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First survey of Interstitial molluscs from Cayo Nuevo, Campeche Bank, Gulf of Mexico

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Abstract

Six sediment samples weighing between 224–735 g were collected in June of 2016 from Cayo Nuevo reef, located at the Campeche Bank, southern Gulf of Mexico. Samples were collected by SCUBA diving, from were two stations at depths of 7.6 and 18.2 m. Sediment was sieved and molluscs (adults and micromolluscs ≤ 10 mm) were sorted, examined, and identified to the lowest taxonomic level. A total of 1,347 specimens was found, of which 224 shells were dead and 1,123 were alive. Thirty-four families, 53 genera, and 67 species were identified. The most abundant families were Chamidae and Arcidae for the Bivalvia class, and Caecidae and Tornidae for the Gastropoda class. The vertical range of *Bentharca* sp. was extended.

Keywords

baseline, inventory, Campeche Bank, Gulf of Mexico, micromolluscs

Introduction

Frequently, species molluscan biodiversity accounts are incomplete because of the lack of some groups such as sea slugs and micromolluscs. Compared with macromolluscs, the study of micromolluscs is still in its infancy, which is probably due not to the difficulty involved in obtaining samples, but difficulties in identification of such small animals and the time-consuming process required to separate specimens from sand or other substrates (e.g., algae or rocks), and photography. In order to get a more realistic picture of the biodiversity for different habitats, micromolluscs should be incorporated into the different studies (Sasaki 2008, and pers. obs.).

The term micromollusc has been applied in arbitrary and non-standardised ways. Micromolluscs are molluscs not visible without some type of artificial assistance, such as a microscope or magnifying glass. The most restrictive definition, or *sensu stricto,* stated that micromollusc size should be less than 5 mm as an adult (Narciso 2005, Geiger et al. 2007). Other authors considered micromolluscs as specimens smaller than 10 mm as an adult (Barrera and Tunnell 2001). Finally, the wider definition of micromollusc, or *sensu lato*, includes molluscs whose size is typically less than 10 mm as an adult and also included juvenile representative of macromolluscs (Moore 1964; García-Cubas 1970; Tunnell 1974; Kay 1980; Vokes and Vokes 1983).

Barrera (2001) stated that within Texas and Mexico, the majority of the studies involved macromolluscs. In Mexico, more than 4,643 species of marine molluscs have been recorded, and approximately 2,067 of them inhabit the Gulf of Mexico (GoMx) and the Mexican Caribbean Sea (Castillo-Rodríguez 2014). Unfortunately, Castillo-Rodríguez did not state which of them were micromolluscs. Important molluscan compilations focusing on these groups include publications by García-Cubas and Reguero (2007) and Vokes and Vokes (1983), although only a few publications have addressed the micromolluscan fauna specifically such as García-Cubas (1963, 1970 and 1971) for lagoons in the Gulf of Mexico and Hicks et al. (2001) at Alacranes reef.

The Campeche Bank is located at the southern GoMx and is composed of several emergent and submerged coral reefs (Tunnell et al. 2010). One of the smaller and most remote reefs is Cayo Nuevo, located between Arenas and Triángulos reefs (190 km offshore) in the GoMx (Fig. 1). Studies on this reef are almost non-existent with the exception of polychaetes (Granados-Barba et al. 2003).

The present work focuses on the molluscs of the Cayo Nuevo sandy bottoms, Gulf of Mexico. In this substrate we could find micromolluscs *sensu stricto* and juveniles of macromolluscs species that inhabit the interstitial as but also empty shells that could be carried by external factors such wind and currents.

Materials and methods

Using SCUBA gear, six sediment samples of coarse sand to fine gravel weighing 224–735 g each were collected by hand at 7.6 and 18.2 m on 19 June 2017 at Cayo Nuevo

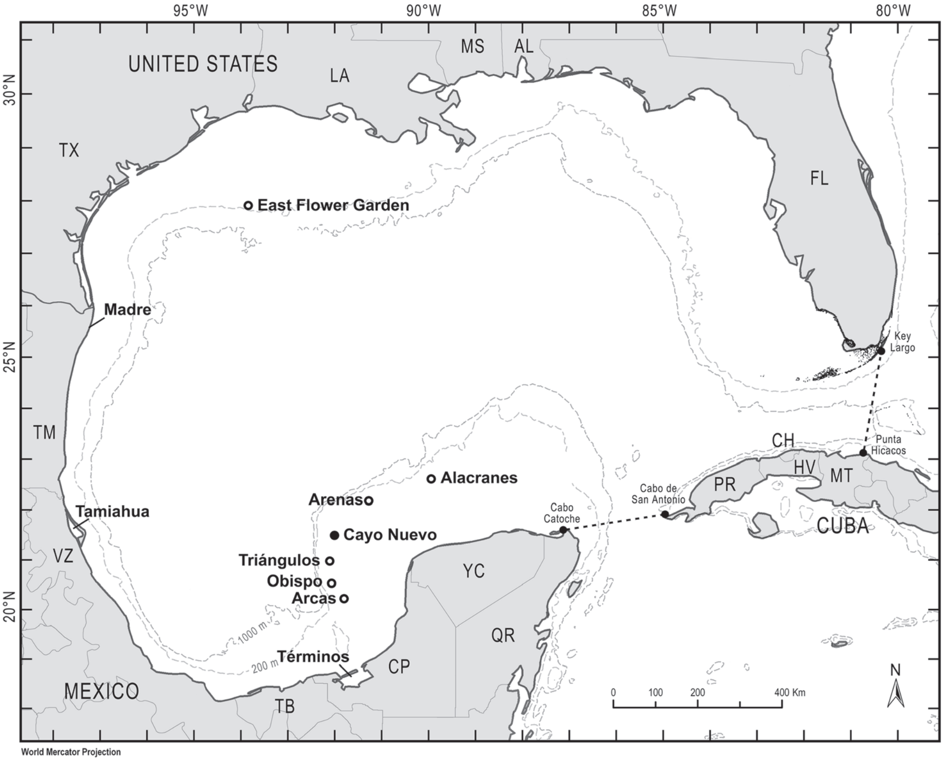

Figure 1. Map of the Gulf of Mexico, with the largest reef in the GoMx and sampling locations where micromolluscs have been documented in the literature and this study (adapted from Felder and Camp 2009).

Table 1. Sampling stations and coordinates at Cayo Nuevo on 18 June 2016.

Station	Depth (m)	Latitude (N)	Longitude (W)	Sample
1	18.2	21°49'40.32"	92°4'37.62"	GoMex-001
				GoMex-002
				GoMex-002
				GoMex-004
2	7.6	21°49'47.82"	92°4'34.32"	GoMex-005
				GoMex-006

reef (Table 1). Each sample was sieved by pouring water through six differently sized sieves (2 mm, 1.4 mm, 1 mm; 710 µm, 500 µm, and 250 µm) (Table 2) and sorted dry using a dissecting Nikon SMZ800 microscope. Specimens were picked out using soft forceps and 000 fine paint brushes. Molluscs were placed into 2 mm tubes and micromolluscs were placed into PCR tubes, both with 70% ethanol for long-term storage. Identification of specimens to species level was based on Abbott (1974), Tunnell et al. (2010) and Redfern (2013). Whenever possible, at least one specimen of each species

Table 2. Weight of each sample per sieve size in grams.

Sieve	GoMex-001	GoMex-002	GoMex-002	GoMex-004	GoMex-005	GoMex-006
2 mm	86.89	335.85	372.2	645.4	679.1	615.19
1.4 mm	46.94	60.04	21.7	46	80.7	91.53
1 mm	34.01	28.43	5.7	27.3	14.8	22.96
710 µm	27.1	11.13	1.3	28.5	2.3	5.32
500 µm	16.04	3.07	0.3	16.7	0.7	0.82
250 µm	13.86	1.2	0.07	2.6	0.6	0.004
Total	224.84	439.72	401.27	766.5	778.2	735.824

or morphotype was photographed. All the specimens were deposited at the "Colección de Moluscos de la Peninsula de Yucatán" (**CMPY**), Unidad Multidisciplinaria de Docencia e Investigación Campus Sisal, Universidad Nacional Autónoma de México.

The nomenclature of the species listed was assigned according to Bouchet and Rocroi (2010) for Bivalvia, Bouchet et al. (2017) for Gastropoda, and Kaas and Van Belle (1985) for Polyplacophora, due to the variability of some categories we only present the Linnaean ones. Abundance categories were assigned following Hicks et al. (2001): Abundant ≥ 50 (A); Common = 6–49 (C); Uncommon = 2–5 (UC), and Rare = 1 (R). Juvenile species are denoted by an asterisk (*).

Results

The results from the analysis of the sediment from Cayo Nuevo reef revealed 67 species of molluscs, from which 50 species are gastropods, 14 species are bivalves and three are chitons. These species belong to 38 different families.

Phylum Mollusca Linnaeus, 1758
Class Polyplacophora Gray, 1821
Polyplacophora sp. 1 * (R) (Fig. 2-1)
Polyplacophora sp. 2 * (R) (Fig. 2-2)

Order Chitonina Thiele, 1909
Family Chitonoidea Rafinesque, 1815
Genus *Ischnochiton* Gray, 1847
***Ischnochiton* sp.** * (UC) (Fig. 2-3)

Class Bivalvia Linnaeus, 1758
Order Arcida Stoliczka, 1871
Family Arcidae Lamarck, 1809
Arcidae sp. (UC) (Fig. 2-4)
Genus *Arca* Linnaeus, 1758
***Arca imbricata* Bruguière, 1789** * (UC) (Fig. 2-5a, b)

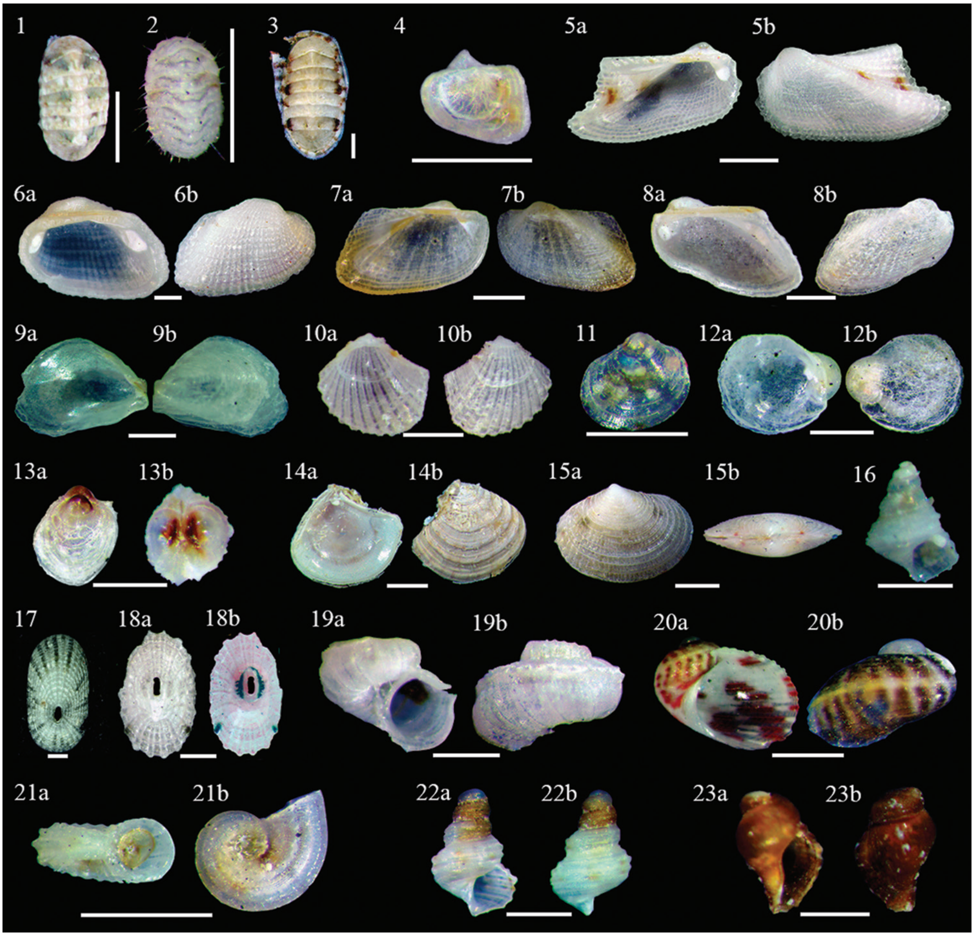

Figure 2. Polyplacophora **1–3**: **1** Polyplacophora sp. 1, dorsal view, scale bar 0.5 mm **2** Polyplacophora sp. 2, dorsal view, scale bar 1 mm **3** *Ischnochiton* sp., dorsal view, scale bar 0.5 mm. Bivalvia **4–15**: **4** Arcidae sp., dorsal view, scale bar 0.5 mm **5** *Arca imbricata* **5a** ventral view **5b** dorsal view, scale bar 1 mm **6** *Barbatia domingensis* **6a** ventral view **6b** dorsal view, scale bar 1 mm **7** *Barbatia* sp. **7a** ventral view **7b** dorsal view, scale bar 1 mm **8** *Bentharca* sp. **8a** ventral view **8b** dorsal view, scale bar 1 mm **9** *Anomia* sp. **9a** ventral view **9b** dorsal view, scale bar 1 mm **10** *Carditopsis smithii* **10a** ventral view **10b** dorsal view, scale bar 1 mm, **11** Lucinidae sp., ventral view, scale bar 0.5 mm **12** *Chama sinuosa* **12a** ventral view **12b** dorsal view, scale bar 0.5 mm **13** Chamidae sp. **13a** ventral view **13b** dorsal view, scale bar 1 mm, **14** *Crassinella lunulata* **14a** ventral view **14b** dorsal view, scale bar 1 mm **15** *Semele bellastriata* **15a** dorsal view **15b** lateral view, scale bar 1 mm. Gastropoda **16–23**: **16** Gastropoda sp., ventral view, scale bar 0.5 mm, **17** *Diodora minuta*, ventral view, scale bar 0.25, **18** *Diodora listeri* **18a** ventral view **18b** dorsal view, scale bar 1 mm, **19** *Scissurella redferni* **19a** ventral view **19b** dorsal view, scale bar 1 mm **20** *Synaptocohlea picta* **20a** ventral view **20b** dorsal view, scale bar 1 mm **21** *Lodderena ornata* **21a** ventral view **21b** Apical view, scale bar 0.5 mm **22** *Cerithium* sp. 1 **22a** ventral view **22b** dorsal view, 1 scale bar 1 mm **23** *Cerithium* sp. 2 **23a** ventral view **23b** dorsal view, scale bar 1 mm.

Genus *Barbatia* Gray, 1842
Barbatia domingensis * (Lamarck, 1819) (UC) (Fig. 2-6a, b)
***Barbatia* sp.** * (C) (Fig. 2-7a, b)

Genus *Bentharca* Verrill & Bush, 1898
***Bentharca* sp.** (R) (Fig. 2-8a, b)

Order Mytilida Férussac, 1822
Family Mytilidae Rafinesque, 1815
Genus *Crenella* T. Brown, 1827
***Crenella* sp.** (R)

Order Pectinida Gray, 1854
Family Anomiidae Rafinesque, 1815
Genus *Anomia* Linnaeus, 1758
Anomia* sp. (R) (Fig. 2-9a, b)

Order Cardita Bruguière, 1792
Family Carditidae Férussac, 1822
Genus *Carditopsis* E. A. Smith, 1881
***Carditopsis smithii* (Dall, 1896)** (C) (Fig. 2-10a, b)

Order Lucinida Gray, 1854
Family Lucinidae J. Fleming, 1828
Lucinidae sp. * (UC) (Fig. 2-11)

Order Venerida Gray, 1854
Family Chamidae Lamarck, 1809
Chamidae sp. (UC) (Fig. 2-12a, b)
Genus *Chama* Linnaeus, 1758
***Chama sinuosa* Broderip, 1835** * (A) (Fig. 2-13a, b)

Family Galeommatidae Gray, 1840
Galeommatidae sp. (R)

Order Carditida Dall, 1889
Family Crassatellidae Férussac, 1822
Genus *Crassinella* Guppy, 1874
Crassinella lunulata (Conrad, 1834) * (R) (Fig. 2-14a, b)

Order Cardiida Férussac, 1822
Family Semelidae Stoliczka, 1870 (1825)
Genus *Semele* Schumacher, 1817
***Semele bellastriata* (Conrad, 1837)** * (UC) (Fig. 2-15a, b)

Class Gastropoda Cuvier, 1795
Gastropoda sp. * (R) (Fig. 2-16)

Order Lepetelloidea Dall, 1882
Family Fissurellidae Fleming, 1822
Genus *Diodora* Gray, 1821
***Diodora minuta* (Lamarck, 1822)** * (UC) (Fig. 2-17)
***Diodora listeri* (d'Orbigny, 1847)** (R) (Fig. 2-18a, b)

Family Scissurellidae Gray, 1847
Genus *Scissurella* d'Orbigny, 1824
***Scissurella redferni* (Rolán, 1996)** (C) (Fig. 2-19a, b)

Order Trochida Rafinesque, 1815
Family Trochidae Rafinesque, 1815
Genus *Synaptocochlea* Pilsbry, 1890
***Synaptocochlea picta* (d'Orbigny, 1847)** (A) (Fig. 2-20a, b)

Family Skeneidae W. Clark, 1851
Genus Lodderena Iredale, 1924
***Lodderena ornata* (Olsson & McGinty, 1958)** (A) (Fig. 2-21a, b)

Family Cerithiidae Fleming, 1822
Genus *Cerithium* Bruguière, 1789
***Cerithium* sp. 1** (R) (Fig. 2-22a, 22b)
***Cerithium* sp. 2** (UC) (Fig. 2-23a, 23b)
***Cerithium atratum* (Borns, 1778)** (R) (Fig. 3-1a, b)

Family Litiopidae Gray, 1847
Genus *Alaba* H. adams & A. Adams, 1853
***Alaba incerta* (d'Orbigny, 1841)** (C) (Fig. 3-2a, b)

Family Scaliolidae Jousseaume, 1912
***Finella* sp.** (UC) (Fig. 3-3a, 3b)

Family Pickworthiidae Iredale, 1917
Subfamily Pickworthiinae Iredale, 1917
Genus *Sansonia* Jousseaume, 1892
***Sansonia tuberculata* (Watson, 1886)** (R) (Fig. 3-4a, b)

Family Hypponicidae Troschel, 1861
***Hipponix* sp.** (C) (Fig. 3-5a, 5b)

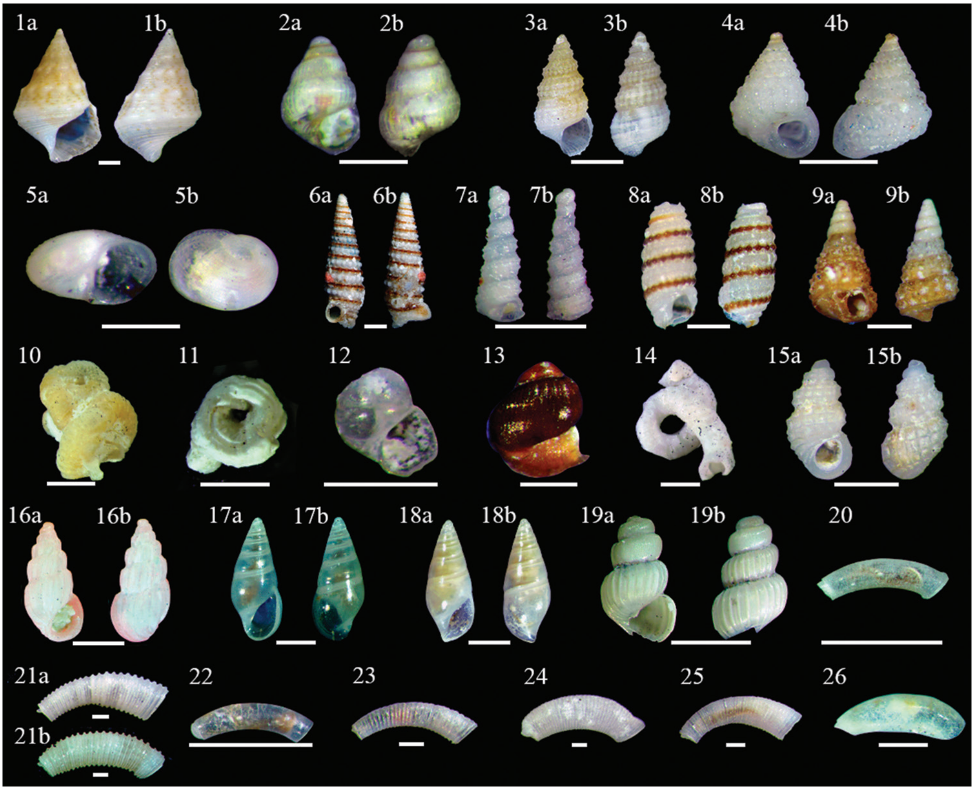

Figure 3. Gastropoda **1–26**: **1** *Cerithium atratum* **1a** ventral view **1b** dorsal view, scale bar 1 mm **2** *Alaba incerta* **2a** ventral view **2b** dorsal view, scale bar 0.25 mm **3** *Finella* sp. **3a** ventral view **3b** dorsal view, scale bar 1 mm **4** *Sansonia tuberculata* **4a** ventral view **4b** dorsal view, scale bar 1 mm **5** *Hipponix* sp. **5a** ventral view **5b** dorsal view, scale bar 0.1 mm **6** *Iniforis turristhomae* **6a** ventral view **6b** dorsal view, scale bar 1 mm **7** *Metaxia rugulosa* **7a** ventral view **7b** dorsal view, scale bar 0.5 mm **8** *Cerithiopsis* sp. **8a** ventral view **8b** dorsal view, scale bar 1 mm **9** *Cerithiopsis* cf. *iuxtafuniculata* **9a** ventral view **9b** dorsal view, scale bar 1 mm **10** Vermetidae *incertae sedis* irregularis scale bar 1 mm **11** *Dendropoma corrodens* scale bar 1 mm **12** Vermetid sp. C, ventral view, scale bar 0.25 mm **13** *Petaloconchus mcgintyi*, ventral view, scale bar 0.25 mm **14** *Thylacodes* sp. scale bar 1 mm **15** *Simulamerelina caribaea* **15a** ventral view **15b** dorsal view, scale bar 1 mm **16** *Schwartziella fischeri* **16a** ventral view **16b** dorsal view, scale bar 1 mm **17** *Zebina* sp. 2 **17a** ventral view **17b** dorsal view, scale bar 1 mm **18** *Zebina* sp. 2 **18a** ventral view **18b** dorsal view, scale bar 0.5 mm **19** *Truncatella* sp. **19a** ventral view **19b** dorsal view, scale bar 0.5 mm **20** *Caecum circumvolutum*, lateral view, scale bar 0.2 mm **21** *Caecum donmoorei* **21a** stage 1, lateral view, scale bar 0.2 mm **21b** adult, lateral view, scale bar 0.2 mm **22** *Caecum johnsoni*, lateral view, scale bar 0.2 mm **23** *Caecum pulchellum*, lateral view, scale bar 0.2 mm **24** *Caecum textile*, lateral view, scale bar 0.2 mm **25** *Caecum* sp. B, lateral view, scale bar 0.2 mm **26** *Meioceras nitidum*, lateral view, scale bar 0.2 mm.

