has been established to support the development of a sustainable community of practice that will ensure that all U.S. biodiversity collections are digitally available for research, education, informed decision-making, and other scholarly and creative activities. The vast majority of entomological specimens have yet to be digitized (Fig. 1, only 7% of the occurrence data in GBIF is entomological despite insects representing well over 75% of all species and specimens in museum collections) and doing so will take many millions of dollars and likely many decades. However, the "writing is on the wall" that the scientific and public community want NHC and taxonomic data to be digitized and freely available online, despite the challenges this entails (Maddison et al. 2012, Roche et al. 2015, Page 2016). The more high-quality NHC data become available, the more they will be used by non-taxonomists, and the more appreciation (and funding) for NHCs and taxonomy will grow.

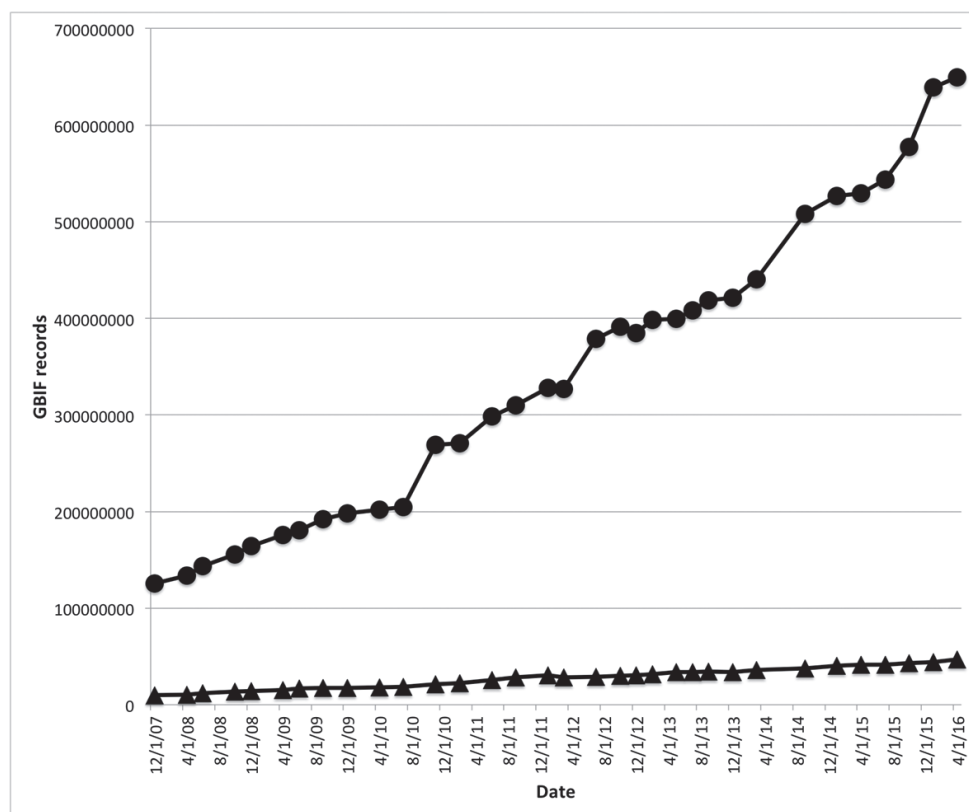


Figure 1. Number of insect records in GBIF.org (triangles) between December 2007 and March 2016, in comparison to all records (circles).

Digital data quality is us

We agree entirely with the sentiment Ferro and Flick promote with their quote of Soberón and Peterson (2004) “*without a strong and active taxonomic community, BI [Biodiversity Informatics] will never be more than a clever set of software tools lacking a substantial factual basis.*” However, we wish to reiterate that biodiversity data are what the taxonomic and museum community produce and are only as good as the effort applied. They are *our data* and making them available online for the scientific community strengthens the taxonomic community. Consequently, we feel it is detrimental to the taxonomic community to produce high-quality data that are not shared with aggregators like GBIF.org.