Family Triphoridae Gray, 1847
Genus *Iniforis* Jousseaume, 1884
***Iniforis turristhomae* (Holten, 1802)** (UC) (Fig. 3-6a, b)

Genus *Metaxia* Monterosato, 1884
Metaxia rugulosa **(C. B. Adams, 1850)** (R) (Fig. 3-7a, b)

Family Cerithiopsidae H. Adams & A. Adams, 1853
Genus *Cerithiopsis* Forbes & Hanley, 1850
Cerithiopsis **sp.** (R) (Fig. 3-8a, 8b)
Cerithiopsis **cf.** ***iuxtafuniculata*** **Rolán, Espinosa & Fernández-Garcés, 2007** (R) (Fig. 3-9a, b)

Family Vermetidae Rafinesque, 1815
Vermetidae ***incertae sedis irregularis*** **d'Orbigny, 1841** (Fig. 3-10)

Genus *Dendropoma* Mörch, 1861
Dendropoma corrodens **(d'Orbigny, 1841)** (R) (Fig. 3-11)
Vermetid sp. C Redfern 2013 (A) (Fig. 3-12)

Genus *Petaloconchus* Lea, 1843
Petaloconchus mcgintyi **(Olsson & Harbison, 1953)** * (C) (Fig. 3-13)

Genus *Thylacodes* Guettard, 1770
Thylacodes **sp.** * (R) (Fig. 3-14)

Family Rissoidae Gray, 1847
Genus *Simulamerelina* Ponder, 1985
Simulamerelina caribaea **(d'Orbigny, 1842)** (UC) (Fig. 3-15a, b)

Family Zebinidae Coan, 1964
Genus *Schwartziella* G. Nevill, 1881
Schwartziella fischeri **(Desjardin, 1949)** (UC) (Fig. 3-16a, b)

Genus *Zebina* H. Adams & A. Adams, 1854
Zebina **sp. 1** (A) (Fig. 3-17a, 17b)
Zebina **sp. 2** (C) (Fig. 3-18a, 18b)

Family Truncatellidae Gray, 1840
Genus Truncatella Risso, 1826
Truncatella **sp.** (R) (Fig. 4-19a, 19b)

Family Caecidae Gray, 1850
Genus *Caecum* Fleming, 1813
Caecum circumvolutum **de Folin, 1867** (C) (Fig. 3-20)
Caecum donmoorei **Mitchell-Tapping, 1979** (C) (Fig. 3-21a, b)
Caecum johnsoni **Winkley, 1908** (A) (Fig. 3-22)

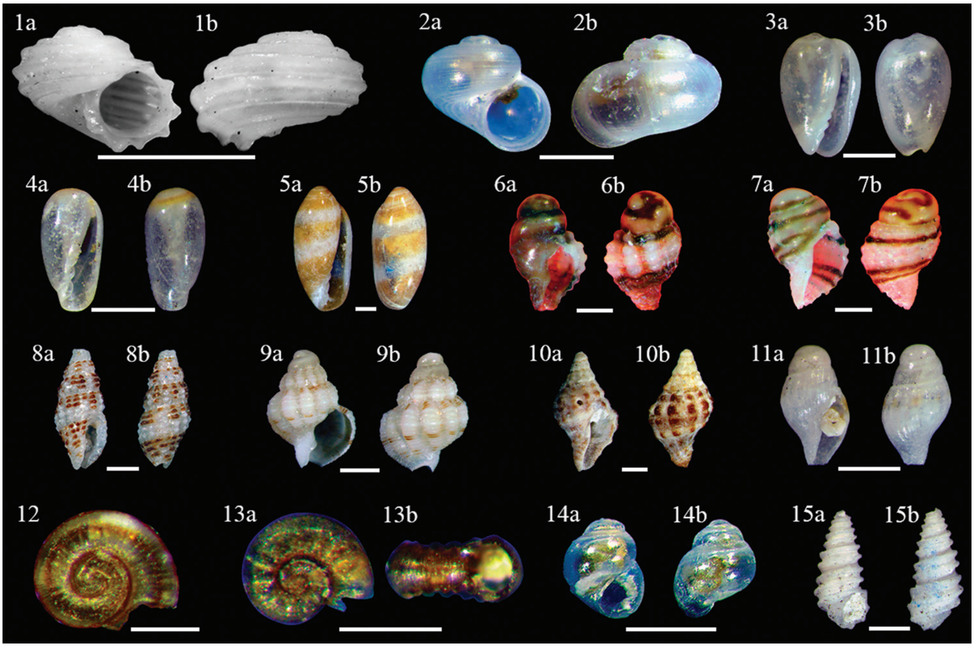

Figure 4. Gastropoda **1–18: 1** *Parviturboides* sp. **1a** ventral view **1b** dorsal view, scale bar 1 mm **2** *Vitrinella* sp. **2a** ventral view **2b** dorsal view, scale bar 0.5 mm **3** *Gibberula lavalleeana* **3a** ventral view **3b** dorsal view, scale bar 1 mm **4** *Volvarina* sp. 1 **4a** ventral view **4b** dorsal view, scale bar 1 mm **5** *Volvarina* sp. 2 **5a** ventral view **5b** dorsal view, scale bar 1 mm **6** Columbellidae sp. 1 **6a** ventral view **6b** dorsal view, scale bar 0.5 mm **7** Columbellidae sp. 2 **7a** ventral view **7b** dorsal view, scale bar 0.5 mm **8** *Steironepion moniferum* **8a** ventral view **8b** dorsal view, scale bar 1 mm **9** *Phrontis* sp. 9a ventral view **9b** dorsal view, scale bar 0.5 mm **10** *Trachypollia* sp. **10a** ventral view **10b** dorsal view, scale bar 1 mm **11** Turridae sp. 1 **11a** ventral view **11b** dorsal view, scale bar 1 mm **12** *Ammonicera lineofuscata*, Apical view, scale bar 0.25 mm **13** *Ammonicera minortalis* **13a** Apical view **13b** ventral view, scale bar 0.1 mm **14** *Rissoella galba* **14a** ventral view **14b** dorsal view, scale bar 0.1 mm **15** *Pseudoscilla* aff. *babylonia* **15a** ventral view **15b** dorsal view, scale bar 1 mm.

***Caecum pulchellum* Stimpson, 1851** (C) (Fig. 3-23)
***Caecum textile* de Folin, 1867** (UC) (Fig. 3-24)
***Caecum* sp. B *sensu* Redfern 2013** (C) (Fig. 3-25)

Genus *Meioceras* Carpenter, 1859
***Meioceras nitidum* (Stimpson, 1851)** (UC) (Fig. 3-26)

Family Tornidae Sacco, 1896 (1884)
Genus *Parviturboides* Pilsbry & McGinty, 1949
***Parviturboides* sp.** (C) (Fig. 4-1a, b)

Genus *Vitrinella* C. B. Adams, 1850
***Vitrinella* sp.** (A) (Fig. 4-2a, b)

Family Cystiscidae Stimpson, 1865
Genus *Gibberula* Swainson, 1840
***Gibberula lavalleeana* (d'Orbigny, 1824)** (UC) (Fig. 4-3a, b)

Family Marginellidae Fleming, 1828
Genus *Volvarina* Hinds, 1844
***Volvarina* sp. 1** (UC) (Fig. 4-4a, 4b)
***Volvarina* sp. 2** (R) (Fig. 4-5a, 5b)

Family Columbellidae Swainson, 1840
Columbellidae sp. 1 (UC) (Fig. 4-6a, b)
Columbellidae sp. 2 (R) (Fig. 4-7a, 7b)
Genus *Steironepion* Pilsbry & H. N. Lowe, 1932
***Steironepion moniliferum* (G. B. Sowerby I, 1844)** (UC) (Fig. 4-8a, b)

Family Nassariidae Iredale, 1916 (1835)
Genus *Phrontis* H. Adams & A. Adams, 1853
***Phrontis* sp.** (UC) (Fig. 4-9a, b)

Family Muricidae Rafinesque, 1815
Genus *Trachypollia* Woodring, 1928
***Trachypollia* sp.** (R) (Fig. 4-10a, b)

Family "Turridae" H. Adams & A. Adams, 1853 (1838)
Turridae sp. 1 (R) (Fig. 4-11a, 11b)

Family Omalogyridae G.O. Sars, 1878
Genus *Ammonicera* Vayssière, 1893
***Ammonicera lineofuscata* Rolán, 1992** (A) (Fig. 4-12)
***Ammonicera minortalis* Rolán, 1992** (A) (Fig. 4-13a, b)

Family Rissoellidae Gray, 1850
Genus Rissoella Gray, 1847
***Rissoella galba* Robertson, 1961** (R) (Fig. 4-14a, b)

Order Aplysiida
Family Aplysiidae Lamarck, 1809
Genus *Aplysia* Linnaeus, 1767
***Aplysia* sp.** (R)

Order Siphonarimorpha
Family Pyramidellidae Gray, 1840
Genus *Pseudoscilla* Boettger, 1901
***Pseudoscilla* aff. *babylonia* (C. B. Adams, 1845)** (R) (Fig. 4-15a, 185b)

Discussion

The most abundant families of gastropods were the Caecidae (456 specimens, seven species), Tornidae (221 specimens, two species), and Omalogyridae (132 specimens, two species). The most abundant families of bivalves were Arcidae (40 specimens, five species) and Chamidae (59 specimens, two species). The most abundant gastropod species were *Caecum johnsoni* (310 specimens), *Vitrinella* sp. (208 specimens), Vermetid sp. C (91 specimens), *Lodderena ornata* (71 specimens), and *Caecum donmoorei* (147 specimens). For the Bivalvia the most abundant species were *Chama sinuosa* (57 specimens), *Barbatia domingensis* (57 specimens) and *Carditopsis smithii* (12 specimens).

From the six sediment samples, the most commonly found molluscs were *Lodderena ornata*, *Caecum johnsoni* and *Ammonicera lineofuscata*, while other species appeared only once: *Leptochiton* sp., Arcidae sp., *Bentharca* sp., *Crenella* sp., *Anomia* sp., *Chama* sp., Galeommatidae sp., *Chione elevata*, *Semele bellastriata*, *Cerithium* sp. 1, Gastropoda sp., *Diodora listeri*, *Cerithium atratum*, *Sansonia tuberculata*, *Iniforis turristhomae*, *Metaxia rugulosa*, *Cerithiopsis* cf. *iuxtafuniculata*, *Cerithiopsis* sp., Vermetidae *incertae sedis* irregularis, *Dendropoma corrondens*, *Thylacodes* sp., *Finella* sp., *Caecum textile*, *Hipponix* sp., *Volvarina* sp. 2, Columbellidae sp. 2, *Aplysia* sp., and *Pseudoscilla* aff. *babylonia*.

This new data becomes a taxonomic reference list for the molluscs that inhabit Cayo Nuevo, GoMx, including micromolluscs as well as juvenile macromolluscs. To place it within a useful context we mention other inventories made in this area: Felder and Camp (2009) recorded some 5,517 species of invertebrates in the GoMx, of which 2,455 were marine molluscs (Moretzsohn et al. 2009). González et al. (1991) recorded 298 species of molluscs and included 33 localities distributed around the coasts of the Yucatan Peninsula and adjacent coral reefs but did not mentioned Cayo Nuevo. García-Cubas et al. (1999) recorded 110 species of gastropods in the northern and northeastern regions of the Yucatan Peninsula. Rice and Kornicker (1962) recorded 130 species for Alacranes reef in the Campeche Bank and later, Hicks et al. (2001) recorded 215 species of molluscs on the same reef. Although earlier articles include reefs or sampling locations within the Bank of Campeche (e.g., Rehder and Abbott 1951, Springer and Bullis 1956, Kornicker et al. 1959), no mention of molluscs from Cayo Nuevo were found. Only Barrera (2001) study focuses on reef micromolluscs, recording 131 species from the East and West Flower Garden Banks (FGB).

The molluscan assemblage at Cayo Nuevo shares many species also present at the FGB (Barrera 2001) and Alacranes Reef (Hicks et al. 2001) (19 families/21 genera and 21 families/22 genera, respectively) (Table 3). The most diverse families recorded by Barrera (2001) and Hicks et al. (2001) were Caecidae (six genera and ten species) and Rissoidae (five genera and seven species) for Gastropoda and Arcidae (four genera and seven species) for Bivalvia.

Barrera (2001) most abundant gastropod species were *Amphithalamus vallei* (672 individuals) and cf. *Vitrinella* sp. (534 individuals); however, at Cayo Nuevo, 208 individuals of sf. *Vitrinella* sp., were found. Differences in the numbers of collected individ-

Table 3. Comparison of molluscs recorded at Cayo Nuevo (present study) and other interstial records at the GoMx; Madre: García-Cubas (1970), Tamiahua: García-Cubas (1971), Términos: García-Cubas (1963, 1981), East Flower Garden: Barrera and Tunnell (2001), Alacranes: Hicks et al. (2001). The maximum recorded size is provided: Key: a) García-Cubas and Reguero (2004), b) Tunnell et al. (2010), c) Redfern (2013).

Systematics	**Lagoons**			**Reefs**			**Maximum recorded size (mm)**
	Madre	**Tamiahua**	**Términos**	**East Flower Garden**	**Alacranes**	**Cayo Nuevo**	
Class Bivalvia							
Arca imbricata			*		*	*	48[c]
Barbatia domingensis				*	*	*	30[b]
Carditopsis smithii				*	*	*	1.5[c]
Chama sinuosa					*	*	76[b]
Crassinella lunulata		*				*	8[b]
Semele bellastriata						*	14[c]
Class Gastropoda							
Diodora minuta						*	10.5[c]
Diodora listeri					*	*	45[b]
Scissurella redferni						*	1[c]
Synaptocochlea picta					*	*	3.5[c]
Lodderena ornata				*	*	*	0.8[c]
Cerithium atratum						*	
Alaba incerta						*	
Sansonia tuberculata						*	1.5[c]
Iniforis turristhomae				*	*	*	6[c]
Metaxia rugulosa						*	7[c]
Cerithiopsis cf. *iuxtafuniculata*						*	3[c]
Vermetidae *incertae sedis irregularis*						*	6[b]
Dendropoma corrodens					*	*	10[b]
Petaloconchus mcgintyi				*	*	*	35[b]
Simulamerelina caribaea						*	3[b]
Schwartziella fischeri						*	3.5[c]
Caecum circumvolutum						*	4[b]
Caecum donmoorei						*	2[c]
Caecum johnsoni			*	*		*	5[b]
Caecum pulchellum	*	*	*	*	*	*	2[a]
Caecum textile					*	*	2[b]
Meioceras nitidum		*	*			*	3[b]
Gibberula lavalleeana		*				*	4[b]
Steironepion moniliferum						*	
Ammonicera lineofuscata						*	0.6[c]
Ammonicera minortalis				*		*	0.5[c]
Rissoella galba				*		*	1[c]
Pseudoscilla aff. *babylonia*				*	*	*	3[b]

uals can be explained by geography, but also by differences in the quantity of sediment collected and processed, fifteen sites with 300 ml sediment sampled at FGB and six samples of 224–735 g at Cayo Nuevo. *Vitrinella* sp. could not be identified to specific level due to the low similarity of characters shown with other described western Atlantic species. Barrera (2001) previously suggested that it could be an undescribed new species, but further detailed studies are required to establish its identity.

Regarding bivalves, the most abundant species reported by Barrera (2001) for the FGB were *Gregariella coralliophaga* (145 individuals) (summing nine identified as *Barbatia domingensis* (102 individuals) and eleven identified as *Barbatia cancellaria* (68 individuals, currently a synonym), and *Carditopsis smithii* (51 individuals). In comparison, 37 individuals of *Barbatia domingensis* and 12 *Carditopsis smithii* were collected at Cayo Nuevo and these were not the most abundant species.

It should come as no surprise that many organisms were not identified to species level (e.g., *Leptochiton* sp., *Bentharca* sp., *Crenella* sp., *Anomia* sp., *Lottia* sp., *Diodora* sp., *Cerithium* sp., *Cerithiopsis* sp., *Thylacodes* sp., *Zebina* sp. 1, *Zebina* sp. 2, and *Phrontis* sp.). We relied on regional and local literature that in fact was scarce. In the case of juveniles, shells within a genus are similar because they share many characters and the differential characters are difficult to discern even as adults and almost impossible in juveniles. Our specimens identified under the name of *Gibberula lavalleeana* could be considerate as a species complex, due to the evidence and description of new species in Cuban waters (Espinosa and Ortea 2007).

These faunistic results from Cayo Nuevo represent the first inventory of molluscs from this remote reef. These findings contribute to record expansions for the southern GoMx of *Bentharca* sp. This contribution highlights the importance of conserving small areas that can harbour a considerable diversity of organisms. Seasonal changes on the mollusc community assemblages were not evaluated but would be an interesting future project, as would collecting growth series of species to assist in confirming identifications. In 2004, González and Torruco stated the importance of the Campeche Bank's reefs and proposed a marine reserve for the reefs located within this area, Cayo Nuevo included. However, this proposal never materialized and, up to now, only Alacranes reef has governmental protection under the status of marine reserve. Species checklists of micromolluscs, as well as other faunal groups, are of vital importance to serve as a baseline data set, due to the proximity to Mexico's offshore oil production area within the GoMx. The soft benthic interstitial mollusc communities are diverse, and their monitoring could well represent ecological indicators of ecosystem health, especially in the light of potential future oil-spills.

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Supplementary material 1

Species abundance per sample

Authors: Deneb Ortigosa, Nancy Yolimar Suárez-Mozo, Noe C. Barrera, Nuno Simões

Data type: occurences

Link: https://doi.org/10.3897/zookeys.779.24562.suppl1

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First report of *Trichogramma danausicida* and *Trichogramma cacaeciae* reared from *Thaumatotibia leucotreta* eggs in Israel

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Abstract

The egg parasitpoids *Trichogramma danausicida* (Nagaraja) and *Trichogramma cacaeciae* (Marchal) (Hymenoptera: Trichogrammatidae), are reported for the first time in Israel. Moreover, our discovery of *T. danausicida* is the first report of this parasitoid species outside of India. The occurrence of those trichogrammatids was first discovered and documented in May 2016 during a survey of egg parasitoids of the False codling moth *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae). The field survey was conducted on castor bean fruits (*Ricinus communis*) in the Israeli central coastal plain. The identity of the parasitoids was revealed by means of sequencing a portion of the cytochrome oxidase I gene (COI) of the studied parasitoids.

Keywords

DNA barcoding, egg parasitoid, False codling moth, *Ricinus communis,* Trichogrammatidae

Introduction

The False codling moth (*Thaumatotibia leucotreta* (Meyrick); i.e., FCM) (Lepidoptera: Tortricidae), native to African regions south of the Sahara, was first reported in Israel in 1984 on macadamia nuts (Wysoki 1986). It is a polyphagous pest that can develop on more than 70 host plants (CABI 2017). Furthermore, FCM is an important economic pest to many crop fruits in its native habitat, such as citrus, macadamia, avocado, peach, plum, corn, cotton, peppers, and more. The annual estimated loss to the Southern African citrus industry alone, caused by this pest, is approximately 8 million USD (Kirkman and Moore 2007). Among wild plants, the castor bean (*Ricinus communis*) serves as a preferred host plant for the FCM, providing fruits nearly all year round for FCM development and survival (Kirkman and Moore 2007, CABI 2017, CABI 2018). One of the most effective parasitoids for controlling FCM in South Africa is the egg parasitoid *Trichogrammatoidea cryptophlebiae* (Nagaraja) (Newton 1988, Bedford et al. 1998, Moore and Hattingh 2012). Moreover, *T. cryptophlebiae*'s natural parasitism level can reach more than 80 percent of the FCM eggs. In such cases, the FCM population level is significantly reduced in citrus orchards (Moore and Hattingh 2012). *T. cryptophlebiae* was introduced to Israel in 1998 for controlling the FCM. More than 300,000 parasitoids were released in the Israeli central coastal plain; however, no recovery was reported to date "(Yael Argov, pers. comm.). Other reported egg parasitoids that attack the FCM are *Trichogrammatoidea fulva* (Nagaraja) and *Trichogrammatoidea lutea* (Girault) (CABI 2017). We were interested in investigating whether *T. cryptophlebiae* was established on FCM eggs in Israel, and if not, are other egg parasitoids attacking FCM eggs? Therefore, the objective of this study was to perform a field survey of FCM egg parasitoids in the Israeli central coastal plain.

Materials and methods

A survey of FCM egg parasitoids was performed on castor bean plants (*Ricinus communis*) in the Israeli central coastal plain (Table 1). The survey sites were determined based on the locations where *T. cryptophlebiae* were originally released in 1998, and where castor bean plants were found. Only sites where FCM eggs were actually found are shown in Table 1. Castor bean fruits were randomly collected from each site and transferred to the laboratory. The fruits were then carefully examined under a stereoscopic microscope, and the number of FCM eggs and their status were recorded. The egg status included: hatched eggs (i.e., egg shells), dead eggs, live eggs, or parasitized eggs (Figs 1–2). Parasitised eggs and suspected as such, were individually confined within petri dishes (55 mm in diameter, 26 mm height), and observed daily for adult emergence. After emergence, the adults were placed in 75% ethanol until their identity was determined using DNA sequencing.

DNA was extracted from single parasitoids in 25 µL lysis buffer (Skaljac et al. 2013). This DNA was used for amplification of 800 bp from the mitochondrial Cytochrome Oxidase I (COI) gene using Polymerase Chain Reaction (PCR). PCR was performed in a total volume of 50 µL containing 25 µL of Ready Mix (HyLabs, Israel), 13.5 µL double distilled water, 0.75 µL of 20 pmole for each primer used, and 10 µL of DNA template (total of 200 ng). The primer sequences used for PCR are LCO_1490F 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO_2198R 5'-TAAACTTCAGGGTGACCAAAAATCA-3'. PCR cycling conditions were 94 °C

Table 1. The Universal Transverse Mercator (UTM) coordinates of nine castor bean collection sites, and the number of FCM eggs that were found in each location.