The data that Ferro and Flick (2015) downloaded from GBIF were all from the Snow Entomological Museum (SEMC, 142 records) but they also borrowed 198 specimens from SEMC and georeferenced them for modeling. Presumably they georeferenced previously georeferenced specimens (i.e. the 142 GBIF records from SEMC were possibly within the set of 198 specimens they borrowed). This raises questions about duplication of data (and hence statistical independence of data points), duplication of effort, and how best to cite online data. Ferro and Flick (2015) listed the data they obtained from GBIF (their table 1) as ‘alternative distribution data’ from GBIF. This is not an ideal way to cite online data. These data were from SEMC which should have been cited as the data provider, with a link (DOI) for the data download from GBIF. To do otherwise is to cheat the data providers of important citations for their contribution to science. We recently searched GBIF for records of *Thoracophorus costalis* (GBIF.org (31st May 2016) GBIF Occurrence Download <http://doi.org/10.15468/dl.1gs48e>) and note that there are now 152 records. New data were added since Ferro’s download by the Yale Peabody Museum of Natural History (YPM) and the Essig Museum of Entomology (EMEC) – with high-quality identifications by Ferro. Again, these data in GBIF should not be called ‘alternative distribution data,’ they should be cited as data from each NHC data provider that is shared via GBIF. A researcher is free to visit each collection’s separate website and download the data closer to the source, rather than from GBIF, but why do this? Some NHCs have data online that are not shared with GBIF. It is worth looking for these, but the different interfaces to these collection websites mean that a researcher will have to learn how to search and download data from each website separately, since each will generally have their own unique ways of presenting and organizing data. A researcher will then have to invest considerable effort converting and aggregating each dataset into a format compatible with the Darwin Core Standard (Wieczorek et al. 2009) that is otherwise shared with, and available from, GBIF.

Taxonomy produces the highest-quality ‘dark data’

We strongly agree with Ferro and Flick (2015) in their condemnation of digitization efforts that provide no funding for curation and identification of specimens. All

natural history museums have misidentified and partially identified specimens, and specimens sorted under junior synonyms (Meier & Dikow 2004, Goodwin et al. 2015 but see also Page 2015). Although the greatest digitization efforts have been to fund museums to database their holdings, the highest quality data with the fewest misidentifications comes directly from taxonomic revisionary work such as Ferro (2015). These datasets are the highest quality but sadly, most fail to be shared digitally and thus join the accumulation of what are called ‘dark data’ (Heidorn 2008). Publishing traditional ‘material examined’ lists in such taxonomic works does not fulfill the expectation of data sharing because these data are not machine-readable nor standardized for easy conversion into a format that is machine readable and often lack geocoordinates. The two most obvious solutions to this issue involve the inclusion of funding for identification verification in all digitization grants (e.g. NSF-funded programs like ADBC and iDigBio) and increased efforts to obtain and share properly-formatted datasets from taxonomists publishing research with occurrence data.

Occurrence data sharing in taxonomy – why so rare?

As remains typical of the majority of taxonomic work currently being published, Ferro (2015) and Ferro and Flick (2015) did not share their specimen data online. The reasons for this are likely varied and include (1) a lack of tradition or expectation to do so, (2) a lack of a user-friendly data-pipeline that taxonomists can use to share and prepare their data in the best format (Darwin Core standard, Wieczorek et al. 2009), (3) a lack of motivation by journal peer reviewers and editors to encourage (or insist) that data be shared, and (4) a lack of perceived reward for doing the extra labor involved in sharing of data. Additionally, we have heard some taxonomists state they do not want to share data with GBIF because they distrust the quality of the data in GBIF. This latter point seems illogical. The data in GBIF are the data from the museums that provide data. If the data in GBIF are not to be trusted then neither are the data in the source museums. It thus seems illogical to be pro-museum and anti-GBIF.