Site	Latitude	Longitude	Elevation (m)	Total number of eggs
1	32°06'55"N	34°54'20"E	22	84
2	32°09'52"N	34°52'49"E	68	15
3	32°09'10"N	34°54'25"E	39	63
4	32°20'44"N	34°53'44"E	32	33
5	32°20'58"N	34°52'30"E	31	764
6	32°00'15"N	34°49'00"E	34	33
7	32°08'50"N	34°53'04"E	33	17
8	31°59'10"N	34°48'06"E	36	87
9	32°08'07"N	34°53'27"E	19	45

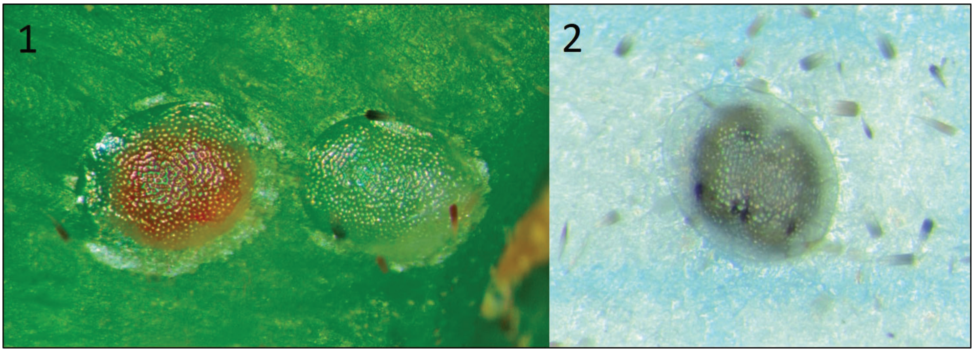

Figure 1–2. *Thaumatotibia leucotreta* eggs. **1** Unparasitised young (clear white) and mature (red) eggs **2** parasitised by *Trichogramma* spp.

for 5 min, followed by 35 cycles of 94 °C for 30 sec, 45 °C for 45 sec, and 72 °C for 1 min, with a final extension at 72 °C for 10 min.

Trichogrammatoidea cryptophlebiae parasitoids obtained from South Africa (from Vital Bugs®, Tzaneen, South Africa) were tested with the same pair of primers mentioned above, however, the obtained sequences did not match any sequences in GenBank (www.ncbi.nlm.nih.gov/Genbank), thus, an additional pair of primers that amplify a portion of the Internal Transcribed Spacer 2 sequences (ITS 2), located in the 5.8S and 28S region of the rDNA complex bordering the ITS 2 region, were used. Their sequences are: ITS2-F 5'-TGTGAACTGCAGGACACATG-3' and ITS2-R 5'-GTCTTGCCTGCTCTGAG-3'. The PCR conditions were as follows: 94 °C for 3 min, followed by 33 cycles of 94 °C for 40 sec, 55 °C for 1 min and 72 °C for 1 min, with a final extension period at 72 °C for 5 min (Wahner et al. 2008). Each PCR reaction was examined by electrophoresis and bands were visualised with UV light. PCR products were excised from the gel and purified using the Nucleospin Gel and PCR Clean-Up Kit (Macherey-Nagel, Germany). Purified PCR products were sequenced in both the forward and reverse directions (HyLabs, Rehovot, Israel).

Sequence alignment and phylogenetic analysis: Sequence alignments for COI gene sequences were performed with MUSCLE 3.7 (Edgar 2004) and the results were adjusted manually where necessary, to maximise alignment. The alignment data for each gene were used in maximum likelihood tree construction, using Kimura-2 parameter model (K2P) genetic distances (Kimura 1980). Both trees were generated using MEGA v.5 (Tamura et al. 2011) and branch support was estimated with 1000 bootstrap replicates. The nucleotide sequences used in this study for generating the phylogenetic tree have been deposited in GenBank under the accession numbers MH102404 to MH102410.

Results

Thaumatotibia leucotreta eggs were found from November 2015 to December 2016 on castor bean fruit in the Israeli central coastal plain. In total, on 2200 fruits, we detected 1141 eggs, of which 449 were alive (i.e., 39.3%). In May 2016, in location number 5 (Table 2), we detected seven parasitised eggs of which only six hatched. These eggs accounted for 3.7 percent of all live eggs that were found during May 2016 in this location.

We sequenced a total of seven wasps (four specimens from Israel and three *T. cryptophlebiae* wasps from South Africa) and obtained their COI sequences. Those sequences were aligned with other Hymenoptera sequences and other outgroup sequences of species from other orders such as the Coleoptera, Diptera and Lepidoptera (obtained from GenBank). All species for which multiple specimens were sampled showed no interspecies variation. The maximum likelihood analysis of the COI gene resulted in a tree typology that showed the presence of two different species of trichogrammatids: *Trichogramma danausicida* (Nagaraja)(3 specimens) (Nagaraja 1996) and *Trichogramma cacaeciae* (Marchal) (one specimen) (Marchal 1927) (Fig. 3), which were clearly separated, but fall within the Hymenoptera.

Discussion

Trichogramma spp. are minute endoparasitoids of insect eggs. Currently, more than 230 species of *Trichogramma* are described worldwide, making them the largest genus in the Trichogrammatidae family. More than 200 insect species are being attacked by different *Trichogramma* species. Moreover, many species of Trichogramma are important biological control agents of numerous agricultural pests (Jalali et al. 2016).Two species of the genus *Trichogramma* were discovered and identified while surveying for egg parasitoids of the FCM *T. leucotreta* in the Israeli central coastal plain. While *T. cacaeciae* is native to Europe and widely distributed around the world (Jalali et al. 2016), the parasitoid *T. danausicida* was reported only in India (Begum and Anis 2014, Yousuf et al. 2015, Jalali et al. 2016). These two egg parasitoids are recorded for the first time in fauna in Israel. Moreover, to the best of our knowledge, this is the first report of *T. danausicida and T. cacaeciae* attacking and developing in the FCM eggs, and the

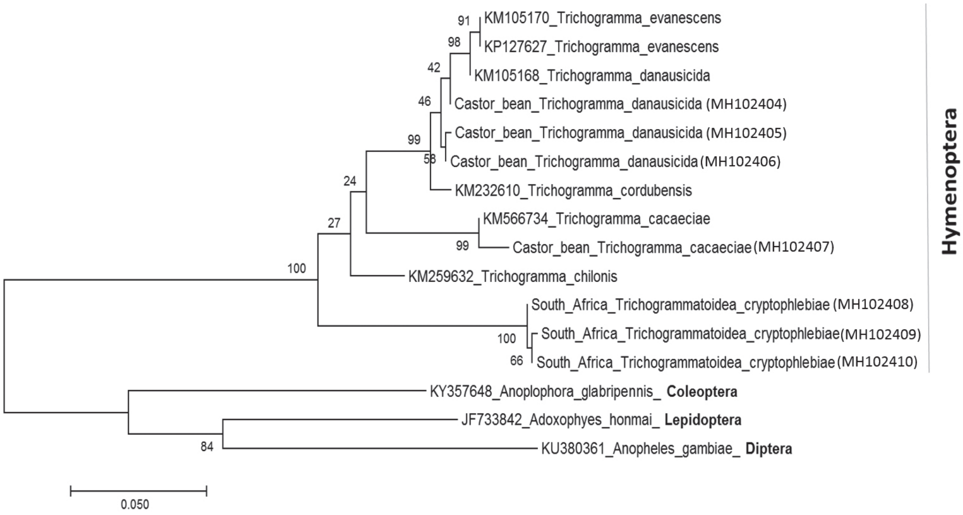

Figure 3. Maximum likelihood tree of COI nucleotide sequences of *Trichogramma danausicida* and *Trichogramma cacaeciae* and other hymenoptera species. Other species from Coleoptera, Lepidoptera and Diptera were used as outgroups to construct the tree. The tree was constructed using Kimura-2 parameter model (K2P) genetic distances with MEGA v.5, and branch support was estimated with 1000 bootstrap replicates. Numbers in parentheses are accessions that were deposited in GenBank.

Table 2. Collection dates, and number of FCM eggs that were found in a field survey, in nine different locations in the Israeli central coastal plain.

Site	Collection date	Number of fruits	Total number of eggs	Number of live eggs	Number of parasitized eggs	Percentage of parasitized eggs from live eggs
1	November 2015	100	28	5	0	0
1	June 2016	100	56	19	0	0
2	April 2016	50	15	7	0	0
3	April 2016	50	9	4	0	0
3	June 2016	100	54	19	0	0
4	May 2016	200	33	16	0	0
5	May 2016	500	518	184	7	3.7
5	June 2016	300	246	161	0	0
6	June 2016	150	29	1	0	0
6	December 2016	100	4	2	0	0
7	July 2016	50	17	6	0	0
8	August 2016	200	62	4	0	0
8	October 2016	100	25	3	0	0
9	November 2016	100	33	10	0	0
9	December 2016	100	12	8	0	0
Total		2200	1141	449	7	

first report of *T. cacaeciae* presence outside of India. The parasitism level of FCM eggs that was found in our study was very low (3.7% only in one site). Both egg parasitoids, *T. danausicida* and *T. cacaeciae*, apparently play only a minor role in keeping FCM population low in castor bean plants, and therefore are not potentially recommended biological control agents for FCM control. Similarly, Pinto et al. (2002) reported that the percentage occurrence of *T. cacaeciae* collected from parasitised tortricid eggs found on pears and apples in North America, was extremely low (less than 1%). However, our findings may contribute to better knowledge of trichogrammatids fauna in Israel and the Middle East. Since information is lacking on those two parasitoids in scientific literature, biological and ecological studies are needed to determine their biology, host list, and their impact on their host biological control.

Acknowledgements

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RESEARCH ARTICLE

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Hippocampus japapigu, a new species of pygmy seahorse from Japan, with a redescription of *H. pontohi* (Teleostei, Syngnathidae)

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Abstract

The pygmy seahorse *Hippocampus japapigu* **sp. n.** is described based on three specimens, 13.9–16.3 mm SL, collected from a mixed soft coral and algae reef at 11 m depth at Hachijo-jima Island, Izu Islands, Japan. The new taxon shares morphological synapomorphies with the previously described central Indo-Pacific pygmy seahorses, *H. colemani*, *H. pontohi*, *H. satomiae*, and *H. waleananus*, including extremely small size, 12 trunk rings, strongly raised continuous cleithral ring, snout spine, large spine on the eighth lateral and fifth and 12 superior trunk ridges, respectively, and unusual wing-like-protrusions immediately posterior to the head. *Hippocampus japapigu* **sp. n.** can be distinguished from all congeners by the following combination of features in the anterodorsal area of the trunk: bilaterally paired wing-like protrusions formed by a single pair of large, truncate spines projecting dorsolaterad on the first superior trunk ridge, followed by a unique elevated dorsal ridge formed by triangular bony mounds dorsally on the second to fourth superior trunk ridges. In contrast, *H. pontohi* possesses a pair of large truncate spines projecting strongly laterad on both the first and second superior trunk ridges followed by flat surfaces dorsally on the third and fourth superior trunk rings. The new species can be further differentiated by genetic divergence from *H. pontohi* (an uncorrected p-distance of 10.1% in the mitochondrial COI gene) and a striking reticulated white and brown lattice pattern on the head, trunk, and tail. *Hippocampus japapigu* **sp. n.** represents the fifth species of pygmy seahorse recorded in Japan.

Keywords

Acanthomorpha, computed tomography, reef fish, new species, systematics, taxonomy, systematics, computed tomography

Introduction

The family Syngnathidae contains 57 valid genera and 300 described predominantly small-bodied and cryptic marine species (Dawson 1985; Froese and Pauly 2018), including the seahorses, pipefishes, pipehorses, and seadragons. The family occurs worldwide in shallow temperate to tropical waters in a range of habitats, including seagrass beds, estuaries, coral and rocky reefs, and mangroves (Foster and Vincent 2004; Kuiter 2009; Froese and Pauly 2018). Pygmy seahorses of the genus *Hippocampus* Rafinesque, 1810 are diminutive in size (13.6–26 mm SL), live in close association with octocorals, colonial hydrozoans, bryozoans, seagrass and algae, and are morphologically distinct from the more numerous and larger species (24–350mm SL) of seahorses in possessing a single rather than paired gill openings and trunk brooding of their young (Whitley 1970; Kuiter 2003; Lourie and Randall 2003; Lourie and Kuiter 2008; Gomon and Kuiter 2009; Lourie et al. 2016).

Fundamental information on the taxonomy, systematics, and distribution of pygmy seahorses is still relatively sparse in comparison to the larger seahorse species. While one species, *H. bargibanti* Whitley, 1970, was described in 1970, all other species have been described since 2000. Most are known from very few specimens and only three species have been analyzed genetically (Hamilton et al. 2017). Six pygmy seahorse species are currently recognized and documented throughout the central Indo-Pacific, ranging from the Coral Triangle, West Pacific, Australia, to central Japan: *H. bargibanti*, *H. denise* Lourie & Randall, 2003, *H. colemani* Kuiter, 2003, *H. pontohi* Lourie & Kuiter, 2008, *H. satomiae* Gomon & Kuiter, 2009, and *H. waleananus* Gomon & Kuiter, 2009 (Whitley 1970; Kuiter 2003; Lourie and Randall 2003; Senou et al. 2006, 2007, 2008; Baine and Harasti 2007; Lourie and Kuiter 2008; Motomura et al. 2010; Allen and Erdmann 2012; Smith et al. 2012). Lourie et al.'s (2016) revision of the genus *Hippocampus* informally placed *H. waleananus* in synonymy with *H. satomiae*. However, we recognize the current taxonomic status of *H. waleananus* as valid based on differences in diagnostic morphological characters, including tail ring counts, coronet profile, and body ornamentation (Tables 1, 3), and host association and diurnal versus nocturnal behavior. Another described pygmy species, *Hippocampus severnsi* (Lourie & Kuiter, 2008), was distinguished from *H. pontohi* primarily by features of coloration in life, however the invalidity of this species was subsequently recognised as coloration is not a reliable morphological diagnostic character in seahorses (Lourie et al. 2004, 2008, 2016). Here, as part of this study, we formally synonymize *H. severnsi* under *H. pontohi* based on mitochondrial COI genetic data. All six pygmy species exhibit similar meristic and morphometric characters, and appear to form two natural groupings (Kuiter 2003; Lourie and Kuiter 2008; Gomon and Kuiter 2009). *Hippocampus colemani*, *H. pontohi*, *H. satomiae* and *H. waleananus* are morphologically highly conserved, with subtle meristic and morphological differences among these species. These four taxa are united by synapomorphies, including 12 trunk rings, strongly raised continuous cleithral ring, snout spine, large spine on the eighth lateral and fifth and 12th superior trunk ridges, wing-like-protrusions immediately posterior to the head, and associations with a wide range of habitat types. In contrast, *H. bargibanti* and *H. denise* are distinct in overall morphological appearance, including the absence of a distinct coronet and presence of large bulbous tubercles (in place of small spines exhibited by the other pygmy seahorse species), absence of a raised cleithral ring, and exclusive habitat association with gorgonian corals.

Japan is recognized as a global hotspot of marine biodiversity (Roberts et al. 2002; Allen 2008; Tittensor et al. 2010; Mittermeier et al. 2011), with 53 recorded species of syngnathids (Senou 2007; Han et al. 2017; Wibowo and Motomura 2017; Froese and Pauly 2018), including ten species of seahorses (Lourie et al. 2016; Han et al. 2017) of which four are true pygmy seahorses from the widely dispersed subtropical island groups Ryukyu, Ogasawara, and Izu: *H. bargibanti*, *H. denise*, and *H. pontohi,* and a fourth species that appears to be *H. colemani* (Senou et al. 2006, 2007; Motomura et al. 2010; Allen and Erdmann 2012). Inshore surveys of the marine ichthyofauna conducted at Hachijo-jima Island, Izu Islands (Senou et al. 2002), approximately 287 km south of Tokyo, have recorded what appears to be an undescribed species of true pygmy seahorse inhabiting mixed soft coral and algae reefs at shallow depths of 5–22 m, which was first brought to our attention from marine life books (*Hippocampus* sp. 7, Kuiter 2009: 57) and online photographs (Smith 2017) before specimens were acquired. This free-living species has been previously observed by local scuba divers from southern to central-eastern Japan at Kashiwa-jima Island, Sukumo Bay; Kushimoto, Kii Peninsula; Osezaki, Izu Peninsula; the Izu islands of Miyake and Hachijo; Sagami Bay; and Chichi-jima, Ogasawara Islands. The new taxon is meristically and morphologically most similar to *H. pontohi* (Tables 1, 3); however, closer examination of a type specimen employing micro-computed tomography (μCT) reveals notable internal differences from a non-type specimen of its congener. Distinguishing characters include the anterodorsal area of the trunk, where bilaterally paired wing-like protrusions are formed by a single pair of large truncate spines projecting dorsolaterad, followed by an elevated dorsal ridge formed by unusual triangular bony mounds. The elevated dorsal ridge is unknown from any other species of seahorse and serves as a key diagnostic morphological character distinguishing *H. japapigu* sp. n. from *H. pontohi*. We can therefore confirm the presence of a 7th species of pygmy seahorse that we hereby describe as the new species *Hippocampus japapigu*, so far only found in subtropical southeast Japan.

Materials and methods

Three specimens of *H. japapigu* were collected with hand nets while scuba diving in less than 15 m depth. Counts and measurements were performed on high-resolution digital images of specimens using ImageJ (Rasband et al. 1997) to the nearest 0.01 mm following

Lourie and Randall (2003) and Lourie and Kuiter (2008). External morphological characters were documented using a dissecting microscope. Live specimens of *H. japapigu* and *H. pontohi* photographed in situ were used to make morphological comparisons of external diagnostic characters only and were not collected as part of this study.

In order to document internal morphological characters, the axial skeleton was examined via non-destructive x-ray micro-computed tomography (μCT) scans at the Karel F. Liem Bioimaging Facility (Friday Harbor Laboratories, University of Washington) using a Bruker Skyscan 1173 scanner (Billerica, MA) with a 1 mm aluminum filter at 60 kV and 110 lA on a 2048 3 2048 pixel CCD at a resolution of 8.8 lm. The specimens were placed inside a 50 ml plastic Falcon tube (Corning, NY), supported by two thin foam pads to prevent movement during scanning and wrapped in ethanol (70%)-infused cheesecloth to prevent desiccation. The resulting CT data were visualized, segmented, and rendered in Horos software (www.horosproject.org).

The holotype (UW 157506) and one paratype (UW 157507) were deposited in the fish collection of the Burke Museum at the University of Washington, the second paratype (KAUM-I. 111770) was deposited at the Kagoshima University Museum (KAUM), and comparative material (one non-type specimen of *Hippocampus pontohi*, AMS I.47833-001, male) was obtained from the Australian Museum (AMS) fish collection. A segment of the mitochondrial cytochrome c oxidase subunit I (COI) DNA was sequenced from the *H. japapigu* paratype (KAUM-I. 111770). DNA extraction, PCR amplification, alignment, and analysis of COI sequence was performed following protocols described in Hamilton et al. (2017). Genetic distances (uncorrected *p*-distances) were calculated based on COI using MEGA v. 7.0.26 (Kumar et al. 2017).

Taxonomy

Hippocampus japapigu sp. n.

http://zoobank.org/F3DC73D6-E040-458E-9648-680EBAC55D20
Figures 1–5, 7–9, Video 1, Tables 1, 2

Holotype. UW 157506, Fig. 1, 16.27 mm SL, off Imasaki, Okago, Hachijo-jima Island, Izu Islands, Japan, 33°08'48"N, 139°44'37"E, depth 10 m, 18 Aug. 2017, collected by Shoichi Kato using a hand net.

Paratypes. UW 157507, Fig. 2A, 15.59 mm SL, off Imasaki, Okago, Hachijo-jima island, Izu Islands, Japan, 33°08'48"N,139°44'37"E, depth 13 m, 18 Aug. 2017, S. Kato; KAUM – I. 111770, Fig. 2B, 14.54 mm SL, Yaene, Okago, Hachijo-jima Island, Izu Islands, Japan, 33°05'47"N, 139°46'10"E, depth 18 m, 12 Jan. 2018, S. Kato.

Other material. Hachijo-jima Island, Izu Islands, Japan, July 2013, 10 to 20 m depth, two photographs of two individuals, R Smith (Figs 4, 5).

Comparative material. *Hippocampus pontohi* AMS I.47833-001, Fig. 6. Data from *H. bargibanti*, *H. denise*, *H. colemani*, *H. satomiae,* and *H. waleananus* also from Kuiter (2003), Lourie and Kuiter (2008), and Gomon and Kuiter (2009).

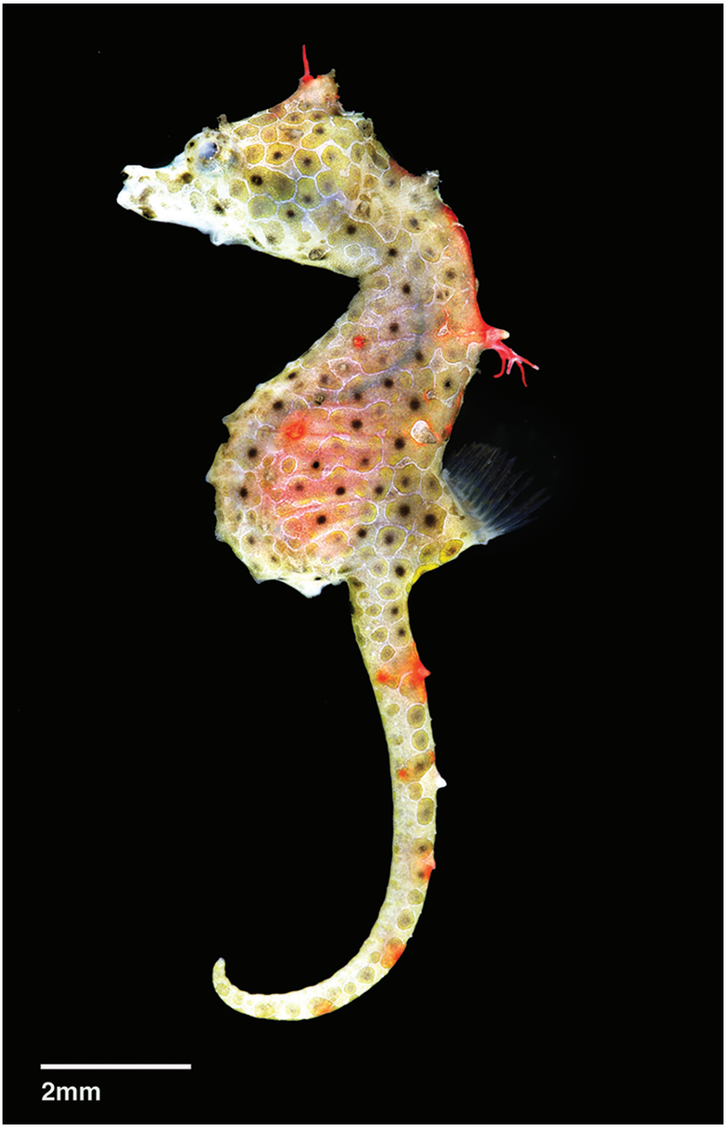


Figure 1. *Hippocampus japapigu*, UW 157506, female holotype directly after collection, 16.33 mm SL, Hachijo-jima Island, Izu Islands, Japan (photograph Hiroyuki Motomura).

Diagnosis. *Hippocampus japapigu* sp. n. differs from its congeners by the following combination of characters: tail rings 28; dorsal fin rays 14; pectoral fin rays nine; subdorsal rings four; bilaterally paired wing-like protrusions formed by a pair of large truncate spines projecting laterad on first superior trunk ridge; elevated dorsal ridge formed by unique triangular bony mounds dorsally on second, third, and fourth trunk rings with the posterior mound less pronounced; large and prominent spine projecting laterad on eighth lateral trunk ridge.

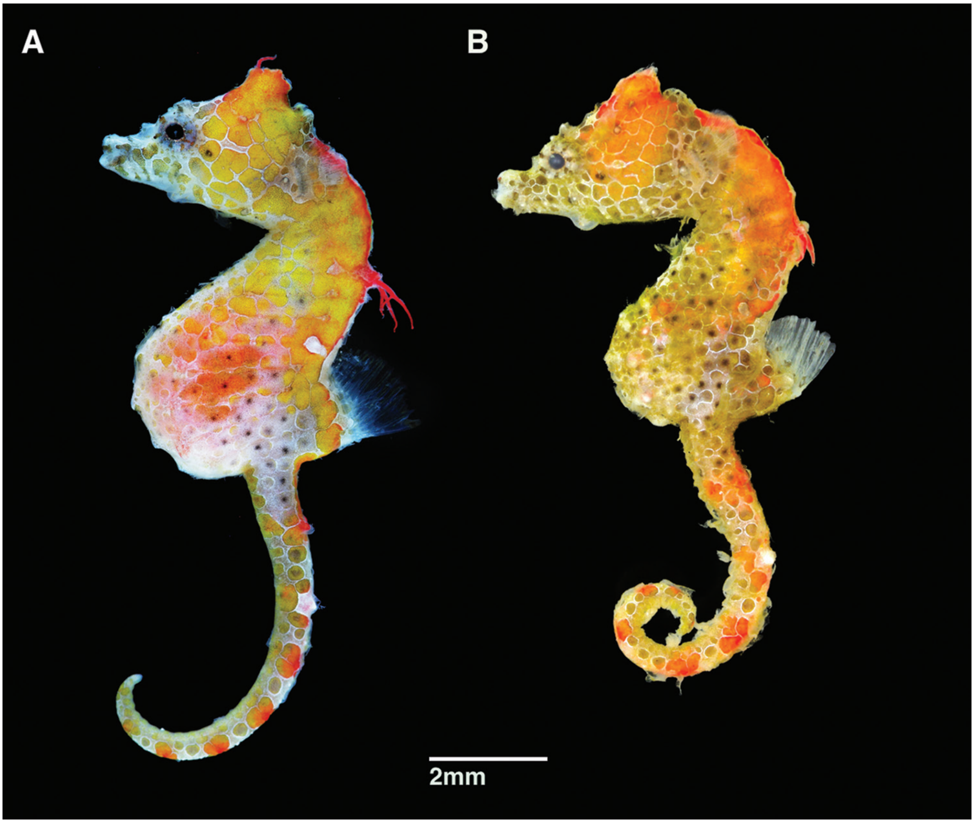


Figure 2. *Hippocampus japapigu*, paratypes directly after collection (**A**) UW 157507, male, 15.59 mm SL (**B**) KAUM-I. 111770, female, 14.54 mm SL, Hachijo-jima Island, Izu Islands, Japan (photographs Hiroyuki Motomura).

Description. General body shape as in Figs 1–5. Morphometric and meristic characters listed in Table 1. Morphometric data ranges for the three type specimens: Head length 17.9–18.74% in SL, head depth 69.9–72.0% in HL; snout length 27.64–28.7% in HL, bulbous tip absent, snout depth 74.0–85.6% in SnL; post-orbital 55.3–49.9% in HL; distinct, angular coronet, coronet height 58.0–55.9% in HL, unbranched dermal appendage attached to anterior part of coronet; single gill-opening on midline behind coronet supported by elevated cleithral ring; dorsal fin 14 rays; pectoral-fin rays nine; anal fin rays four; trunk rings 12, trunk length 32.49–32.64% in SL, trunk depth just anterior to dorsal fin base 18.4–20.32% in SL; dorsal fin base strongly raised dorsally; subdorsal rings four, dorsal fin base starting immediately posterior to ninth trunk ring and ending immediately posterior to first tail ring; no external pouch visible; tail rings 28, tail length 48.73–49.42 % in SL. Body ornamentation: prominent spine dorsal of eye, small spine ventroposterior to eye; lateral head spine ventral of coronet; two moderately large spines on cleithral ring, upper spine at level of last pectoral fin ray, lower spine at ventral extent of ring; snout spine on midline between eyes; nape spine

Figure 3. *Hippocampus japapigu*, UW 157506, preserved female holotype, 16.33 mm SL (left), and UW 157506, 15.59 mm SL, male paratype (right), Hachijo-jima Island, Izu Islands, Japan (photograph Graham Short).

absent; subdorsal spines four, superior trunk ridge ending with three rounded spines protruding laterally, the posterior spine greatly enlarged on 12th trunk ring; superior trunk ridge with large truncate spines, connected by a solid bony ridge, projecting dorsolaterad on first trunk ring, unique triangular bony mounds arched dorsally on second, third, and fourth trunk rings with the posterior mound less pronounced, trunk appearing denticulate in lateral view, very large bilaterally paired spines on fifth trunk ring, and small spines on sixth trunk ring; lateral trunk ridge with small spine on fifth trunk ring and very large spine on eighth trunk ring; inferior trunk ridge with spines of moderate size beginning on fifth trunk ring and ending on 12th trunk ring; superior tail ridge spines well developed anteriorly, becoming smaller posteriorly, with enlarged spines on fifth, ninth, 12th, and 16th tail rings; inferior tail ridge spines absent; caudal fin absent.

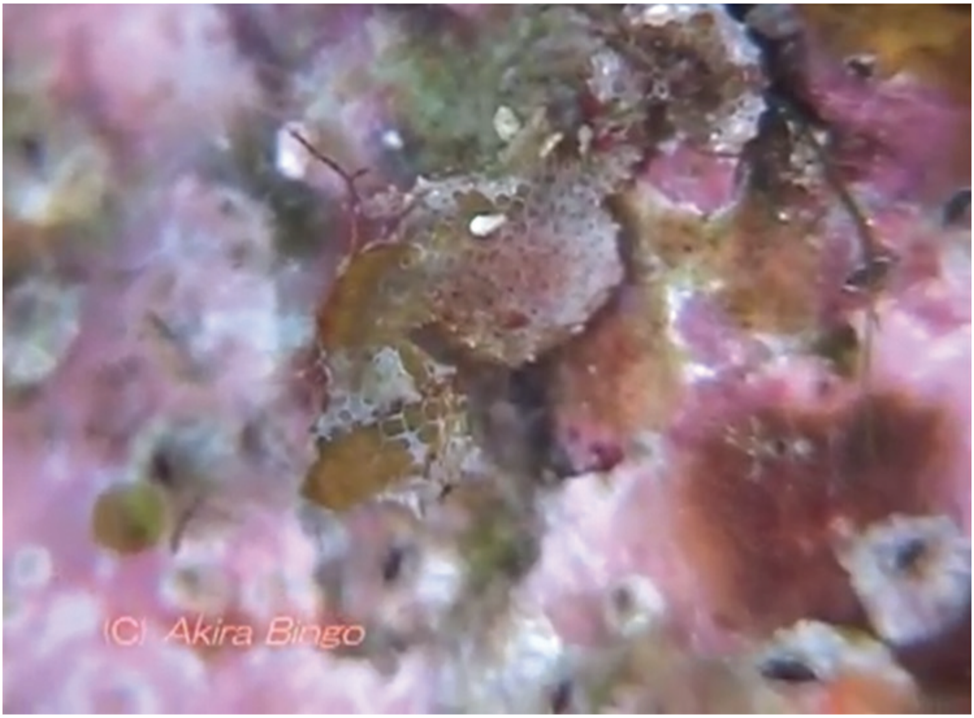
Color in life. *Hippocampus japapigu* (Figs 4, 5, and 7) exhibits cryptic coloration: head**,** trunk and tail, brown, with overlay of reticulate (net-like) irregular quadrilateral and pentagonal skin formations, brown or white, white outline, entire surface of head and body peppered with tiny black dots; elevated dorsal ridge on second to fourth

Table 1. Morphometric measurements and counts of Hippocampus pygmy seahorse species based on holotype specimens. Abbreviations: SnD (snout depth), SnL (snout length), CH (coronet height), HL (head length), HD (head depth), PO (post-orbital length), TrL (trunk length), TaL (tail length), SL (standard length). Numbers separated by a colon represent proportions. Lines present, from top to bottom, numbers for standard length (SL), proportions, and counts for trunk rings, tail rings, dorsal and pectoral fins. The first column is the species holotype.

	H. japapigu	*H. pontohi*	*H. colemani*	*H. satomiae*	*H. waleananus*	*H. bargibanti*	*H. denise*
Voucher number/ data source	UW 157506	Lourie and Kuiter 2008	Kuiter 2003	Lourie and Kuiter 2008	Gomon and Kuiter 2009	Lourie and Kuiter 2008	Lourie and Kuiter 2008
SL (mm)	16.3	16.7	26.9	13.6	17.8	24.5	15.7
SnD:SnL	74.06	84.2	70.5	86	95.2	100.3	73.6
CH:HL	58.1	47.4	45.6	40.2	48.3	57.3	42.6
HD:HL	69.9	60.6	62.6	51.8	67.9	65.7	48.1
SnL:HL	28.7	23.2	27.7	27	26.8	21.8	32.8
PO:HL	55.3	51.2	52.1	45	51.5	56.9	42.0
HL:SL	18.0	21.7	18.1	22	17.7	16.7	19.9
TrL:SL	32.6	33.3	32.0	30	31.3	27.6	27.7
TaL:SL	49.4	45	50	48	63.4	55.7	52.5
TD9:SL	18.9	13.5	19.2	13	15	12.8	9.3
Trunk rings	12	12	12	12	12	12	12
Tail rings	28	28–30	26–28	27–28	32	31–33	27–28
Dorsal fin rays	14	12	14	13	12	14	13–14
Pectoral Fin rays	9	10	9	9	9	10–11	10–11

Figure 4. *Hippocampus japapigu* in situ, Hachijo-jima Island, Izu Islands, Japan at 15 m depth (photograph Richard Smith).

Figure 5. *Hippocampus japapigu* in situ, Hachijo-jima Island, Izu Islands, Japan from 10 m depth (photograph Richard Smith).



Video 1. YouTube video of a pair of specimens of *Hippocampus japapigu* on rocky reef wall (video by Akira Bingoeral 2007).

Table 2. List of pygmy seahorse specimens, including species, collection locality, voucher number, and COI GenBank accession numbers.

	Species	Locality	Voucher	COI Genbank no.
1	*Hippocamus pontohi*	Indonesia	AM I.47833-001	MH645117
2	*Hippocamus pontohi*	Indonesia	AM I.47831-001	MH645118
3	*Hippocamus pontohi*	Indonesia	AM I.47831-001	MH645119
4	*Hippocamus pontohi*	Indonesia	AM I.47831-003	MH645120
5	*Hippocamus pontohi*	Indonesia	AM I.47831-004	MH645121
6	*Hippocamus pontohi*	Indonesia	AM I.47960-001	MH645122
7	*Hippocamus pontohi*	Indonesia	AM I.47960-002	MH645123
8	*Hippocamus pontohi*	Indonesia	AM I.47832-001	MH645124
9	*Hippocamus pontohi*	Indonesia	AM I.47834-001	MH645125
10	*Hippocamus pontohi*	Indonesia	AM I.47834-002	MH645126
11	*Hippocamus pontohi*	Indonesia	AM I.47834-003	MH645127
12	*Hippocamus pontohi*	Indonesia	AM I.47834-004	MH645128
13	*Hippocamus pontohi*	Indonesia	MZB 3597	KY066111
14	*Hippocampus severnsi*	Indonesia	AM I.47960-003	MH645129
15	*Hippocampus severnsi*	Indonesia	AM I.47960-004	MH645130
16	*Hippocampus severnsi*	Indonesia	AM I.47960-005	MH645131
17	*Hippocampus severnsi*	Indonesia	AM I.47961-001	MH645132
18	*Hippocampus severnsi*	Indonesia	AM I.47833-002	MH645133
19	*Hippocampus severnsi*	Indonesia	AM I.47834-006	MH645134
20	*Hippocampus severnsi*	Indonesia	AM I.47833-003	MH645135
21	*Hippocampus severnsi*	Indonesia	AM I.47834-005	MH645136

superior trunk rings, engorged red, reticulate color pattern diffuse or absent; dorsal fin base, red, reticulate pattern absent; tail rings with one row of rounded quadrilaterals present, one quadrilateral per ring, brown with white outline; fifth superior ridge spine red; fifth and eighth lateral trunk ridge spines, white; eighth inferior trunk ridge spine, red; fifth, ninth, 12th superior tail ridge spines, red, every fourth ring thereafter with two dorsolateral color spots, red; dermal appendages on coronet anteriorly.

Color in alcohol. Light brown in holotype, pale brown in paratype, with black dots scattered over head, trunk, and anterior to tail.

Hippocampus pontohi Lourie & Kuiter, 2008

http://zoobank.org/853548F1-CEF4-47CD-8A15-7F225B73BCFC

Figures 6–8, 10, Table 1, 2

Pontoh's Pygmy Seahorse

Hippocampus severnsi Lourie & Kuiter, 2008: figs. 2B-4B (Bunaken, North Sulawesi, Indonesia); Reijnan et al. 2011: fig. 2B (Siladen I, SE Siladen).

Material. AMS I.47833-001. 13.9 mm SL, GenBank accession number KY066111, Cape Kri, Raja Ampat, Indonesia. 0°33'23.5"S 130°41'25.0"E, depth 6 m, collected

Figure 6. *Hippocampus pontohi*, AMS I.47833-001, preserved male non-type, 13.9 mm SL, Cape Kri, Raja Ampat, Indonesia (photograph Graham Short).

by Otto Awom, Gerry Allen, and Mark Erdmann using hand net in small clump of algae and hydroids on vertical surface, 1 January 2007. Mitochondrial COI sequence data and corresponding Genbank accession numbers for additional vouchered specimens of *H. pontohi* (Table 2).

Diagnosis. *Hippocampus pontohi* differs from its congeners by the following combination of characters: subdorsal rings 4; two pairs of bilaterally wing-like protrusions formed by a pair of large truncate spines projecting laterad on both first and second superior trunk ridges; laterodorsal surface flat on the third and fourth trunk rings; tail rings 28; dorsal fin rays 12; pectoral fin rays ten.

Description. General body shape as in Figure 6. Morphometric characters listed in Table 1. Head length 21.6% in SL, head depth 64.4% in HL; snout length 24.1% in HL, bulbous tip absent, snout depth 89.0% in SnL; post-orbital 50.9% in HL; distinct, angular coronet, coronet height 46.2% in HL, unbranched dermal append-

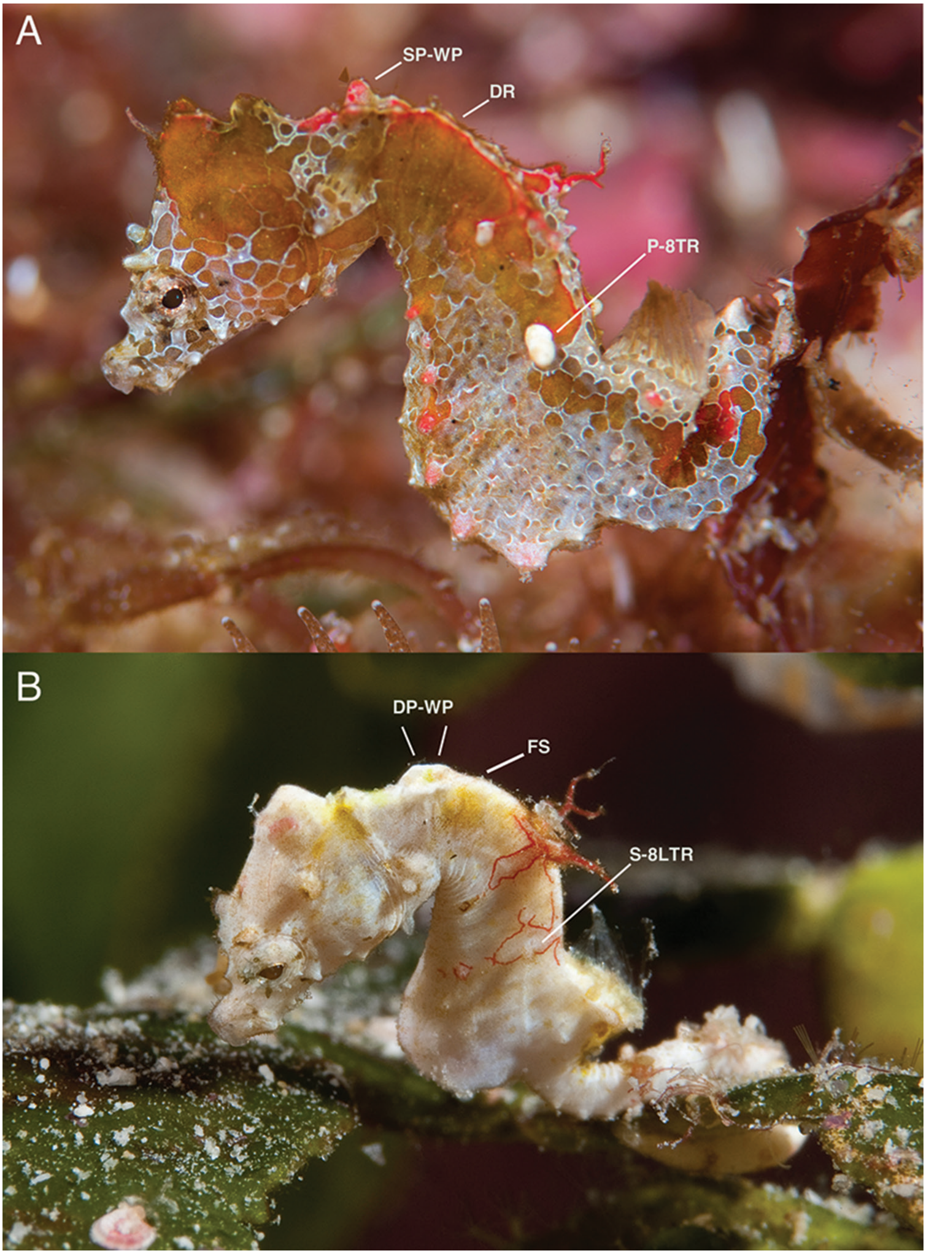


Figure 7. Comparison of live specimens of **A** *Hippocampus japapigu* photographed off Hachijo-jima Island, Japan (Richard Smith), and its most similar congener **B** *Hippocampus pontohi* photographed off Tomia Island, southeast Sulawesi, Indonesia (Richard Smith). Note the differences in the anterodorsal area of the trunk in *H. japapigu* vs. *H. pontohi*: single vs. double pair of bilaterally paired wing-like protrusions behind the head, raised dorsal ridge vs. laterodorsal flat surface, and large and prominent vs. small eighth lateral trunk ridge spine. Abbreviations: SP-WP, single pair of bilaterally paired wing-like protrusions; DP-WP, double pair of bilaterally paired wing-like protrusions; DR, raised dorsal ridge; FS, flat dorsal surface; P-8LTR, prominent eighth lateral trunk ridge spine; S-8LTR, small eighth lateral trunk ridge spine.