Data quality, mapping, and efficiency of production

Via communication with Ferro (in lit.) during which we asked about the sharing of his dataset, we learned that Ferro felt his data were not produced in a manner ideal for sharing with GBIF. Ferro also commented on the lack of a user-friendly data-pipeline to prepare and upload data to share (more on this issue below). Ferro explained that he georeferenced records using the centers of counties for each locality record rather than georeferenced them following the best practices suggestions in Chapman and Wieczorek (2006). As a result, although Ferro and Flick felt their data were of high enough quality to publish and analyze, they felt their data were not of high enough quality to share with the wider scientific community. We find this puzzling. Conse-

quently, if we are to ever have open access to high quality digital data for the species they studied, *Thoracophorus costalis*, someone will have to georeference those 4,900+ specimens again. This will most likely be done by the staff at the NHCs which house these specimens. This is obviously not an efficient use of the limited funds available to NHCs and taxonomists. We believe, and hope most readers do too, that once data have been typed into a computer they should never need to be re-typed. By using their unique georeferencing methods certainly some time and money was saved – but was it worth it? It might be argued that sub-optimal data are better than none. Certainly Ferro and Flick (2015) thought their data were of high enough quality on which to base their analyses and publish. We are not singling Ferro and Flick (2015) out for their choice of cost-saving methods or lack of data sharing (the majority of recently published taxonomic papers that we have seen did not share their specimen data). But we do use their work and their critique of GBIF to highlight these general challenges the entire taxonomic community faces.

Distribution mapping is changing. Such maps are not constellations of the maximum number of georeferenced occurrences, but effectively projected, modeled areas where species are thought to occur with a certain uniform or changing probability. Williams et al.'s (2014) guide to the Bumble Bees of North America relied heavily on shared data for its production and is an excellent example of the use of this form of mapping. For such maps, one needs enough data to make reliable predictions, not necessarily coordinates from every known specimen, as Ferro and Flick (2015) point out. The best available – and most cost efficient – data also means not too much: at a certain level, the price of enlarging one's dataset will continue to go up without improving the estimate of the species' distribution. However, with less well-studied taxa like rove beetles, currently the quality of predictions generally improves with every new observation. In principle, the data accumulation curves will all flatten, even within Coleoptera (Hof & Svahlin 2015, Beck et al. 2014, Fourcade et al. 2014, García-Roselló et al. 2014).

Data quality warnings and peer review

GBIF is currently working to improve the representation of available data to make the completeness and fitness for use of any dataset as transparent to the user as possible. We agree that GBIF.org can include clearer text and information about both the context and limitations of data accessible through GBIF. Data will always be of variable completeness and precision, and GBIF's approach should be to ensure that users such as distribution modelers can easily restrict searches to data fit for their use, while not excluding other data that may still be useful for other purposes. However, taxonomists who are well aware that museum collections are rife with misidentifications and data quality issues such as collector bias (Hjarding et al. 2015, Goodwin et al. 2015 but see also Page 2015), should not be surprised when these issues are present in data aggregated by GBIF. Should all museums post similar warnings inside their collections?

Of course, online data are available to a much wider and less well-trained user audience than physical specimens in NHCs. Thus, we agree it is wise to warn naïve users of potential data quality issues.

Indeed, not all scientific users understand that globally aggregated data always need filtering and post processing, as well as dealing with data gaps. A constructive alliance would enlist experts to help address quality issues in the process of global data aggregation. For example, despite the increasing fraction of wrongly annotated fungal sequences in GenBank, the trustworthy ones (Nilsson et al. 2012; Hyde et al. 2013) are dynamically reflected in the UNITE database (Köljalg et al. 2013). From the UNITE webpage: “*We aim at including only high-quality sequences of well identified fungi, hence initially sacrificing quantity for quality.*”