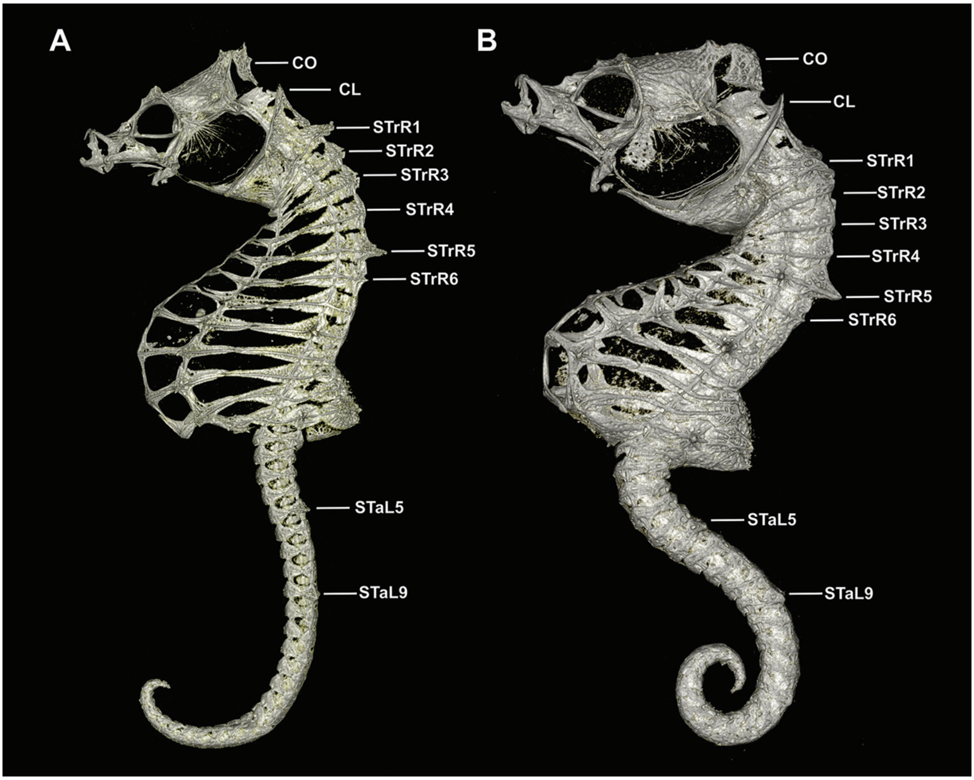


Figure 8. Comparison of the lateral view of reconstructed µCT scans of skeletons of A *Hippocampus japapigu*, UW 157506, preserved male holotype, 16.33 mm SL, Hachijo-jima Island, Japan, and its most similar congener B *Hippocampus pontohi*, AMS I.47833-001, preserved male non-type 13.9 mm SL, Cape Kri, Raja Ampat, Indonesia (photographs Graham Short).

age attached to anterior part of coronet; single gill-opening on midline behind coronet supported by elevated cleithral ring; dorsal fin 14 rays; pectoral-fin rays nine; anal fin rays four; trunk rings 12, trunk length 33.0% in SL, trunk depth just anterior to dorsal fin base 14.4% in SL; dorsal fin base strongly raised posterodorsad; subdorsal rings four, dorsal fin base starting immediately posterior to ninth trunk ring and ending immediately posterior to first tail ring; no external pouch visible; tail rings 28, tail length 45.3% in SL. Body ornamentation: prominent spine dorsal of eye, small spine ventroposterior to eye; lateral head spine ventral of coronet; two moderately large spines on cleithral ring, upper spine at level of last pectoral fin ray, lower spine at ventral extent of ring; snout spine on midline between eyes; nape spine absent; subdorsal spines four, superior trunk ridge ending with three rounded spines protruding laterad, the posterior spine greatly enlarged on 12th trunk ring; superior trunk ridge with large bilaterally paired truncate spines projecting laterad on first and second trunk rings, laterodorsal surface flat on second, third, and fourth trunk rings, large bilaterally paired spines on fifth trunk ring, and small pair of spines dorsally on sixth trunk ridge; lateral

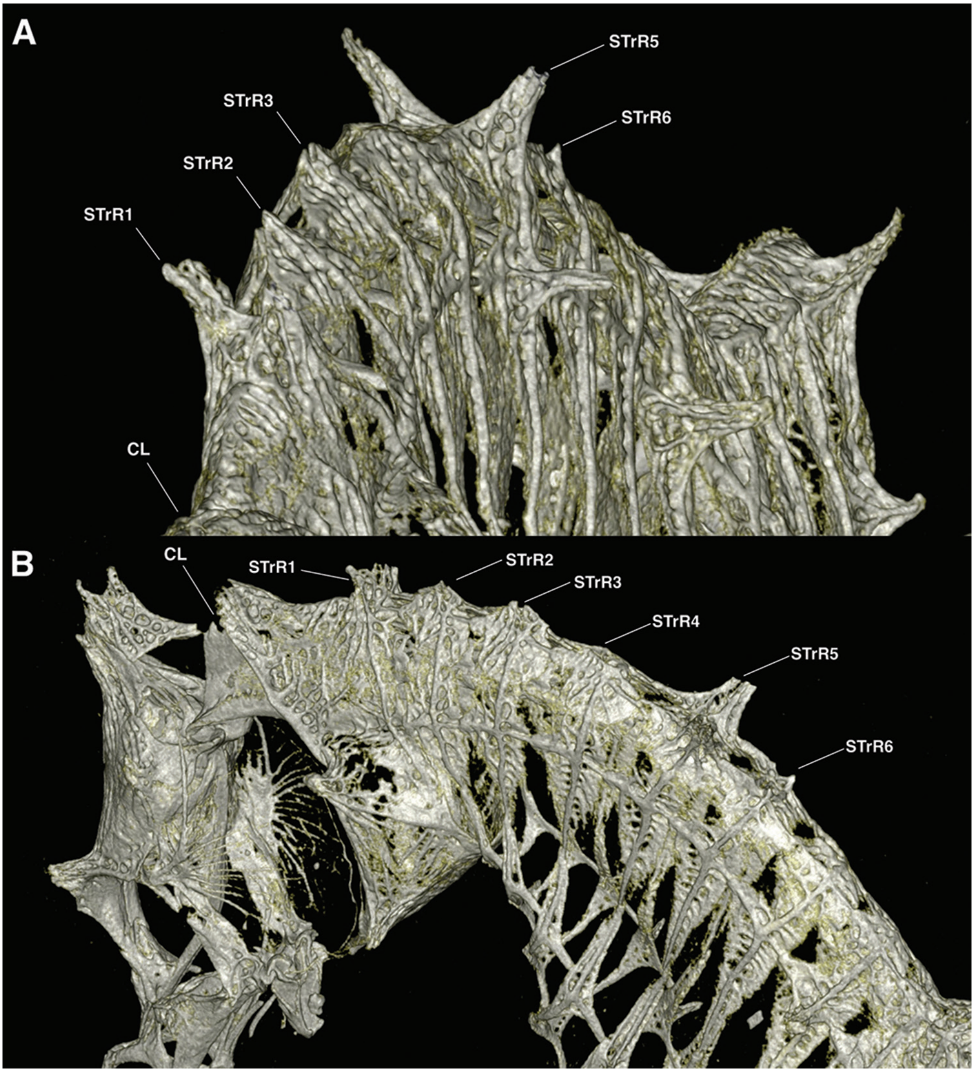


Figure 9. Computed tomography scanned anterior trunk area of *Hippocampus japapigu*, UW 157506, male holotype, 16.33 mm SL, Hachijo-jima Island, Japan (photograph Graham Short). **A** Anterolateral view **B** Lateral view. Note the pair of spines projecting dorsolaterad on STrR1 and triangular bony mounds arched dorsad on STrR2, STrR3, and STrR4. Abbreviations: CL, cliethral ring; STrR1, first superior trunk ridge; STrR2, second superior trunk ridge; STrR3, third superior trunk ridge; STrR4, fourth superior trunk ridge; STrR5, fifth superior trunk ridge; STrR6, sixth superior trunk ridge.

trunk ridge with small spine on fifth trunk ring and large spine on eighth trunk ring; inferior trunk ridge with spines of moderate size beginning on fifth trunk ring and and ending on 11th trunk ring; superior tail ridge spines well developed anteriorly, becoming smaller posteriorly, with enlarged spines on fifth and ninth tail rings; inferior tail ridge spines absent; caudal fin absent.

Remarks. Although *Hippocampus pontohi* was distinguished from *H. severnsi* primarily by features of coloration (Lourie and Kuiter 2008), meristic, morphometric, and diagnostic morphological characters in the original description did not support the separation of these seahorses into two species. The invalidity of *H. severnsi* was subsequently recognised due to the unreliability of employing coloration as a useful diagnostic character in order to distinguish between species of seahorses (Lourie et al. 2016). Here we further support the synonymization of *H. severnsi* under *H. pontohi* based on partial mitochondrial COI genetic data collected from additional 21 vouchered specimens of *H. pontohi* and those referred to as *H. severnsi* (Table 2). Genetic distance analysis (uncorrected p distances) failed to discriminate *H. severnsi* from *H. pontohi* (Suppl. material 1), which revealed an average intraspecific divergence of 0.2%. A neighbour joining tree of the COI sequence data, including COI data from *H. bargibanti*, *H. denise*, and *H. japapigu*, is supplied here as Suppl. material 2.

Comparative remarks. The combination of characters provided in the diagnosis that distinguishes *H. japapigu* from all congeners are presented in Table 3 and summarized below. The new species is unique in *Hippocampus* in possessing a distinct elevated dorsal ridge internally formed by triangular bony mounds in the anterodorsal area of the trunk directly posterior to the head, which we propose as an apomorphy for this species. All currently recognized seahorse species, including the pygmy seahorse congeners, differ in the absence of triangular bony mounds and the presence of typical flat surfaces dorsally on the second to fourth superior trunk rings. *Hippocampus japapigu* is most similar to *H. pontohi* (Fig. 8, Table 3) in meristics, overall body ornamentation, and the presence of a distinct coronet. They differ primarily on the basis of bilaterally paired wing-like protrusions directly posterior to the head, which are internally formed by a single connected pair of large, truncate spines projecting dorsolaterad on the first superior trunk ridge in *H. japapigu*, as opposed to a double pair of large truncate spines projecting strongly laterad on both the first and second superior trunk ridges in *H. pontohi*. Additional distinctions include patterns of the anterodorsal trunk rings (elevated dorsal ridge formed by triangular bony mounds dorsad on the second to fourth trunk rings in *H. japapigu*, laterodorsal surface flat on the third and fourth trunk rings in *H. pontohi*); eighth lateral trunk ridge spine (very large and prominent spine projecting laterad in *H. japapigu*, small in *H. pontohi*); color pattern (brown with white reticulation, thin red line tracing the superior trunk ridge anterior to dorsal fin base in *H. japapigu*, white, brown, or black color with elliptical markings, each outlined with thin red lines, tracing the entire superior trunk ridge and extending ventrally around the fifth superior and eighth lateral trunk ridge spines in *H. pontohi*). *Hippocampus japapigu* and *H. pontohi* can be further be distinguished by the number of tail rings (28 vs. 28–30), dorsal fin rays (14 vs. 12), and pectoral fin rays (9 vs. 10).

Several other pygmy seahorse species are morphologically similar to *Hippocampus japapigu*, including *H. colemani*, *H. satomiae*, and *H. waleananus*. The following characters support the distinctions among these species: number of tail rings (28 in *H. japapigu* vs. 26 in *H. colemani*, 27–28 in *H. satomiae*, 32 in *H. waleananus*); dorsal fin rays (14 in *H. japapigu* vs. 13 in *H. satomiae,* 12 in *H. waleananus*); coronet (distinct in

Table 3. Comparison of morphological characters in *Hippocampus japapigu*, *H. pontohi*, *H. colemani*, *H. satomiae*, and *H. waleananus*.

	H. japapigu	***H. pontohi***	***H. colemani***	***H. satomiae***	***H. waleananus***
Voucher number/data source	WA 41200	Lourie and Kuiter 2008	Kuiter 2003	Lourie and Kuiter 2008	Gomon and Kuiter 2009
Single gill opening	present	present	present	present	present
Strongly raised cleithral girdle	present	present	present	present	present
Coronet	distinct and angular	distinct and angular	low and rounded	distinct and angular	low double mound
Cleithral spines	pectoral fin base, ventral	pectoral fin base, ventral	pectoral fin base, ventral	pectoral fin base, ventral	pectoral fin base
Subdorsal rings (3+1)	present	present	present	present	present
Lateral head spine	present	present	present	present	present
Snout spine	present	present	present	present	present
Eye spine dorsal	present	present	present	present (double)	present
Eye spine ventral	present	present	present	absent	present
first superior trunk ridge spines	present	present	present	present	present
second superior trunk ridge spines	absent	present	present	present	†present
Elevated ridge dorsal of trunk	present	absent	absent	absent	absent
fifth superior trunk ridge spines	present	present	present	present	present
fifth lateral trunk ridge spines	present	present	present	present	present
eighth lateral trunk ridge spines (large)	present	present	present	present	present
eighth inferior trunk ridge spines	present	present	present	present	present
12th superior trunk ridge subdorsal spine (large)	present	present	present	present	present
Superior tail ridge spines	5,9,12,16	5,9,12	absent	5,9,12	4,8,12
Inferior tail ridge spines	absent	absent	absent	absent	posterior 28 rings

H. japapigu vs. low and rounded in *H. colemani,* low double mound in *H. waleananus);* cleithral ring spines (at pectoral fin base and ventral of head in *H. japapigu* vs. pectoral fin base in *H. waleananus)*; eye spine dorsally (double spine in *H. satomiae*), eye spine ventrally (absent in *H. satomiae*); superior tail ridge spines (fifth, ninth, 12th vs. absent in *H. colemani*, fourth, eighth, 12th in *H. waleananus*); inferior tail ridge spines (absent vs. present on last 28 tail rings in *H. waleananus*).

Hippocampus japapigu shares with *H. pontohi*, *H. colemani, H. satomiae,* and *H. waleananus* 12 trunk rings, strongly raised continuous cleithral ring, and presence of diagnostic body ornamentation (snout spine, eye spines, two cliethral spines, lateral head spine, large spine on fifth superior trunk ridge, large spine on eighth lateral trunk ridge, small spine on fifth lateral trunk ridge, Table 2), including wing-like-protrusions imme-

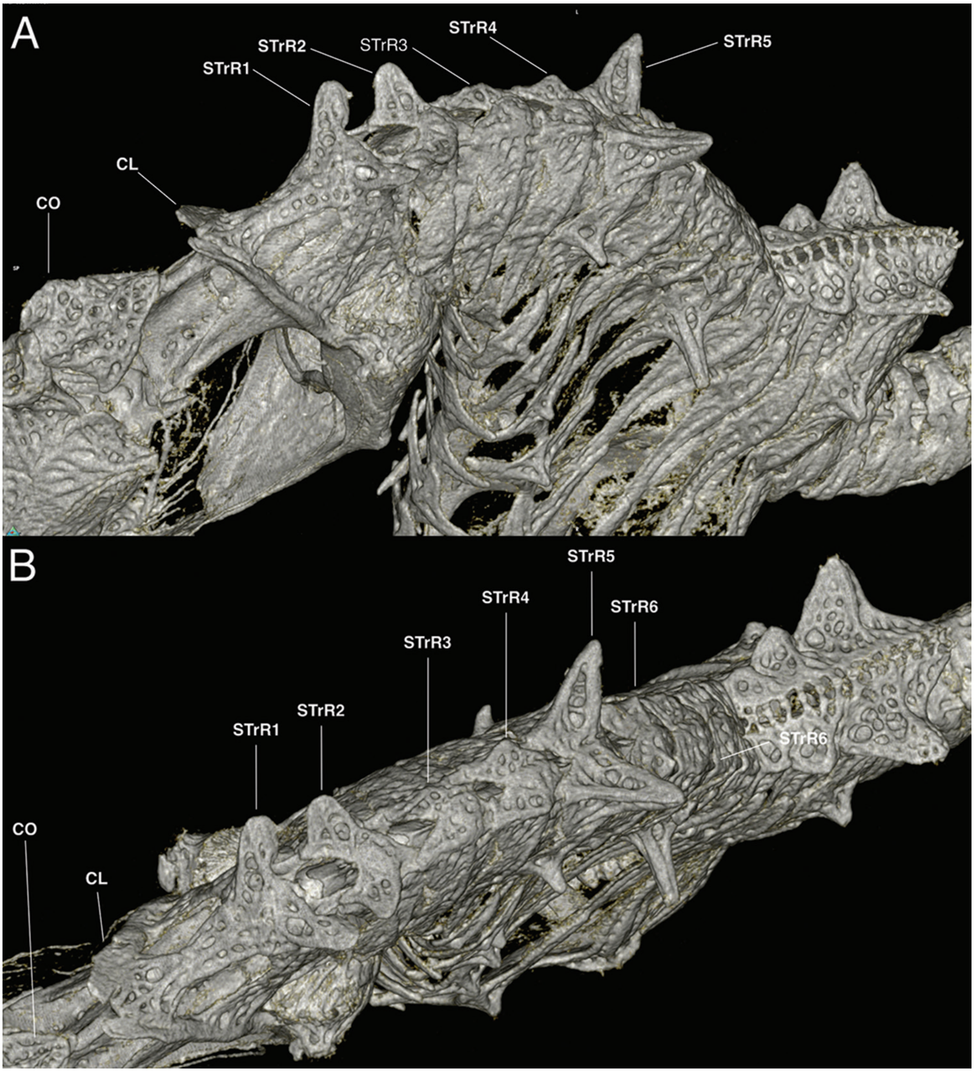


Figure 10. Computed tomography scanned anterior trunk area of *Hippocampus pontohi*, AMS I.47833-001, preserved male non-type, 16.33 mm SL, Cape Kri, Raja Ampat, Indonesia (photograph Graham Short). **A** Anterolateral view **B** Dorsal view. Note the double pair of spines projecting dorsolaterad on STrR1 and STrR1, respectively, and laterodorsal surface flat on STrR3, and STrR4. Abbreviations: CO, coronet; CL, cliethral ring; STrR1, first superior trunk ridge; STrR2, second superior trunk ridge; STrR3, third superior trunk ridge; STrR4, fourth superior trunk ridge; STrR5, fifth superior trunk ridge; STrR6, sixth superior trunk ridge.

diately posterior to the head. Based on careful visual examinations of *in situ* underwater photographs, x-rays, and type material (Kuiter 2003; Lourie and Kuiter 2008; Gomon and Kuiter 2009; Smith 2017), it appears *H. japapigu* shares with *H. waleananus* a single pair of bilaterally paired wing-like protrusions (vs. double pair in *H. colemani* and *H. satomiae)*. In the original description of *H. colemani* (Kuiter, 2003), the number of trunk rings was diagnosed as 11, however in our comparative analysis of trunk ring counts, we detected the presence of 12 trunk rings in the x-ray of the holotype of *H. colemani* (Gomon & Kuiter, 2009, AMS I.41181-001, fig. 3B). Furthermore, we noted 4 subdorsal rings (three trunk and one tail rings) via μCT in *H. japapigu* and the non-type *H. pontohi*, and similarly four subdorsal rings in our examination of the radiographs of *H. colemani*, *H. pontohi,* and *H. satomiae* (Kuiter 2003, fig. 3B; Lourie and Kuiter 2008, figs 2A,C; Gomon and Kuiter 2009, fig. 3B). In contrast, three subdorsal trunk rings were noted in the original diagnoses of these pygmy seahorses. *Hippocampus japapigu* and *H. pontohi* retain the ring and ridge structure of larger seahorses, and with μCT scans, we can detect well-developed ossification of the skeleton, including the strong ossification of the inferior and ventral trunk area (Figs 8–10). Similarly, Gomon & Kuiter (2009, fig. 3B) detected a well-formed skeleton in *H. colemani* via x-ray. In contrast, *H. bargibanti* and *H. denise* reveal incomplete ossification of the inferior and ventral trunk ridges anteriorly, the ridges reduced to star-shaped dermal ossifications (Gomon 1997; Lourie and Randall 2003; Gomon and Kuiter 2009).

Genetic comparisons. Suppl. material 1 shows genetic distance analysis at the COI gene (uncorrected p distances) between *H. japapigu* and previously sequenced non-type specimens of the pygmy seahorses *H. pontohi*, *H. bargibanti*, and *H. denise* (Hamilton et al. 2017), and additional vouchered specimens of *H. pontohi*. *Hippocampus japapigu* differs from *H. pontohi* by 10.1%, from *H. bargibanti* by 13.0%, and *H. denise* by 10.1%. Reported mtDNA clock rates of approximately 1.2% per million years in marine teleosts (Reece et al. 2010) indicate divergence between *H. japapigu* and *H. pontohi* approximately 8.4 million years ago.

Distribution and habitat. *Hippocampus japapigu* sp. n. is only known to occur in Japan, from scattered localities including Kashiwa-jima Island, Sukumo Bay; Kushimoto, Kii Peninsula; Osezaki, Izu Peninsula; the Izu Islands of Miyake and Hachijo; Sagami Bay; and Chichi-jima, Ogasawara Islands. The specimens described herein were found off the northwest coast of Hachijo-jima Island at a depth of 10–13 m, and have been anecdotally reported elsewhere at 5–22 m by local divers. Owing to its diminutive size and extraordinary crypsis, this species may have a wider distribution within Japan. The new taxon is not associated with a particular host, and has been observed in association with mixed soft coral, the coralline algae *Halimeda* sp., and hydroids on rocky reef walls and large boulders in both exposed and semi-sheltered locations. During 15 dives initially spent searching ad hoc for this species by the second author in July 2013, 13 individuals were observed in an approximately 100 m stretch of rocky reef. These ranged in depth from 10 to 20 m and water temperature fluctuated between 19–24°C over 6 days. When one individual was discovered, another was often found in close proximity and appeared to represent male-female pairs. Returning in June 2015 with a larger group of experienced dive guides, with 10 dives searching for the species, only a single individual was found, possibly suggesting fluctuations in the abundance of the species. Several pregnant males were observed in July 2013, but it is unknown whether reproduction occurs seasonally or year-round.

Etymology. The specific epithet is from the colloquial Japanese name of the new species, Japan Pig, Japapigu, or 日本のピグミータツノオトシゴ.

Common name. New common English and Japanese names, Japanese Pygmy Seahorse and Hachijo-tatsu, respectively, are proposed here for *Hippocampus japapigu.*

Discussion

Here we consider *Hippocampus japapigu* as a valid species due to its morphological uniqueness; however, a more detailed phylogenetic and systematic study is necessary to understand its evolutionary relationship to other pygmy seahorses. Using µCT, we have identified key diagnostic characters in the anterodorsal area of the trunk that differentiate *H. japapigu* from the morphologically similar *H. pontohi*. Unequivocally, the most noticeable skeletal characters of *H. japapigu* are the unusual triangular bony mounds that serve as a structural basis for the elevated dorsal ridge along the trunk, and the presence of a single pair of large truncate and connected spines projecting dorsolaterally of the trunk that form the bilateral wing-like protrusions behind the head. In the previous diagnoses of *H. colemani*, *H. pontohi*, *H. satomiae* and *H. waleananus*, these dorsolateral truncate spines were difficult to discern via traditional photography and radiographs (Kuiter 2003; Lourie and Kuiter 2008; Gomon and Kuiter 2009). In *H. colemani* (Kuiter 2003) these spines were not noted; in *H. pontohi* (Lourie and Kuiter 2008, fig. 2A) these spines were described as dorsolateral expansions of the first and second superior trunk rings with no mention of the presence of spines; in *H. satomiae* (Lourie and Kuiter 2008, fig. 2C) these spines were diagnosed as fused spines on the first and second superior trunk ridges, however, in the radiograph provided in the description they appear to be two pairs of spines, and separated; in *H. waleananus* (Gomon and Kuiter 2009, fig. 3A) these spines were described as wing-like protuberances on the second superior trunk ridge with no diagnosis of spines. In the latter, we detect a pair of large spines on the first superior trunk ridge (Gomon and Kuiter 2009, fig. 3A). Additional characters that were not detected via microscopy or high-resolution photography include a small pair of spines dorsally on the sixth superior trunk ridge in both *H. japapigu* and *H. pontohi* (Figs 8–10). Therefore, µCT offers new avenues for enhancing taxonomic descriptions by documenting otherwise difficult or indiscernible diagnostic skeletal features in small specimens.

Hippocampus japapigu is known to occur throughout subtropical southeast Japan where investigations of inshore marine ichthyofauna (Senou et al. 2006) have recorded similar species compositions between southern Honshu and the Izu and Ogasawara Islands, suggesting a passive and long distance recruitment and dispersal due to the influence of the Kuroshio Current (Kuriiwa et al. 2014). This north-flowing ocean current, which originates east of the Philippine coast, flows alongside Taiwan to the south coast of the major islands of Japan, including Honshu, and extends northward and southward between the Izu and Ogasawara Islands, is likely to act as an important conduit to transport tropical fishes from the south to these islands. *Hippocampus japapigu* has not been observed in the Ryukyu Islands to date despite these islands being located in the Kuroshio Current. Given time, scuba divers may observe the new species in the Ryukyu Islands, and even as far south as Taiwan where *H. bargibanti* (Kwang-Tsao et al. 2008), *H. colemani,* and a species appearing to be *H. pontohi* have been observed by local scuba divers.