The issue of data quality will never, and should never, go away. All data need vetting. The study of Hjarding et al. (2014) compared expertly vetted data obtained from various NHCs, many of which didn’t share data with GBIF, to unvetted data available from GBIF and, not surprisingly, found the unvetted data to be unreliable. They wrote “*Our results suggest that before conducting desktop assessments of the threatened status of species, aggregated museum locality data should be vetted against current taxonomy and localities should be verified. We conclude that available online databases are not an adequate substitute for taxonomic experts in assessing the threatened status of species and that Red List assessments may be compromised unless this extra step of verification is carried out.*” We agree. This study, and the consequent discussions on iPhylo and Taxacom covered many of the same concerns seen in Ferro and Flick (2015). These include issues such as how to best correct taxonomy and locality data, sharing of data, and georeferencing. One of the larger issues, which parallels Ferro and Flick (2015), is the lack of sharing of the expertly vetted data. Most of the NHCs from which the vetted data were obtained do not have sharing agreements with GBIF and the authors of Hjarding et al. (2015) did not share the vetted data. GBIF and similar data aggregators are not going to go away. They will improve with time but if those who have control of the highest quality data don’t share their data with GBIF, this improvement will be slow, to the detriment of all. The taxonomic community has the ability to overwhelm and replace any low-quality data in GBIF with data of the highest quality – to work together as part of the solution, rather than contribute to the problem. Researchers who work frequently with GBIF-mediated data often make suggestions for improvements to the error-reporting system itself. The GBIF community shares the desire to make it easy to report corrections and annotations in ways that the providers of the source data can see, handle and respond to. A key to making this happen is the wider adoption of consistent specimen and record level identifiers.

It is worth considering an analogy with GenBank regarding Ferro and Flick’s (2015) statement “*Online databases offer an opportunity for naïve or lethargic researchers to quickly produce poor quality research with little effort.*” Yes, a naïve user could download sequence data from a variety of genes for a small subset of the known species in a group, feed these into an automated alignment program and then without inspecting the alignment, generate a distance tree. The results would be a poor to worthless

estimate of the group's phylogeny. No one expects GenBank to warn users to prevent such poor science, and even less, no one would publish a critique of GenBank arguing GenBank is not to be trusted because it doesn't have all genes for all species. Peer and editorial review are the gatekeepers that prevent poor science from being published. Reviewers and editors of work based on downloaded data from GBIF and other aggregators should be appropriately critical of authors' methods. If an author was foolish enough to attempt to publish a niche model analysis of *T. costalis* using only data from GBIF, we would hope that peer reviewers of such a manuscript would recommend rejection of the work, not because GBIF data were used, but rather because any reliable reviewer or editor should know that most entomological specimen data are not yet in GBIF. And conversely, if an author attempted to conduct a niche model analysis of this species and ignored the abundant and easily obtained high-quality data in GBIF for this species, we hope reviewers would require the authors to include the GBIF data or at least provide a rational justification for not doing so. Vetting GBIF data is now easier than before because of a new GBIF service that provides DOIs for any data download, which enables reviewers to easily examine the raw data on which analyses are based.

To carry the GenBank analogy a little further, imagine a researcher who assembled via their own lab-work a thorough genetic dataset to do a proper phylogenetic analysis of a taxon and then did the following (1) published a critique of GenBank complaining it lacked most of the data that the researcher had to generate and (2) held their data back rather than shared it with the scientific community. It is generally a requirement by journals for authors publishing on newly obtained genetic data to deposit their data with GenBank. It is our hope that the taxonomic community will see the benefits of treating specimen data the way most journals treat genetic data - as an investment in the greater good, as a way of raising the standards of taxonomic research, as a way of saving future generations the time and effort of digitizing specimens (again), as a way of making taxonomic research more useful for non-taxonomic researchers, and as a way of meeting the expectations of funding agencies. We need a GenBank for specimen data – a point made by Meier and Dikow (2004) who discuss the enormous potential value to conservation biology of the data published as part of taxonomic revisions.

Biodiversity conservation

The taxonomic community is often quite vocal about conservation of biodiversity. Many conservation efforts are based on geo-political regions, be they nations, states, parks, or refuges. However, because taxonomy organizes data by taxon rather than region, it is easier to determine where a species occurs than to determine how many and which species occur in a region. For entomology, most of these data are found only on labels on pins scattered among various NHCs and scattered literature organized by taxon, not region. As a result, most regional checklists are usually limited in taxonomic scope (e.g. one large order or family).

If these data are shared globally they can be used for conservation of biodiversity related to land preservation or in analyses of shifting distributions resulting from climate change (e.g. Kerr et al. 2015). For example, to investigate the response of bumble bees to climate change, Kerr et al. (2015) were able to compile a georeferenced dataset for 67 species from Europe and North America that spanned 110 years. Records came from GBIF (171,479 North American and 192,039 European records), Bumblebees of North America (153,023 records), and the Status and Trends of European Pollinators Collaborative Project (237,586 records). These data came from institutions and organizations that digitized and shared their data. How many digitized and unshared records, or undigitized records that were not included in Kerr et al.'s analysis is unknown, but it is likely to be a very substantial number. We are headed towards a future in which specimen data that are not shared digitally will be increasingly overlooked. With the current re-evaluation of the Collections in Support of Biological Research (CSBR) program by the NSF, it is examples like this study of Kerr et al. (2015) that help illustrate the importance of NHCs to addressing big questions of global science. NHCs that refuse to (or are unable to) share their data will find themselves left out of such large collaborative studies and find it harder to justify future funding from programs like the NSF's CSBR.

Any taxonomist who publishes new occurrence records but fails to share these data is, in effect, handicapping conservation efforts by hiding their taxa "in the dark" from geographically based searches. In particular, newly described species are often highly localized endemics known from few localities, or just the type locality. These species are of great interest to conservationists but it is the rarest of exceptions in entomology for occurrence data for these species to be shared with GBIF. Given the conservation importance of these species, and often the relatively few specimens involved, it is unfortunate that more such small and easily-prepared datasets are not shared.

Identifying and prioritizing more collections for digitization and publication through GBIF.org would serve the long-term needs of conservationists, while providing collections with greater visibility and return on investment because funding agencies are more likely to make awards to NHCs that are digitizing their holdings. A task force convened by GBIF is currently investigating how this can be best achieved through wide consultation with the global collections community (<http://www.gbif.org/newsroom/news/accelerating-discovery-of-biocollections-data>).

Natural history collections digitization efforts

Imagine if all the NHCs from which Ferro and Flick (2015) had borrowed specimens had already databased and georeferenced their specimens and shared the data with GBIF? This would have reduced the time and cost of their study considerably. First, the task of verifying identifications would be easier. Most records shared with GBIF include the names of the determiners and the dates of determination, which enables evaluation of the trustworthiness of the records. Having a full dataset prior to bor-

rowing any specimens would allow them to select only specimens that were identified by people that Ferro and Flick did not trust, were outliers in the distribution, or were needed for morphological study. This would reduce the number of loaned specimens and data capture efforts considerably. Incidentally, Ferro could improve the dataset by the correction of identifications. Secondly, while taxonomists are the best qualified to identify specimens they are not necessarily the best qualified to georeference specimens. Museum curators and collection managers who know the history, languages, and geography of the regions best represented in their collections, and the history of the collectors involved, can bring to bear far more knowledge for accurate georeferencing than can taxonomists who borrow specimens from various NHCs. A partnership between taxonomists and museums towards the creation of high-quality data is ideal.

Conclusions and solutions

Historian J. J. O'Donnell, in his book *Avatars of the Word* (1998), notes the striking similarities between our current concerns about the internet (and digital data) and the responses of Medieval monks to the invention of the printing press. Their primary concern was that errors could creep into the bible and be duplicated hundreds of times, with no hope of gathering and destroying all erroneous copies. O'Donnell's two main conclusions were (1) all technological change has consequences, good and bad, but (2) there is no stopping it. Ferro and Flick highlight the bad and provide useful caveats and warnings, but taxonomists should not turn their backs on this new reality. They should instead work to shape and improve it.

We welcome and appreciate the great effort invested by Ferro and Flick (2015) to compile and curate their dataset for *T. costalis*. Coleopterists and other users of biodiversity data in modeling and research could benefit from wider access to such data. Working with scientific publishers, GBIF has strongly advocated for the broader use and acceptance of data papers as a means of gaining academic recognition of activities necessary for data collection, curation and publication (see <http://www.gbif.org/mendeley/data-paper>). We encourage Ferro to publish what is described as the “most comprehensive collection of distributional data for the species to date” (Ferro 2015), as a data paper (Costello et al. 2013, Costello and Wieczorek 2014). Datasets published through GBIF are automatically assigned a DOI and URL. Alternatively, in the spirit that any digital data are better than none, systematists can archive datasets with figshare.com or the Dryad Digital Repository (e.g. Sikes & Venables 2013a, b). Data in Dryad are not automatically shared with GBIF but are at least accessible openly for free download and use. Once permission is provided by each respective museum, a dataset like that produced by Ferro and Flick (2015), with records from various museums, can be archived directly in GBIF (e.g. Dikow 2012, Sikes & Mousseau 2013a,b). However, at present, GBIF and its participants only publish data from organizations — that is, institutions, networks, and societies — rather than individuals. Individuals wishing to publish data must work through their affiliated organizations