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Supplementary material 1

Genetic distance analysis (uncorrected p distances) of COI sequence data from 21 specimens of *H. pontohi* and those referred to *H. severnsi*

Authors: Graham Short, Richard Smith, Hiroyuki Motomura, David Harasti, Healy Hamilton

Data type: molecular data

Link: https://doi.org/10.3897/zookeys.779.24799.suppl1

Supplementary material 2

NJ tree of COI sequences from 21 specimens of *H. pontohi* and those referred to *H. severnsi*

Authors: Graham Short, Richard Smith, Hiroyuki Motomura, David Harasti, Healy Hamilton

Data type: phylogenetic tree

Link: https://doi.org/10.3897/zookeys.779.24799.suppl2

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Tapping into technology and the biodiversity informatics revolution: updated terrestrial mammal list of Angola, with new records from the Okavango Basin

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Abstract

Using various sources, including the Global Biodiversity Information Facility (GBIF), published literature, recent (2015–2017) collections, as well as bat detector and camera trap surveys with opportunistic sightings and live capture in the upper Okavango catchment in central Angola, we present an updated mammal checklist of 275 species from 15 different orders for Angola (including the Cabinda region). Recent surveys (captures and bat detectors) of small mammals from the upper Okavango catchment yielded 46 species (33 species of bats, ten species of rodents and three species of shrews). One bat (*Pipistrellus rusticus*, rusty pipistrelle); two rodents (*Mus setzeri*, Setzer's mouse and *Zelotomys woosnami*, Woosnam's broad-faced mouse) and one shrew (*Suncus varilla*, lesser dwarf shrew) were captured for the first time, in Angola. While our species lists of bats conformed to predicted totals, terrestrial small mammals were under sampled, with only 13 species recorded by our trapping survey compared to a total of 42 shrew and rodent species expected based on GBIF records for the central Angolan highlands. Seven terrestrial small mammal species (one shrew and six rodents) are endemic to the central and western Angolan highlands but none of these were captured in our survey. The bat detector surveys added three further bat species to the country list: *Pipistrellus hesperidus*, *Kerivoula argentata*, and *Mops midas*. Camera trap surveys and opportunistic sightings in the upper Okavango catchment in 2016 yielded a total of 35 species of medium-large mammals, from 17 families, although all of these had been reported previously in Angola. GBIF proved to be an excellent source of biodiversity data for Angolan mammals, most importantly for documenting dramatic historical range changes of larger mammals such as the sable (*Hippotragus niger niger*), Kirk's sable (*H. niger kirkii*) and the giant sable (*H. niger variani*).

Keywords

Angola, checklist, Global Biodiversity Information Facility, mammals, Okavango Basin, scientific collections

Introduction

Country species checklists and distribution maps for key taxa such as mammals represent a critical step in national efforts towards reaching international (e.g., the Convention on Biological Diversity) and national biodiversity targets and planning for conservation management and sustainable development at regional and local levels. Rapid advances in biodiversity informatics leading to huge volumes of reliable historical and recent occurrence data through public portals such as the Global Biodiversity Information Facility (https://www.gbif.org) make it possible to conduct taxonomic and conservation biodiversity assessments and compile reliable annotated species lists even for poorly known countries and regions (Soberón and Peterson 2004; Beaman and Cellinese 2012; Coetzer 2012; Wieczorek et al. 2012).

At the same time, advances in technology such as camera traps and microphones (including bat detectors) and associated analytical tools are facilitating rapid and efficient field inventories of groups such as larger mammals, bats, birds, crickets, and amphibians. In many cases, acoustic systems have been developed for automated species classification of huge volumes of call data. In the case of bats, echolocation calls are not songs, making the identification to species from bat calls a challenging exercise that requires suitable cross-testing of results using reliably identified calls, e.g., from captured and released individuals (Barclay 1999; Taylor et al. 2013; Monadjem et al. 2017; Rydell et al. 2017). Similarly, camera traps have enabled efficient and comprehensive surveys of medium and large-sized mammals and other groups (Stein et al. 2008; Tobler et al. 2008; Rovero and Marshall 2009; Rovero et al. 2014).

The objectives of this study were firstly to consolidate available data to compile an updated species checklist of terrestrial mammals of Angola, and secondly to add to this list the results of recent surveys of mammals in the poorly surveyed Okavango catchment area of the central highlands of Angola, using both live capture and remote camera trap and acoustic techniques.

Brief historical overview of mthe south-western quarter of Angola in the American Museum of Natural History from the Vernay-Lang (conducted in 1925) and Phipps-Bradley (in 1932) expeditions, Hill and Carter (1941) listed a total of 223 species of mammals for Angola, including 13 shrews (Soricomorpha), one hedgehog (Erinaceomorpha), one golden mole and one otter shrew (Afrosoricida), two elephant shrews (Macroscelidea), one aardvark (Tubulidentata), two pangolins (Pholidota), 53 bats (Chiroptera), 10 primates (Primates), two hares (Lagomorpha), 66 rodents (Rodentia), 36 carnivores, one elephant (Proboscidea), two hyraxes (Hyracoidea) and 32 ungulates (four Perissodactyla and 28 Artiodactyla). Subsequent to this publication, Angola has been largely neglected in terms of mammal survey effort. For example, those who led the Smithsonian Institution's ambitious African Mammal Project (1961–1972), which collected 63,213 voucher specimens from throughout Africa and led to the definitive "Mammals of Africa: An Identification Guide" (Meester and Setzer 1971), did not visit Angola at all (Schmidt et al. 2008). Crawford-Cabral and co-authors compiled a database of just under 10,000 records (hosted by the University of Lisbon, Instituto de Investigação Científica Tropical in Portugal) mainly from 1930–1980 of 140 species and subspecies of carnivores, ungulates, and rodents collected from Angola (Crawford-Cabral 1998; Crawford-Cabral and Simões 1987, 1988; Crawford-Cabral and Veríssimo 2005).

The Lubango Museum, originally housed by Instituto de Investigação Científica de Angola (IICA) and currently housed at the the Instituto Superior da Ciências e Educação (ISCED) comprises about 4,000 mammal specimens of at least 123 species from Angola (https://www.gbif.org/publisher/975daf99-f28c-4201-86f2-2bfce0cba085). Another important museum in Angolan history is that established in Dundo. This was a relatively well-stocked museum and was in an important location (in the far northeast) for tropical species. There are at least two important papers by A Monard in 1931 and 1935 (cited in Hayman 1963) that detail the bat species in that collection (as well as other mammals). The small mammals in this collection were reviewed by Hayman (1963) and he mentioned 602 specimens that he examined (apparently these were shipped to him in London) belonging to 91 species and subspecies (including some 14 not previously reported for Angola).

It is little appreciated that Angola was actually relatively well known compared with East Africa until the turn of the 20th century. Early explorers and scientists such as Bocage made enormous contributions. Bocage described 25 Angolan taxa based on new collections between 1878 and 1890, and seven Angolan taxa were named after him by other scientists (Hill and Carter 1941). Of the taxa described by Bocage, although most have been relegated to synonyms or subspecies in current lists, at least eight currently recognized Angolan mammals were named by him: the Angolan fruit bats *Epomophorus angolensis* and *Epomops dobsoni*, the murid rodents *Myomyscus*

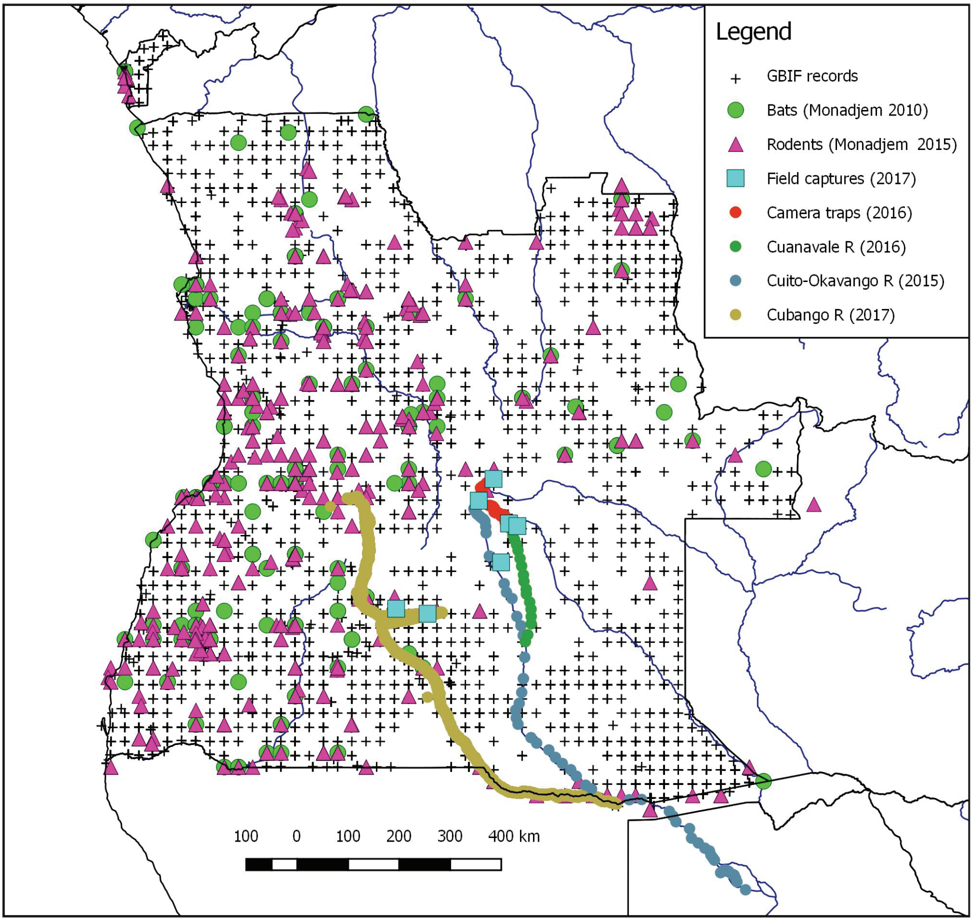

Figure 1. Map of Angola showing spatial occurrence of mammal records obtained from various sources, including the present study which reported on captures of small mammals and acoustic recordings of the echolocation calls of bats. Although the Cuito-Okavango River trip of 2015 extended beyond Angola into Namibia and Botswana, there were no species identified from acoustic data in Namibia and Botswana that were not also detected in Angola.

angolensis and *Otomys anchietae*, the squirrel *Funisciurus bayoni*, the gerbil *Gerbilliscus validus*, the genet *Genetta angolensis* and the mongoose *Herpestes flavescens*.

Notwithstanding changes in taxonomy and the relative lack of survey effort, the list of species known to occur in Angola has increased, particularly in the case of small mammals. A relatively recent synthesis of Angolan murid rodents was published by Crawford-Cabral (1998) after an older synthesis of bats (Crawford-Cabral 1986). More recently, biogeographical and taxonomic syntheses of African bats (Monadjem et al. 2010a) and rodents (Monadjem et al. 2015) have listed 60 bat species and 78 rodent species from Angola, representing species richness increases of 13% and 18%, respectively, in comparison to Hill and Carter (1941).

Materials and methods

Updating of mammal list for Angola

We combined records from the literature (Hill and Carter 1941; Hayman 1963; Crawford-Cabral 1986, 1998; Crawford-Cabral and Simões 1987, 1988; Crawford-Cabral and Veríssimo 2005; Monadjem et al. 2010a, 2015) with records obtained from a search of the GBIF portal (www.gbif.org) conducted on 18 December 2017 (GBIF.org 2017), which yielded 14,275 records based on 31 databases (Suppl. material 1). The two main institutions contributing data were the Instituto de Investigação Científica Tropical in Portugal (8,977 records) which incorporates the works of Crawford-Cabral (1998); Crawford-Cabral and Simões (1987, 1988) and Crawford-Cabral and Veríssimo (2005), and the American Museum of Natural History (2,240 records), which incorporates the survey of Hill and Carter (1941). Other important contributors include the Field Museum of Natural History (1,223 records) and The Natural History Museum in London (895 records). Together, these four databases comprised 93% of all records. We added records from the 2016 collection of 68 small mammals (bats, rodents and shrews) from the Okavango catchment of Angola deposited in the Durban Natural Science Museum (DNSM). The DNSM mammal collection also yielded an additional 14 records of Angolan rodents collected by S. Eiseb and J. Jarvis. Finally, we also added records of bat species determined by acoustic bat detector and capture surveys, shrews and rodents by live trapping methods and medium to large mammals recorded by camera traps supplemented with verifiable opportunistic sightings or signs.

To compile an updated species list based on the above sources, we adopted the taxonomy of Wilson and Reeder (2005) or more recent taxonomic treatments for certain groups, e.g., Monadjem et al. (2010a) and the 2016 African Chiroptera Report for bats (ACR 2016), Monadjem et al. (2015) and Denys et al. (2017) for rodents, and species accounts from all volumes of the series on Mammals of Africa (Kingdon et al. 2013a). We also tested the current taxonomic validity of each name using the Interagency Taxonomic Information System (www.itis.org) and the Mammal Diversity Database of the American Society of Mammalogists (Mammal Diversity Database 2018 https://mammaldiversity.org). Species of dubious occurrence were defined as those having only a single record and/or collector, no clear basis for identification, occurring well outside their known range, and not included in authoritative texts for Angola (Hill and Carter 1942; Hayman 1963; Crawford-Cabral 1986; Crawford-Cabral 1998; Crawford-Cabral and Simões 1987; 1988; Crawford-Cabral and Veríssimo 2005; Monadjem et al. 2010a, 2015). Dubious species were flagged as such, including the reason for their exclusion. As mentioned above, bat species records based only on acoustic data were also added to this list.

Sampling of upper Okavango catchment region

Sampling of small mammals from the highlands of Angola using both capture and acoustic techniques was carried out in 2013 (acoustic only), 2015 (acoustic only), 2016 (capture and acoustic) and 2017 (acoustic only).

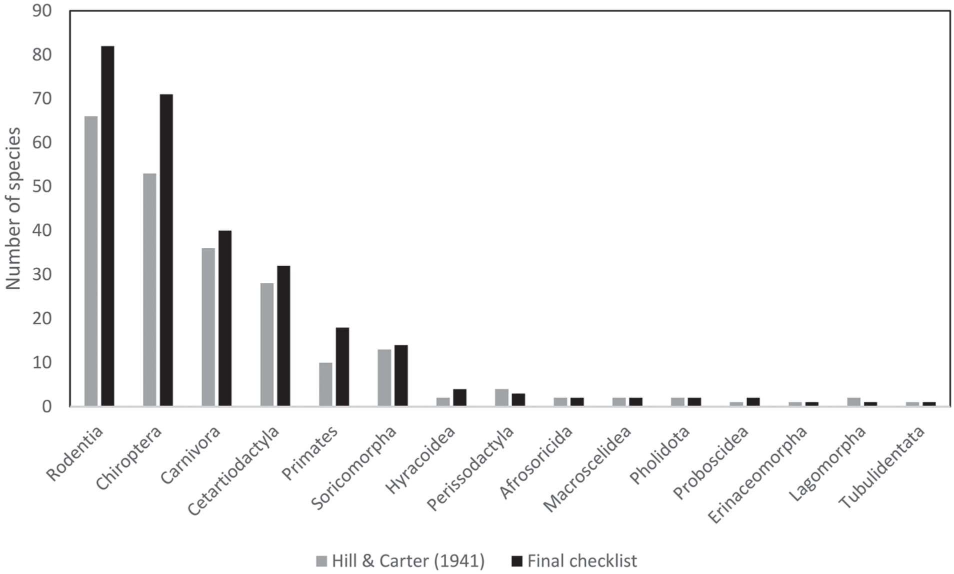

Figure 2. Histogram summarising species number of Angolan terrestrial mammals per order based on Hill and Carter (1943) and the current study.

Capture survey. Between 16 and 22 March 2016, and 29 October and 4 November 2016, bats were captured using one to three mist nets (Ecotone; 6 m, 9 m and 12 m) set per night, a two-bank harp trap ("Austharp", Faunatech), and via searches for roosting bats, e.g., under the loose bark of trees. During the two 2016 field periods, as well as opportunistically on other occasions, shrews and rodents were captured using standard Sherman live-traps, usually 25–30 per night, set 5–10 m apart in a line. Some shrews were captured in the herpetofauna drift fence arrays. Two bats were collected opportunistically in 2013 and 2016 by W. Conradie.

Acoustic recordings of bats. Using four different bat detectors, we obtained recordings for a total of 208 detector-nights between 2015 and 2017. Apart from monitoring of several point localities, regular (mostly nightly) acoustic monitoring was carried out during three canoe river journeys down the length of the Cuanavale, Cuito, Cubango and Okavango Rivers, totalling a 2,744 km transect. During the 2015 field season, passive acoustic recordings were obtained with an EM3 bat detector (Wildlife Acoustics, Concorde, USA) for 75 nights (between 23 May and 18 September 2015) during a river expedition starting at the Cuito source and continuing to the Okavango Delta. During the early 2016 field season, passive acoustic recordings of bat echolocation calls were obtained using two Song Meter SM2BAT+ bat detectors (Wildlife Acoustics) and an ANABAT SD2 bat detector (Titley Electronics). Passive recording was carried out for six nights in March 2016 with an SM2BAT+ detector and three nights with ANABAT SD2 (in both cases between 15 and 21 March 2016), from the Cuito and Cuanavale source areas, Cuchi Gorge and Samboana Village. Additionally, recordings were obtained for 43 nights in total (between 17 February and 4 April 2016), of which 37 nights yielded calls, during a river trip from the source of Cuanavale River to its confluence with the Cuito River. Passive recordings were obtained between 21 October and 4 November 2016 (eight nights) using an SM2BAT+ detector, from 27 October to 4 November 2016 (10 nights) with an ANABAT SD2 detector and from 27 October to 2 November 2016 (six nights) with an EM3 detector. During the late 2016 season, recordings focussed on the Cuanavale and Saliakwembo source areas. During 2017, recordings were taken for a period of 1–2 hours nightly for a total of 51 nights with an ANABAT SD1 detector during a river trip down the Cubango River.

The following approach was used to identify bat calls to species or species-groups. Call analysis and identification was undertaken independently by three observers (PJT, MK and DP) although consensus was later obtained on the definition of call parameters for each species after extensive consultation and comparison of calls by PJT. Based on this, a final species list was derived by PJT (Table 1). Using Kaleidoscope Pro software (Wildlife Acoustics), the wave files collected with two SMBAT2+ detectors during the early 2016 season (February to April 2016), both from the Cuanavale River transect and from recordings by PJT in the Cuanavale and Cuito source areas, were firstly converted to zero-crossing (ANABAT) files and then identified manually by PJT using AnalookW software (version 4.1t, Chris Corbin, www.hoarybat.com), for comparison with zero-crossing ANABAT calls obtained directly from ANABAT SD1 and SD2 detectors. The latter included recordings from a SD2 bat detector from the Cuanavale and Cuito source lakes area and Cuchi Gorge in early 2016, as well as those from an Anabat SD1 detector during the 2017 transect of the Cubango River. Identifications were based on reference calls obtained both from ANABAT calls from captured and field-identified individuals that were subsequently released, as well as from standard references (Monadjem et al. 2010, 2017; Happold and Happold 2013; Taylor et al. 2013) and unpublished call data (Taylor, unpublished, Monadjem, unpublished). Using the same identification criteria, calls recorded from the Cuanavale and Saliakembo source lakes in late 2016 using EM3, SM2+ and Anabat SD2 detectors were identified by MK, while calls recorded with the EM3 detector during the 2015 transect of the Cuito and Okavango Rivers were identified by DP using a different software (SonoBat). After extensive comparisons of calls and consultation between PJT, MK, and DP, PJT derived a standard list of putative bat species. This library of identified calls is available on request from PJT.

Camera trapping. The medium and large mammal surveys were conducted using two systematic camera-trapping assessments during 2016. The first camera trapping survey ran between February and March 2016 (936 trap nights) and the second ran between July and November 2016 (1,349 trap nights).

In the first camera trapping survey, two sites near the southern end of the Bie and Moxico provinces were sampled using linear transects. Transect 1 (north-west orientation along the Cuanavale and Cuando Rivers) was approximately 56 km long and was separated by ca. 20 km from transect 2 (south-east orientation just south of the town of Munhango) which was 50 km long. We sampled 19 camera stations along transect 1 and 20 camera stations along transect 2, with each station spaced ca. 3 km (range: 2–4 km) apart. One camera trap was placed at each station and was either a Cuddeback C2 (n = 11), E3 (n = 8) (Non-Typical, Inc. Green Bay, Wisconsin) or Bushnell Trophy Cam Aggressor HD (n = 20) (Bushnell Outdoor Products, Cody, Kansas). Cameras were placed on animal paths about 30cm high on the base of the tree or on a stake placed in the ground to maximize photographic captures of the full range of mammalian body sizes (Mann et al. 2015). Cameras were set to take three sequential photographs per trigger, with an interval of one minute between trigger events. Sensitivity of the sensor was set on medium (normal) and picture quality was set to 5MP. Cameras were operational for 24 hours a day and were checked only upon collection.

The second camera trapping survey employed a similar approach. However, in this survey 17 camera stations were sampled along the Cuanavale River south-east from the village of Tchijanga towards the Quembo River. The transect was approximately 120 km long and camera traps (13 Cuddeback and four Bushnell) were again placed on well used animal paths, but with no specific distance between each camera station (range: 1–20 km).

The date and GPS locations of any verifiable sightings and signs of medium and large mammals were recorded during each expedition. Analyses of photographs from each camera trap survey were limited to those photographs taken from 12:01am the day after setting up a camera trap until 12:00pm of day before camera trap stopped recording. To ensure independence of capture events for each camera trap, images of individuals of the same species were ignored if captured within one hour of a previous sighting (O'Brien et al. 2003). For each photograph we recorded the site, date, time, and species. We excluded sightings of birds, small (< 1 kg) mammals, domestic animals, people, and any unidentifiable images. The total number of capture events (n) per species was tallied and their percentage contribution (spp %) to the total number of photographs was calculated. The capture frequency (CF) for each species was calculated as the number of capture events (n) per 100 camera-trapping days (Tobler et al. 2008).

Results

Mammal list for Angola

An updated species checklist of 275 species of Angolan mammals is presented in Suppl. material 1, comprising 245 species records obtained from GBIF and an additional 30 species added from the literature and recent surveys (additional records marked in bold in Suppl. material 1). The list excludes commensal and domesticated species. Of this total, nine bat species were added to the revised GBIF list by Monadjem et al. (2010a), four rodent species were added by Monadjem et al. (2015), and additional bat, rodent and shrew species were added by the current survey (see paragraph below).

New small mammal capture data from Okavango Basin

We collected 68 specimens of 24 species of small mammals (three shrew, ten rodent, and 11 bat species; Table 1). Of these species, one bat (*Laephotis angolensis*, the Angolan long-eared bat) and one rodent (*Otomys anchietae*, the Angolan vlei rat) are Angolan endemics (Table 1). One bat (*Pipistrellus rusticus*, rusty pipistrelle), two rodent species (*Mus setzeri* Setzer's mouse and *Zelotomys woosnami* Woosnam's broad-faced mouse) and one shrew (*Suncus varilla*, lesser dwarf shrew) were recorded for the first time in Angola, all listed as Least Concern, by the IUCN Red List. Two of the bat species were listed as Data Deficient (*Plerotes anchietae*, Anchieta's broad-faced fruit bat and Angolan long-eared bat) and one is classified as Near Threatened (*Epomophorus angolensis*, Angolan epauletted fruit bat), while one was not assessed.