or through journals (for example, Pensoft journals <http://www.pensoft.net/journals>), or GBIF nodes like Canadensys (<http://www.canadensys.net/>), which will publish data directly to GBIF from individual authors associated with a Canadian collection or organization (e.g. Schwarzfeld 2016). Ideally, such a service would be available to people from any nation, as GenBank is. It remains the case that many taxonomic organizations are not registered data providers, which is clearly a barrier to progress.

Because datasets generated from taxonomic revisionary work are the most thorough and high quality datasets available, we hope to see changes that enable these datasets to be more easily archived and shared. It is unrealistic and not efficient to expect all specimen digitization efforts to be performed by museums – especially when so much digitization is already being performed by taxonomists who borrow specimens. The changes necessary to realize this goal are both technological (e.g. easy access to data templates that can be filled in and user-friendly methods to share data) and behavioral (e.g. rewards for authors who take the extra effort to archive data, Chavan & Penev 2011). Scientific societies and journal editorial boards should encourage authors to deposit digital data. We direct readers interested in how to share data to a simple 10-step guide to data sharing written by Goodman et al. (2014) and the best-practices guide written by Costello and Wieczorek (2014). Dikow and Agosti (2015) recently published a valuable and relevant overview of new methods for sharing taxonomic data, with introduction of the term ‘cybercatalog’, and a description of Plazi, an effort to retroactively digitize taxonomic data by extracting it from legacy literature.

By publishing data papers and sharing their high-quality data, taxonomic experts critical of the quality of GBIF-mediated data can contribute constructively to improvements and at the same time gain wider visibility and recognition of their professional efforts. It has been asserted many times – the future of taxonomy is decline or digital renaissance (Godfray 2002, Maddison et al. 2012). Taxonomists and data aggregators should work together to maintain and advance the profile of biodiversity sciences. We know that to some this treatise probably sounds more like the Borg of Star Trek declaring “resistance is futile, prepare to be assimilated,” and that is the nature of O’Donnell’s conclusions. However, it need not be so bleak – GBIF is not the Borg, it is merely a data aggregator that helps users access data from various NHCs. We envision a bright future of well-maintained and well-digitized, growing Natural History Collections, and a thriving taxonomic community that continues to document our planet’s endless forms of most beautiful and wonderful life.

Acknowledgments

We thank Ferro and Flick for their thoughtful insight and concern in raising these issues. We thank Tim Robertson, Head of Informatics, at GBIF for preparation of the figure. We also thank reviewers who made helpful suggestions to improve the manuscript.