There was a very marked difference in bat activity between March 2016 (which was characterised by heavy rainfall), when only 13 bats were caught, and October/November 2016 (less frequent rainfall) when 151 bats were collected, in spite of very similar trap/net effort and six nights sampled for both periods. Captures in late 2016 were dominated by *Pipistellus rusticus*, *Neoromicia capensis*, and *N. zuluensis*.

New acoustic data from the Okavango Basin

From manual identification of zero-crossing calls, we identified a total of 29 putative species of insectivorous bats (Tables 2, Suppl. material 2).

Diversity and importance of small mammals in Angola and the Okavango source lakes region

The current capture survey adds one bat species, two rodent species and one shrew species to the country lists above, bringing to 67 and 87 the total number of Angolan bat and rodent species. From our current survey, we recorded 13 non-volant small mammals from the upper Okavango catchment of Angola, and some 33 species of bats, based on the estimate of insectivorous species from acoustic data (29 species; see above) combined with one rare species (*Mimetillus thomasi*, Thomas's flat headed bat), for which we have no echolocation data, and three additional species of fruit bats captured with mist nets/harp-traps (Table 1).

Camera trap data from the Okavango source lakes region

A total of 35 species of medium-large mammals, from 17 families were recorded in 2016 through opportunistic sightings and two formal camera-trapping surveys (Table 3). Ten species were recorded by both camera surveys and opportunistic sightings (common

Table 1. List of small mammal species collected in the central region of Angola in 2013 (four specimens) and 2016 (64 specimens) and deposited in the Durban Natural Science Museum. All specimens were checked by PJT based on cleaned skulls and skins in ethanol.

Species	Common name	IUCN status	Localities recorded
Order Chiroptera			
Plerotes anchietae	Anchieta's Broad-Faced Fruit Bat	Data Deficient	Cuanavale Source
Epomophorus angolensis	Angolan Epauletted Fruit Bat	Near Threatened	Cuchi Gorge
Epomops dobsonii	Dobson's Epauletted Fruit Bat	Least Concern	Sambojana, Saliakembo
Hypsugo anchietae	Anchieta's pipistrelle	Least Concern	13 km north of Chett
**Pipistrellus rusticus*	Rusty Pipistrelle	Least Concern	Cuito Source, Sambojana, Cuanavale Source, Saliakembo
Neoromicia zuluensis	Zulu Pipistrelle	Least Concern	Cuanavale Source, Saliakembo Source
Neoromicia capensis	Cape Serotine	Least Concern	Cuanavale Source, Saliakembo Source
***Laephotis angolensis*	Angolan Long-Eared Bat	Data Deficient	Cuanavale Source, Sambojana, Saliakembo Source
Scotophilus leucogaster	White-bellied House Bat	Least Concern	Saliakembo Source
Mimetillus thomasi	Thomas's flat-headed bat	Not assessed	Cuanavale Source, Saliakembo Source
Chaerephon nigeriae	Nigerian Free-Tailed Bat	Least Concern	Cuanavale Source
Order Soricomorpha			
Crocidura fuscomurina	Bicolored musk shrew	Least Concern	Mupapa Falls
Crocidura hirta	Reddish-Grey Musk Shrew	Least Concern	Cuanavale Source, Saliakembo Source
* *Suncus varilla*	Lesser Dwarf Shrew	Least Concern	En route to Sambojana
Order Rodentia			
***Otomys anchietae*	Angolan Vlei Rat	Least Concern	Cuito Source
Rhabdomys cf. *dilectus*	Striped Mouse	Least Concern	Cuanavale Source
Mastomys natalensis	Multimammate mouse	Least Concern	Cuanavale Source Lake
**Mus setzeri*	Setzer's Mouse	Least Concern	Cuanavale Source, Cuito Source Cunde Falls
Lemniscomus griselda	Single-Striped Mouse	Least Concern	25 km west of Menongue
**Zelotomys woosnami*	Woosnam's Broad-Faced Mouse	Least Concern	Cuito Source
Gerbilliscus leucogaster	Lowveld Gerbil	Least Concern	Cuanavale Source, Cunde Falls
Graphiurus kelleni	Dormouse	Least Concern	Cuito Source
Saccostomus campestris	Pouched mouse	Least Concern	Cuanavale Source, Cuito Source, Sambojana
Steatomys krebsii	Fat Mouse	Least Concern	Cuanavale Source, Cuito Source, Mupapa Falls

* New record for Angola ** Angola endemic.

duiker, large-spotted genet, honey badger, side-striped jackal, serval, spotted hyena, porcupine, scrub hare, and vervet monkey; see Table 3 for scientific names). Eleven species were recorded by the camera traps only (greater bushbaby, tree squirrel, springhare, aardwolf, caracal, African wild cat, lion, aardvark, blue duiker and steenbok). Five species were only detected opportunistically during expeditions (African elephant, oribi, roan, lechwe and sitatunga).

Common duikers and large-spotted genets were the most frequently photographed species in both camera surveys (Table 3). Interestingly, leopards were only recorded in the second camera survey and not the first, but a total of four per 100 days of sampling would likely yield a single leopard photographic event, higher than any other carnivore recorded in our study (Table 3). Other species which stood out during one or both of the camera surveys were vervet monkeys, side-striped jackals, bushpigs, silver-backed duiker and blue duiker. All other species, although detected during the course of the study, were recorded in less than one per 100 days of sampling (Table 3). Unfortunately, 15% of the photographic events in the first camera survey were unidentifiable due mostly to poor placement of cameras (Table 3).

Discussion

Surveys of Okavango source lakes

Based on both acoustic and trapping surveys, at least 46 species of small mammals occur in the upper Okavango catchment region, including several rare and endemic species. This diversity compares favourably with studies reviewing the diversity of terrestrial small mammals (Taylor et al. 2015) and bats (Schoeman et al. 2013; Taylor et al. 2013; Cooper-Bohannon 2016; Herkt et al. 2016) in African highlands generally. However, the estimate of terrestrial small mammal richness is probably grossly under-estimated as a GBIF search of central Angola yielded 42 species, compared with our list of 13 species of shrews and rodents based on captures. Many of the small mammals recorded in our survey such as shrews, Anchieta's vlei rat and fruit bats are habitat specialists which would be adversely affected by deterioration of wetlands due to anthropogenic effects such as extensive fires, tree clearing, wetland drainage and overgrazing, typical in the Miombo woodlands (Syampungani et al. 2009; Jew et al. 2016). Fruit bats provide valuable ecosystem services through seed dispersal and pollination, Anchieta's broad-faced fruit bat possesses whiskers which are thought to be involved in pollination (Monadjem et al. 2010). Bats of the genera *Eidolon*, *Epomophorus*, and *Rousettus* are known to pollinate baobab trees (*Adansonia digitata*) over much of Africa (Baum 1995).

Mammal checklist

Afrosoricida

This order is represented by two families in mainland Africa: Chrysochloridae (golden moles) and Potamogalidae (otter-shrews). Angola harbours just one species of golden mole, the Congo golden mole *Huetia leucorhina* (previously *Calcochloris leucorhinus*, see Asher et al. 2010). This group of subterranean fossorial mammals is therefore either poorly represented in Angola, or it has been greatly overlooked in the country. The species *H. leucorhina* is classified as Data Deficient and is known from just 10 scattered

Table 2. Putative bat species definitions based on analysis of bat calls from various acoustic surveys in the Okavango catchment of Angola between 2015 and 2017. Although the Cuito-Okavango River trip of 2015 extended beyond Angola into Namibia and Botswana, there were no species identified in Namibia and Botswana that were not also detected in Angola. Matching of calls with species was based on release calls from bats captured and released in Angola (for *Laephotis angolensis*, *Neoromicia capensis*, *Pipistrellus rusticus* and *N. zuluensis*) as well as Monadjem et al. 2010a, Happold and Happold 2013, Taylor et al. 2013a and unpublished data from PJT and AM. Known occurrence of species in Angola is shown based on previous evidence from specimens (based on the current survey, GBIF records or listed as such by Monadjem et al. 2010) or only from the literature. The occurrence of a species is shown as "predicted" where records are known from adjacent countries and high probabilities of occurrence were indicated for any part of Angola in Maximum Entropy Modeling (MaxEnt for short) species models depicted in Monadjem et al. (2010a). R = River.

Species	Functional group (Monadjem et al. 2010a)	Overlap species	Evidence for occurrence in Angola	Caught in current survey	No. calls (Cuanavale R)	No. nights (Cuanavale R)	Cuito-Okavango R (2015)	Cuanavale R (2016)	Source lakes (early 2016)	Source lakes and Cuchi Gorge (2016)	Source Lakes (late 2016)	Cubango R. (2017)
Family Emballonuridae												
Coleura afra	Open-air	Possibly *T. perforatus* (unrecorded)	Specimen	No	29	7	√	√	√			√
Taphozous mauritianus	Open-air	*C. pumilus*, *T. aegyptiaca*	Specimen	No	272	24		√			√	√
Family Hipposideridae												
Macronycteris vitattus	Clutter	None	Specimen	No	0	0					√	
Family Rhinolophidae												
Rhinolophus fumigatus	Clutter	None	Specimen	No	14	5		√	√		√	
Family: Miniopteridae												
Miniopterus cf. *fraterculus*	Clutter-edge	*M. fraterculus*	Acoustic evidence only	No	20	12	√	√	√	√	√	√
Miniopterus natalensis	Clutter-edge	*P. rusticus*	Specimen	No	55	19	√	√				
Family Molossidae												
Chaerephon ansorgei	Open-air	*T. ventralis*, *C. nigeriae*, *T. fulminans*	Specimen	No	0	0	√					
Chaerephon nigeriae	Open-air	*T. ventralis*, *C. ansorgei*, *T. fulminans*	Literature	Yes	6	3		√			√	√
Chaerephon pumilus	Open-air	*T. aegyptiaca*, *M. condylurus*, *T. mauritianus*	Specimen	No	79	12	√	√		√	√	√
Mops condylurus	Open-air	*C. pumilus*, *T. aegyptiaca*	Specimen	No	21	9	√	√		√	√	√

Species	Functional group (Monadjem et al. 2010a)	Overlap species	Evidence for occurrence in Angola	Caught in current survey	No. calls (Cuanavale R)	No. nights (Cuanavale R)	Cuito-Okavango R (2015)	Cuanavale R (2016)	Source lakes (early 2016)	Source lakes and Cuchi Gorge (2016)	Source Lakes (late 2016)	Cubango R. (2017)
Mops midas	Open-air	*C. nigeriae*	Predicted	No	2	2	√	√			√	√
Otomops martiensseni	Open-air	None	Specimen	No	1	1		√				√
Tadarida aegyptiaca	Open-air	*T. mauritianus*, *C. pumilus*	Specimen	No	101	18	√	√		√	√	√
Mops cf. *condylurus*	Open-air	*M. condylurus*	Calls do not match any known species	No	23	3		√		√	√	
Family: Vespertilionidae												
Eptesicus hottentotus	Clutter-edge	*S. dinganii*, *M. welwitschii*	Specimen	No	8	4		√			√	√
Hypsugo anchietae	Clutter-edge	*M. natalensis*	Specimen	Yes	80	22	√	√	√	√	√	√
Kerivoula argentata	Clutter-edge	None	Predicted	No	0	0		√			√	√
Kerivoula lanosa	Clutter-edge	None	Specimen	No	0	0					√	
Laephotis angolensis	Clutter-edge	*P. hesperidus*	Specimen	Yes	2	2					√	√
Myotis welwitschii	Clutter-edge	*M. bocagii*	Specimen	No	16	9		√	√		√	
Neoromica zuluensis	Clutter-edge	*P. hesperidus*	Specimen	Yes	43	10		√	√	√	√	√
Neoromicia capensis	Clutter-edge	*S. viridis*, *Scotoecus hindei/albigula*, *N. schlieffeni*	Specimen	Yes	29	13	√	√		√	√	√
Neoromicia nana	Clutter-edge	None	Specimen	No	0	0			√	√		√
Neoromicia cf. *nana*	Clutter-edge	*N. nana*	Calls do not match any known species	No	59	4	√		√	√		√
Nycticeinops schlieffeni	Clutter-edge	*S. viridis*, *S. hindei/albigula*, *N. capensis*	Specimen	No	124	14	√	√	√	√	√	√
Pipistrellus hesperidus	Clutter-edge	*N. zuluensis*, *L. angolensis*	Predicted	No	805	25	√	√	√	√	√	√
Pipistrellus rusticus	Clutter-edge	*M. natalensis*	Predicted	Yes	161	25	√	√	√	√	√	√
Scotophilus dinganii	Clutter-edge	*E. hottentotus*, *L. botswanae*	Specimen	No	28	7		√	√	√	√	√
Scotophilus leucogaster	Clutter-edge	*N. schlieffeni*, *S. viridis*, *N. capensis*	Specimen	Yes	28	17		√	√	√		

Table 3. All medium-large mammal species detected opportunistically (see methods) and during two formal camera-trapping surveys of the south-eastern highlands of Angola in 2016. Where applicable, the total number of photographic events per species (n), their percentage contribution (Spp. %) to the total number of photographic events, and the capture frequency (CF) (number of events/100 camera days) is shown. V = verified opportunistic sighting, C1 = camera trap survey 1, C2 = camera trap survey 2. If a species was recorded in both camera-trapping surveys, the values for both surveys are separated by a backslash (i.e., C1/C2).

Family	Species	Common name	V	C1	C2	n	Spp. %	CF
Galagidae	*Otolemur crassicaudatus*	Greater bushbaby		√		3	2.5	0.3
Cercopithecidae	*Chlorocebus cynosuros*	Vervet monkey	√	√	√	10/3	8.5/0.6	1.1/0.2
Leporidae	*Lepus victoriae*	Africa savanna hare	√	√	√	8/7	6.8/1.5	0.9/0.5
Sciuridae	*Paraxerus cepapi*	Tree squirrel		√		1	0.9	0.1
Pedetidae	*Pedetes capensis*	Springhare			√	11	2.3	0.8
Hystricidae	*Hystrix africaeaustralis*	Porcupine	√	√	√	2/8	1.7/1.7	0.2/0.6
Protelidae	*Proteles cristatus*	Aardwolf		√		1	0.9	0.1
Hyaenidae	*Crocuta crocuta*	Spotted hyena	√	√	√	2/6	1.7/1.2	0.2/0.4
Felidae	*Acinonyx jubatus*	Cheetah		√	√	3/1	2.5/0.2	0.3/0.1
	Caracal caracal	Caracal			√	3	0.6	0.2
	Felis lybica	African wildcat			√	1	0.2	0.1
	Leptailurus serval	Serval	√	√	√	1/1	0.9/0.2	0.1/0.1
	Panthera leo	Lion			√	1	0.2	0.1
	Panthera pardus	Leopard	√		√	48	10.0	3.6
Canidae	*Lycaon pictus*	African wild dog	√		√	8	1.7	0.6
	Canis adustus	Side-striped jackal	√	√	√	4/13	3.4/2.7	0.4/1.0
Mustelidae	*Mellivora capensis*	Honey badger	√	√	√	1/4	0.9/0.8	0.1/0.3
	Ictonyx striatus	Striped polecat (Zorilla)	√		√	1	0.2	0.1
Viverridae	*Civettictis civetta*	African civet	√	√		3	2.5	0.3
	Genetta maculata	Large-spotted genet	√	√	√	25/3	21.2/0.6	2.6/0.2
Herpestidae	*Atilax paludinosus*	Marsh mongoose	√		√	1	0.2	0.1
	Ichneumia albicauda	White-tailed mongoose		√	√	2/9	1.7/1.9	0.2/0.7
	Mungos mungo	Banded mongoose		√		1	0.9	0.1
Orycteropodidae	*Orycteropus afer*	Aardvark			√	4	0.8	0.3
Elephantidae	*Loxodonta africana*	African elephant	√			–	–	–
Suidae	*Potamochoerus porcus*	Bushpig	√	√	√	1/24	0.9/5.0	0.1/1.8
	Phacochoerus africanus	Warthog	√		√	5	1.0	0.4
Bovidae	*Cephalophus silvicultor*	Silver-backed duiker		√	√	5/14	4.2/2.9	0.5/1.0
	Philantomba monticola	Blue duiker		√	√	4/19	3.4/3.9	0.4/1.4
	Sylvicapra grimmia	Common duiker	√	√	√	23/285	19.5/59.1	2.5/21.1
	Ourebia ourebi	Oribi	√			–	–	–
	Raphicerus campestris	Steenbok			√	2	0.4	0.1
	Hippotragus equinus	Roan	√			–	–	–
	Kobus leche	Lechwe	√			–	–	–
	Tragelaphus spekii	Sitatunga	√			–	–	–
Unidentifiable	Unidentifiable	Unidentifiable		√		18	15.2	–

locations including one from Angola where a series of seven specimens from the Field Museum of Natural History were collected by H. R. Heinrich in 1954–1955 from Canzele, 30 km west of Camabatela in central Angola. Otter-shrews are represented by just three species, all of them associated with tropical forests in Africa. Of these, the largest and most widely distributed *Potamogale velox* has been recorded from numerous localities in forested regions of northern Angola.

Carnivora

In addition to 37 species of Angolan carnivores in the GBIF database, reliable literature records (Kingdon and Hoffmann 2013a) add three species, including the Congo clawless otter *Aonyx congicus* and the African golden cat *Profelis auratus* from N Angola, and the black-footed cat *Felis nigripes* from extreme south-east Angola. The king genet *Genetta poensis*, the servaline genet *G. servalina*, the central African linsang *Poiana richardsonii*, the long-nosed mongoose *Xenogale naso* and the black-legged mongoose *Bdeogale nigripes* all have distributions apparently encompassing or bordering Cabinda but without any specific mention of their occurrence in Cabinda, Angola (Kingdon and Hoffmann 2013a) so we do not add them to the checklist.

This order is represented by seven terrestrial families in Africa: Canidae (dogs), Mustelidae (weasels, polecats and allies), Nandiniidae (palm civet), Felidae (cats), Viverridae (genets and civets), Hyaenidae (hyenas and aardwolf) and Herpestidae (mongooses). Five canid species have been recorded in Angola. However, only two species (African wild dog *Lycaon pictus* and side-striped jackal *Canis adustus*) were photographed and/or sighted in the Okavango catchment during our 2016 assessment. Nevertheless, the relatively frequent rate with which the wild dogs were photographed on the camera traps (capture frequency = 0.6) is pleasing given their Red List Endangered status on the continent.

Eight felid species occur in Angola of which six (all except *P. auratus* and *F. nigripes*) were recorded during our 2016 camera trapping, suggesting that these species may have relatively cosmopolitan distributions across the country. However, our field data indicates that the largest of these obligate carnivores (the lion *Panthera leo*) occurs at much lower densities than the other felids. The lion is often one of the first species to be lost from the large carnivore guild when prey becomes limiting and/or conflict with humans escalates (*sensu* the effects of war). Thus, their presence in the south-east of Angola (albeit patchy) suggests some post-civil war recolonization. Interestingly, leopards *P. pardus* were the most frequently encountered felid during the 2016 camera trapping (capture frequency = 3.6) supporting the notion that leopards are adaptable generalists.

Two hyena species and the aardwolf *Proteles cristatus* have been recorded in Angola. However, only the aardwolf and the spotted hyena *Crocuta crocuta* were photographed in our 2016 assessment. The latter finding is somewhat unsurprising given that brown hyena *Parahyaena brunnea* density is known to be negatively correlated with that of spotted hyenas (Mills 2015).

The Herpestidae family is presented by 12 Angolan species, with several of these species occurring throughout much of sub-Saharan Africa. Three species of the mongoose family were formally recorded in the Okavango catchment survey of 2016, the marsh mongoose, *Atilax paludinosus* was recorded from a visual and camera survey, with banded mongoose, *Mungos mungo* and white-tailed mongoose *Ichneumia albicaudata* just recorded by camera surveys. The banded mongoose occurs in a broad range of habitats, with extensive range throughout Angola (Gilchrist and Do Linh San 2016). Selous's Mongoose, *Paracynictus selousi* occurs in savanna and woodland in central and southern parts of Angola (Stuart and Stuart 2013a). The black-legged mongoose, *Bdeogale nigripes* occurs in far north-west Angola (van Rompaey and Colyn 2013a). Yellow mongooses *Cynictis penicillata* occur marginally in southern Angola (Taylor 2013a). The highly social meerkat, *Suricata suricatta*, is endemic to the more arid open regions of western parts of southern Africa, and has a marginal intrusion in to SW Angola (Macdonald 2013). The dwarf mongoose (*Helogale parvula*, and a subspecies *H. parvula mimetra* (sometime treated as valid species *H. mimetra*) are reported to occur in Angola (Creel 2013), with *H. parvula* reported to occur in open woodlands, thickets and often associated with termite mounds etc., allowing for suitable dens (Creel 2013). Creel (2013) also indicates that there is a subspecies *H. p. varia* that occurs in north-east Angola.

Three species of *Herpestes* occur in Angola, the Egyptian mongoose, *H. ichneumon* (Do Linh San et al. 2016), the common slender mongoose *H. sanguineus* (Hoffmann and Taylor 2013), and the Kaokoveld slender mongoose, *H. flavescens* from SW Angola including Benguela Province (Taylor 2013c). The taxonomic history of *H. flavescens* is very confusing. It has been included as a chestnut-coloured race of different species included in either *H. pulverulentus* or *H. ochraceus* but these two last-mentioned species are now considered to have much more restricted distributions in South Africa and S Namibia and Somalia and Ethiopia respectively. Crawford-Cabral (1996) demonstrated that *flavescens* was conspecific with the blackish-coloured *nigrata* race from north-east Namibia, a view that we follow. A chestnut-coloured form from Caconda near Benguela in Angola, *H. ansorgei* Thomas & Schwann, 1905 should be assigned to *H. flavescens* on colour and geographic grounds, and not to *H. ochraceus* as proposed by Taylor (2013b).

Ansorge's Crusimanse *Crossarchus ansorgei* is a cryptic Angolan Herpestidae species known from a single location from the proposed Angolan distribution collected in 1908 (Angelici and Do Lin San 2015 citing Crawford-Cabral 1989; Van Rompaey and Colyn 2013b).