References

- Beck J, Böller M, Erhardt A, Schwanghart W (2014) Spatial bias in the GBIF database and its effect on modeling species' geographic distributions. *Ecological Informatics* 19. doi: 10.1016/j.ecoinf.2013.11.002
- Berlin Declaration on Open Access to Knowledge in the Sciences and Humanities 2003. 2003. Table of signatories @ 2015 <http://oa.mpg.de/lang/en-uk/berlin-prozess/signatoren>
- Chapman AD, Wicczorek J (Eds) (2006) Guide to best practices for georeferencing. Copenhagen: Global Biodiversity Information Facility. 90 pp. <http://www.gbif.org/resource/80536>
- Chavan V, Penev L (2011) The data paper: a mechanism to incentivize data publishing in biodiversity science. *BMC Bioinformatics* 12(Suppl. 15): S2. doi: 10.1186/1471-2105-12-S15-S2
- Costello MJ, Michener WK, Gahegan M, Zhang ZQ, Bourne PE (2013) Biodiversity data should be published, cited, and peer reviewed. *Trends in Ecology & Evolution*, 28(8): 454–461. doi: 10.1016/j.tree.2013.05.002
- Costello MJ, Wicczorek J (2014) Best practice for biodiversity data management and publication. *Biological Conservation*, 173: 68–73. doi: 10.1016/j.biocon.2013.10.018
- Dikow T (2012) Review of *Namibimydas* Hesse, 1972 and *Nothomydas* Hesse, 1969 (Diptera: Mydidae: Syllegomydinae: Halterorchini) with the description of new species. *African Invertebrates*, 53(1): 79–111. doi: 10.5733/afin.053.0105
- Dikow T, Agosti D (2015) Utilizing online resources for taxonomy: a cybercatalog of Afro-tropical apiocerid flies (Insecta: Diptera: Apioceridae). *Biodiversity data journal*, (3). doi: 10.3897/BDJ.3.e5707
- Ferro ML (2015) Review of the Genus *Thoracophorus* (Coleoptera: Staphylinidae: Osoriinae) in North America North of Mexico, with a Key to Species. *The Coleopterists Bulletin* 69(1):1–10. doi: 10.1649/0010-065X-69.1.1
- Ferro ML, Flick AJ (2015) “Collection Bias” and the importance of natural history collections in species habitat modeling: A case study using *Thoracophorus costalis* Erichson (Coleoptera: Staphylinidae: Osoriinae), with a critique of GBIF.org. *The Coleopterists Bulletin*, 69(3):415–425. doi: 10.1649/0010-065X-69.3.415
- Fourcade Y, Engler JO, Rödder D, Secondi, J (2014) Mapping species distributions with MAXENT using a geographically biased sample of presence data: A performance assessment of methods for correcting sampling bias. *PLoS ONE* 9(5): e97122. doi: 10.1371/journal.pone.0097122
- García-Roselló E, Guisande C, Manjarrés-Hernández A, González-Dacosta J, Heine J, Pelayo-Villamil P, González-Vilas L, Vari RP, Vaamonde A, Granado-Lorencio C, Lobo JM (2014) Can we derive macroecological patterns from primary Global Biodiversity Information Facility data? *Global Ecology and Biogeography* 24(3): 335–347. doi: 10.1111/geb.12260
- Godfray HCJ (2002) Challenges for taxonomy. *Nature* 417(6884): 17–19. doi: 10.1038/417017a
- Goodman A, Pepe A, Blocker AW, Borgman CL, Cranmer K, Crosas M, Di Stefano R, Gil Y, Groth P, Hedstrom M, Hogg DW, Kashyap V, Mahabal A, Siemiginowska A, Slavkovic A (2014) Ten simple rules for the care and feeding of scientific data. *PLoS Comput Biol* 10(4): e1003542. doi: 10.1371/journal.pcbi.1003542

- Goodwin ZA, Harris DJ, Filer D, Wood JR, Scotland RW (2015) Widespread mistaken identity in tropical plant collections. *Current Biology* 25(22): R1066–R1067. doi: 10.1016/j.cub.2015.10.002
- Heidorn PB (2008) Shedding light on the dark data in the long tail of science. *Library Trends* 57(2): 280–299. doi: 10.1353/lib.0.0036
- Hjarding A, Tolley KA, Burgess ND (2015) Red List assessments of East African chameleons: a case study of why we need experts. *Oryx* 49(4):652–658. doi: 10.1017/S0030605313001427
- Hof AR, Svahlin A (2015) The potential effect of climate change on the geographical distribution of insect pest species in the Swedish boreal forest. *Scandinavian Journal of Forest Research*. doi: 10.1080/02827581.2015.1052751
- Hyde KD, Udayanga D, Manamgoda DS, Tedersoo L, Larsson E, Abarenkov K, Bertrand YJK, Oxelman B, Hartmann M, Kausarud H, Ryberg M, Kristiansson E, Nilsson RH (2013) Incorporating molecular data in fungal systematics: a guide for aspiring researchers. *Current Research in Environmental and Applied Mycology* 3: 1–32. <http://arxiv.org/abs/1302.3244v1>
- Kaiser J (2015) U.S. agencies fall in line on public access: Major research funders move to make papers free to all. *Science* 348 (6231): 167. doi: 10.1126/science.348.6231.167
- Kerr JT, Pindar A, Galpern P, Packer L, Potts SG, Roberts SM, Rasmont P, Schweiger O, Colla SR, Richardson LL, Wagner DL, Gall LF, Sikes DS, Pantoja A (2015) Climate change impacts on bumblebees converge across continents. *Science* 349 (6244): 177–180. doi: 10.1126/science.aaa7031
- Köljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AF, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM, Douglas B. (2013) Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology*. 22(21): 5271–5277. doi: 10.1111/mec.12481
- Maddison DR, Guralnick R, Hill A, Reysenbach AL, McDade LA (2012) Ramping up biodiversity discovery via online quantum contributions. *Trends in Ecology & Evolution* 27(2): 72–77. doi: 10.1016/j.tree.2011.10.010
- Meier R, Dikow T (2004) Significance of specimen databases from taxonomic revisions for estimating and mapping the global species diversity of invertebrates and repatriating reliable specimen data. *Conservation Biology* 18: 478–488. doi: 10.1111/j.1523-1739.2004.00233.x
- Nilsson RH, Tedersoo L, Abarenkov K, Ryberg M, Kristiansson E, Hartmann M, Schoch CL, Nylander JA, Bergsten J, Porter TM, Jumpponen A. (2012) Five simple guidelines for establishing basic authenticity and reliability of newly generated fungal ITS sequences. *MycKeys* 4: 37–63. doi: 10.3897/mycokeys.4.3606
- O'Donnell JJ (1998) *Avatars of the Word*. Harvard University Press, Cambridge, Mass., USA.
- Page RDM (2015) Comments on “Widespread mistaken identity in tropical plant collections” <http://iphylo.blogspot.co.uk/2015/11/comments-on-mistaken-identity-in.html> [accessed 7 Jan 2016]
- Page RDM (2016) Surfacing the deep data of taxonomy. In: Michel E (Ed.) *Anchoring Biodiversity Information: From Sherborn to the 21st century and beyond*. *ZooKeys* 550: 247–260. doi: 10.3897/zookeys.550.9293

- Roche DG, Kruuk LEB, Lanfear R, Binning SA (2015) Public data archiving in ecology and evolution: How well are we doing? PLoS Biol 13 (11): e1002295. doi: 10.1371/journal.pbio.1002295
- Schwarzfeld MD (2016) Specimen occurrence data for the *Ophion scutellaris* species group. doi: 10.5886/y2bbsq5 [accessed on 13 Sep 2016]
- Sikes DS, Mousseau T (2013a) Description of *Nicrophorus effereus*, new species, from Bougainville Island (Coleoptera, Silphidae, Nicrophorinae). ZooKeys 311: 83–93. doi: 10.3897/zookeys.311.5141
- Sikes DS, Mousseau T (2013b) ZooKeys: Type specimen data for *Nicrophorus effereus* Sikes & Mousseau, 2013-05-28. <http://www.gbif.org/dataset/8fd60078-620a-40a6-b565-0b093778e0fe> [accessed 7 Jan 2016]
- Sikes DS, Venables C (2013a) Molecular phylogeny of the burying beetles (Coleoptera: Silphidae: Nicrophorinae). Molecular Phylogenetics and Evolution 69(3): 552–565. doi: 10.1016/j.ympev.2013.07.022
- Sikes DS, Venables C (2013b) Data from: Molecular phylogeny of the burying beetles (Coleoptera: Silphidae: Nicrophorinae). Dryad Digital Repository. doi: 10.5061/dryad.mr221
- Soberón J, Peterson AT. (2004) Biodiversity informatics: managing and applying primary biodiversity data. Philosophical Transactions of the Royal Society of London B 359: 689–698. doi: 10.1098/rstb.2003.1439
- Wieczorek J, Döring M, De Giovanni R, Robertson T, Vieglais D (2009) Darwin Core Terms: A quick reference guide. <http://rs.tdwg.org/dwc/terms> [Accessed 2016-05-30]
- Williams PH, Thorp RW, Richardson LL, Colla SR (2014) Bumble Bees of North America: An Identification Guide. Princeton University Press, New Jersey, 208 pp.