Mustelidae. The Zorilla, *Ictonyx striatus*, ranges across a broad range of habitats of sub-Saharan Africa, and is common throughout its range, yet reported to be locally uncommon (Stuart and Stuart 2013b). Our upper Okavango catchment study of 2016 recorded this species by visual sightings and camera trap surveys. African clawless otters, *Aonyx capensis*, are medium sized otter species, and occur widely, associated with seasonal rivers, and can occur in fresh and marine water. The clawless otter is reported to have a distribution range in the central and southern parts of Angola (Somers and Nel 2013), yet was not recorded in the 2016 assessment. The spotted necked otter, *Hydrictis maculicollis* appears to be closely linked to permanent freshwater systems with abundant food resources (fish) and shoreline cover. Their continental distribution is considered to be wide. However, there is a paucity of information for this otter species in Angola (Reed-Smith et al. 2015). The ratel, *Mellivora capensis*, occurs across Africa in most habitat types, from deserts to forests. This species is reported to exist in low densities, and is often regarded to be rare throughout its range (Begg et al. 2013). This species was recorded in the Okavango catchment expedition through visual observation and camera trap survey. African striped weasels, *Poecilogale albinucha*, are thought to be uncommon and rare, throughout their extensive range in sub-Saharan Africa (Stuart and Stuart 2013c), and are delineated to occur in the northern parts of Angola.

Nandiniidae. The two-spotted Palm Civet, *Nandinia binotata*, are arboreal and found across the west and central African forest belt, likely to only be found in the North east of Angola (Gaubert 2013).

Viverridae. The African civet, *Civettictis civetta*, is distributed throughout sub-Saharan Africa, with suitable habitat from 29°S latitude extending north to south of 15°N latitude, and a commonly encountered carnivore (Ray 2013). This species was recorded from visual observation and through camera trap surveys in our upper Okavango catchment expedition. The Miombo genet, *Genetta angolensis*, originally known from three adult specimens, lost in a fire in 1978 (Crawford-Cabral 2013) has a broad distribution from Angola, Democratic Republic of Congo, Mozambique, Malawi, Zambia and Tanzania. This species is reported to be similar in size to the common genet, and occurs in "open miombo woodlands" and is reported to be locally common in some areas (e.g., the Luando Strict Nature Reserve (Central Angola) (Crawford-Cabral 2013, Gaubert et al. 2016). The common genet, *Genetta genetta*, is a widespread and locally common genet, and in its southern distribution range occur from central and south Angola through Botswana, Namibia, Zimbabwe and South Africa, replaced to the east by the large spotted genet (Delibes and Gaubert 2013). *Genetta maculata*, along with *G. tigrina* and *G. pardina* form part of the 'large spotted genet complex' (Gaubert and Dunham 2013). The species is very common and widespread with over 50 localities recorded by GBIF records. Based on camera trap images and visual sightings, *G. maculata* was present during the Okavango catchment expeditions in 2016. The servaline genet *G. servalina* is depicted as occurring in Cabinda by Van Rompaey and Colyn (2013c) but without any specimen or sighting records, its occurrence there is dubious albeit possible.

Certartiodactyla

This order is represented by five indigenous families in Africa and Angola: Suidae (pigs), Hippopotimidae (hippopotamuses), Tragulidae (chevrotains), Giraffidae (giraffes) and Bovidae (bovines and antelopes). Following the taxonomy of Kingdon and Hoffman (2013b), a total of 32 artiodactlys are recognised in Angola, of which 26 are bovids (Suppl. material 1). Following the taxonomy of Groves and Grubb (2011), the list would result in 35 Angolan Artiodactyla including 29 bovids. This increase of three bovid species is because these latter authors regard Lichtensteini's harbertebeest *Alcelaphus lichtensteini* as a separate species from the red hartebeest *A. buselaphus*; furthermore, two species (a tropical forest Kabinda species and a southern savanna species) are recognised each within the African buffalo (*Syncerus nanus* and *S. caffer* respectively) and the bushbuck (*Tragelaphus ornatus* and *T. phaleratus* respectively) populations from Angola. The taxonomy of Groves and Grubb (2011) doubled the number of recognised African ungulate species. This species-splitting process would raise several local Angolan subspecies to species rank and result in name changes for several of the Angolan ungulates. It would make two bovid species endemic to Angola, the Angolan blue duiker *Philantomba anchietae* and the Angolan springbok *Antidorcas angolensis*. The klipsringer *Oreotragus tyleri* would become near-endemic to Angola, extending marginally to north-west Namibia. Some conservationists have opposed the taxonomy of Groves and Grubb (2011); for recent debates see: Gippoliti and Groves (2012), Heller et al. (2013), Zachos et al. (2013), Cotterill et al. (2014), Heller et al. (2014), Garnett and Christidis (2017), Cotterill et al. (2017), and Groves et al. (2017).

GBIF yielded 5,821 species-identified historical records of Artiodactyla dating back to 1889, of which 5,438 were compiled by the IICT in Portugal, and 385 were preserved specimens, predominantly from the American Museum of Natural History and Field Museum of Natural History. These records provide valuable insights into the historical distribution of the larger ungulates, some of which have become extinct or almost extinct in the wild in Angola. For example, the GBIF database provides 13 georeferenced records of Lichtenstein's hartebeest from 13 localities throughout E Angola, whereas this species is now thought to be extinct in Angola (Gosling and Capellini 2013). The GBIF database contains 213 records (including 25 museum specimens dating back to 1925) of the black-faced impala (*Aepyceros melampus petersi*) from 78 georeferenced localities throughout S Angola whereas the status of the species in Angola is currently uncertain. It occurs at Iona National Park and possibly two other national parks (Fritz and Bourgarel 2013). The case of the three sable subspecies, the nominate form (*Hippotragus niger niger*), Kirk's sable (*H. niger kirkii*) and the giant sable *H. n. variani* is also remarkable. All three subspecies were widespread in central, south-eastern, and eastern Angola (Figure 3) but are currently much more restricted. The giant sable came close to extinction during the Angola civil war and is currently known from just two reserves in central Angola, the Cangandalo National Park and the Luando Reserve (Figure 3) where its survival continues to be threatened by poaching and hybridization with roan antelope *Hippotragus equinus* (Vaz Pinto 2006, 2018; Estes 2013). In the case of Kirk's sable from eastern Angola, (Figure 3), there have been no observations for 40 years (Cabral and Veríssimo 2005). However the nominate race (*H. n. niger*) is still known to occur in the region between the Cuito and Cuando Rivers in southeastern Angola, including the Luengue-Luiana and Mavinga National Parks (Funston et al. 2017, Figure 3). However no sable were detected in the present camera trap survey or ad hoc observations conducted in the upper Okavango catchment.

A dubious record of the common reedbuck *Redunca redunca* from Calunga in SE Angola in the Los Angeles County Museum must be a misidentified *R. arundinum* since *R. redunca* is not known to occur anywhere near Angola (Kingdon and Hoffman 2013b).

As one of the most speciose certartiodactyl families in Africa, it is unsurprising that 26 bovid species occur in Angola. Only eight of these species were recorded during our 2016

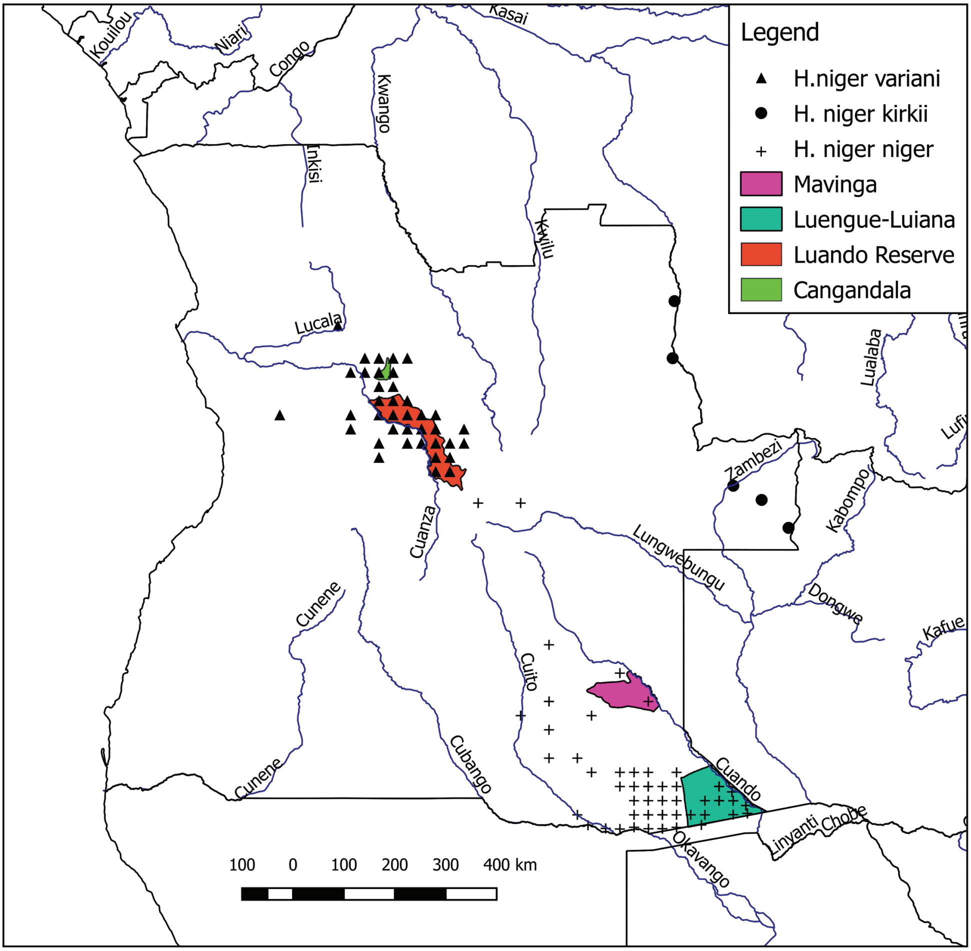

Figure 3. Historical distribution of the nominate sable subspecies (*Hippotragus niger niger*), Kirk's sable (*H. niger kirkii*) and the giant sable (*H. niger variani*) in Angola based on records of the Instituto de Investigação Científica Tropical (IICT) in Portugal (obtained via GBIF search). Protected areas where *H. n. niger* and *H. n. variani* currently known to occur are indicated in the legend. The subspecies *H. n. kirkii* from eastern Angola has not been recorded during the past 40 years.

survey in the south-east (*Cephalophus silvicultor*, *Philantomba monticola*, *Sylvicapra grimmia*, *Ourebia ourebi, Raphicerus campestris*, *Hippotragus equinus*, *Kobus leche* and *Tragelaphus spekii*). Anecdotal data from our camera trapping and discussions with local Angolans suggests that the bovids are likely the most targeted mammalian group when it comes to poaching for bushmeat that is routinely traded commercially in many parts of the country.

Three pig species have been recorded in Angola. The common warthog *Phacochoerus africanus* and the bushpig *Potomochoerus larvatus* have been collected/sighted from numerous localities across Angola. However, the red river hog *Potomochoerus porcus* is restricted to the forested northern regions of the country based on 12 GBIF records (seven museum specimens and five additional historical records from IICT). Although not formally recorded during our 2016 camera trapping survey, hippopotamuses *Hippopotamus amphibius* have been regularly recorded across Angola wherever suitable water and nocturnal grazing is available. Only one chevrotain species *Hyemoschus aquaticus* has been recorded in Angola and this species occurs in the forested north of the country. The giraffe *Giraffa camelopardalis* has been recorded at numerous localities in Angola but was not formally recorded in the upper Okavango catchment during our 2016 assessment. However, what may have been giraffe tracks/spoor were noted.

Chiroptera

The GBIF search alone reported 59 species of Angolan bats while Monadjem et al. (2010a) reported 64, seven of which were not included in the GBIF search. The current survey added one new Angolan species record based on captures (*Pipistrellus rusticus*) and three tentative species records based on acoustic identification (*Kerivoula argentata*, *Mops midas*, *Pipistrellus hesperidus*; Table 2). Happold and Happold (2013) added one species (*Triaenops afer*). Merging these three sources resulted in a combined list of 71 species (Suppl. material 1). Over 220 species of bats have been recorded in mainland Africa of which over 75% occur in Sub-Saharan Africa (Happold and Happold 2013). Of these, 116 species occur in southern Africa defined by Monadjem et al. (2010a), a region which includes Angola. Within this region, the more diverse countries have bat species richness typically between 60–70 species (Monadjem et al. 2010b). For example, 67 species have been recorded in Mozambique, 65 species in Zambia, and 62 species each in Malawi and Zimbabwe (Monadjem et al. 2010b). Therefore, the current total of 71 species that we report for Angola in this study, is on the higher end of richness for the region, in spite of the fact that Angola remains one of the most poorly known southern African countries with respect to bats (and mammals in general). For example, during the course of just a few weeks of fieldwork we were able to add a new species of bat to the country list, as well as additional (possible) new records of a further three species, based on echolocation calls, which would bring the total up to 71 species (making it the most diverse country in the region). We suspect that there are numerous species of bat that still await discovery in Angola, and we further predict that a number of new (and possibly endemic) species will be eventually recorded from the western escarpment region that is high in bird endemism (Mills et al. 2011, 2013). To the best of our knowledge, no recent bat (or other small mammal) surveys have been conducted in any of the remaining Afromontane forests and adjacent grasslands along this escarpment, and this must remain a critical zone for future surveys.

Two other regions are likely to be fruitful survey locations for bats. One is Kabinda which is on the northern side of the Congo River; this river forms a substantial barrier for African mammals resulting in it forming the southern limit for many species. To the best of our knowledge, Cabinda has not been surveyed specifically for bats before. We suspect that a large number of tropical rainforest species (e.g., *Myopterus whitleyi*, *Mops annulus*, *Mops thersites* and *Nycteris grandis*) will be shown to occur in Cabinda. The second is the extreme south-western arid zone on the Namibian border (part of which is included in Iona National Park). This region has also not been the focus of dedicated bat surveys, but may harbour arid zone specialist species (e.g., *Sauromys petrophilus* and *Rhinolophus denti*) that have not yet been recorded from Angola. We suspect that these three regions (western escarpment, south-western arid zone and Kabinda) will also harbour new country records of other mammal groups, and therefore encourage mammalogists to specifically target these areas.

One reason for this high bat diversity is the considerable range in habitats from lowland desert to high altitude Afromontane forest, and from open grassland to tropical rainforest. Despite the high diversity, no bat species are endemic to Angola. This is rather surprising considering the large number of endemic birds and reptiles (Mills et al. 2011). We suspect that future research will uncover endemic bat species to Angola, most probably along the western escarpment, and particularly on isolated mountain tops coated in forest. By way of example, *Rhinolophus eloquens* has been collected from Jau, Huila Province, a location that is over 2,000 km away from the closest records in the east of the Democratic Republic of Congo (DRC) and Rwanda. These specimens from the American Museum of Natural History are worth re-examining as we suspect that this may refer to a new species within the *R. eloquens*/*R. hildebrandtii* group.

Hyracoidea

Four species of hyraxes are confirmed in Angola including two species of tree hyraxes (Kingdon et al. 2013a). A large number of records of the southern tree hyrax *D. arboreus* in the GBIF database come from 36 georeferenced localities in north-east Angola. The western tree hyrax *D. dorsalis* is known only from Cabinda in Angola (Shulz and Roberts 2013) and is vouched for by two specimens from the collection of the Royal Belgian Institute of Natural Sciences.

Lagomorpha

Only one lagomorph species (*Lepus victoriae*, *African savanna hare*) is known from Angola. GBIF records assigned to *L. capensis*, *L. saxatilis* and *Poelagus marjorita* must be mis-identifications as there is no valid modern records or voucher specimens for the occurrence of these species in Angola.

Macroscelididae

Only two species of sengis or elephant shrews are known to occur in Angola, *Elephantulus brachyrhynchus* and *E. intufi* (Suppl. material 1). Based on proximity to the border, *Macroscelides proboscideus* and *Elephantulus rupestris* may eventually be shown to occur in south-west Angola.

Erinaceomorpha

Only one widespread species in known, the southern African hedgehog, *Atelerix frontalis*.

Perissodactyla

Three native species can be confirmed for Angola (Suppl. material 1). This order is represented by two families in Africa: Equidae (zebras) and Rhinocerotidae (rhinoceroses). Two zebra species can be confirmed to occur in Angola. The threatened Hartman's zebra *Equus zebra hartmannae* is known from Iona National Park while the plain's zebra *E. quagga* is more widely distributed across the country. A single Grevy's zebra *E. grevyi* specimen housed by UCM is from an unknown collector and the locality is simply given as Angola and is likely misidentified since the species is known only from Kenya and Ethiopia (Williams 2013). Only the black rhinoceros *Diceros bicornis* has been recorded in Angola and they have all but been extirpated from the country due to the civil war. No zebras or rhinoceroses were recorded during our 2016 camera trapping assessment.

Pholidota

Four species of pangolin are present in Africa, of which two have been confirmed within the borders of Angola (the ground pangolin *Smutsia temmincki* and the tree pangolin *Phataginus tricuspid* (Hill and Carter 1941; Kingdon and Hoffman 2013b) and two (*Smutisa gigantea* and *Phataginus tetradactyla*) may possibly occur in Cabinda (the Congo River is the southern limit for both these species); this makes Angola possibly the only country on the continent where all pangolin species probably occur.

Primates

After correcting for taxonomic changes, the GBIF database listed 17 species of primates occurring in Angola. Based on species distributions in Butynski et al. (2013), two of these GBIF records could not be verified. Although three un-dated specimens from the Royal Belgian Institute of Natural Sciences from Angola (no specific locality) were labelled as the grey-cheeked mangabey *Lophocebus albigena*, according to Butynski et al. (2013) the species occurs only north of Cabinda. While it could conceivably occur in Cabinda, until we have clear evidence, we regard the Angolan GBIF records of *L. aligena* as dubious. Likewise, although the Red-capped Mangabey *Cercocebus torquatus* is not documented as occurring in Angola by Ehardt (2013), two undated GBIF specimens from Cabinda in the Royal Belgian Institute of Natural Sciences collected by Serge M. Frechkop are labelled as *C. torquata*. Although the species is known to occur near to the Cabinda border, for the moment we treat this record as uncertain. Although the Kinda baboon *Papio kindae* from Angola was included as a synonym of the yellow baboon *P. cyanocephalus* by Butynski et al. (2013), it was shown to be distinct by Mittermeier et al. (2013). Therefore *P. kindae* and not *P. cyanocephalus* is found in Angola. One species not included in the GBIF database but validated by Goossens et al. (2002) and Butynski et al. (2013) as occurring in Cabinda, is the robust chimpanzee *Pan troglodytes*. Considering the above, we consider there to be 16 species of primates occurring in Angola.

Cercopithecidae. The red-tailed monkey *Chlorocebus ascanius* (synonym *Cercopithecus ascanius*) occurs through most of central and north Angola into DRC and other countries. With a highly fragmented distribution, three subspecies are considered to occur in parts of Angola, *C. a. katangae*, *C. a. atrinasus*, and *C. a. ascanius* (Oates et al. 2008a). *Chlorocebus ascanius atrinasus* is reportedly found in northwest Lunda district of northeast Angola, and known from nine specimens (Oates et al. 2008b). GBIF records account for one 1925 Angolan specimen of *C. ascanius* in the American Museum of Natural History collected by Lang, and four specimens from the Field Museum collected in 1954 by G. H. Heinrich from Canzele, 30 km west of Camabatela. The range of the moustached monkey *Cercopithecus cephus* extends from Cameroon southwards into parts of far north west of Angola (likely Cabinda province) (Oates et al. 2008c). The gentle monkey, or blue monkey *Cercopithecus mitis* has great variation with disputed taxonomy but the nominate subspecies *C. m. mitis* is generally considered to be endemic to Angola (Lawes et al. 2013). The De Brazza's Guenon *Cercopithecus neglectus* is found in NE Angola associated with riverine forest habitats, common in many parts of its range (Struhsaker et al. 2008). The Malbrouck monkey *Chlorocebus cynosuros* is part of the *C. aethiops* (vervet) group but was elevated to full species by Groves (2001). *Chlorocebus cynosuros* extends throughout Angola, Zambia and parts of the DRC (Sarmiento 2013). A total of 25 GBIF records from six widely separated localities in west and central Angola document its widespread presence within the country.

The Angola colobus, *Colobus angolensis*, is considered to be endemic to equatorial Africa. The Sclater's Angola colobus, *C. a. angolensis* occur throughout the north-east part of Angola, with populations restricted to forest fragments (Bocian and Anderson 2013).

The southern Talapoin monkey *Miopithecus talapoin*, or Angolan Talapoin monkey is regarded as endemic to Northern Angola and south-west DRC.

The Kinda baboon occurs in central and northern parts of Angola (north of the Cunene river) ranging eastwards through DRC, Zambia. The preferred habitat of this species is reported to be Miombo (*Brachystegia*) woodland in the fire-climax stage (Kingdon 2016). The Chacma baboon *Papio ursinus*, is sympatric in Angola in some part of the range of *Papio kindae*, with the Chacma considered endemic to southern Africa. The species is considered to have three subspecies, of which two inhabits Angola: *P. u. ruacana* ranging into south and central Angola from its Namibian distribution and the *P. u. griseipes* ranging through Zimbabwe, Botswana, and Mozambique with a south eastern intrusion to Angola (Cowlishaw 2013). The chacma baboon occurs in a broad range of habitats within Angola (Hoffman and Hilton-Taylor 2008).

The southern lesser galago *Galago moholi*, ranges broadly across southern Africa, Angola in the North-western extension of their range, reportedly being from the subspecies *G. m. bradfieldi* that occur in north Namibia and south central Angola through to southern DRC and then Zambia and Tanzania, south into northern South Africa (likely the *G. m. moholi*). The southern lesser galago prefers semi-arid woodland and savanna habitats (Pullen and Bearder 2013). The Demidoff's Dwarf Galago, *Galagoides demidoff*, also referred to *G. demidovii*, are recorded in the northern parts of Angola extending northwards and north-eastwards into the DRC Rainforest and Afromontane–Afroalpine forests zones (Bearder 2016). The large eared greater Galago, *Otolemur crassicaudatus,* (synonym *Galago crassicaudatus*) is found throughout most of southern Africa, ranging from Angola in the west, to Tanzania in the east, extend south into KwaZulu-Natal, South Africa. The proposed subspecies, the Miombo silver Galago *Otolemur c. monteiri* (or often elevated to species level *O. monteiri*) are found widely across the range in Angola (Bearder and Svoboda 2013). We photographed what we believe was *Otolemur c. monteiri* during one of the camera trap surveys in the Okavango catchment expedition in 2016.

The western Gorilla *Gorilla gorilla*, extends only into the Cabinda province of Angola.

The potto, *Perodicticus potto*, is considered to have several subspecies and even groups that can be elevated to species designation. The potto, *P. potto edwardsi* occurs in the north-east of Angola and in Cabina, occurring in a range of habitats (Pimley and Bearder 2013). Two undated specimens from Cabinda are located in the Royal Museum of Central Africa.

Proboscidea

There are two elephant species in Africa, *Loxodonta africana* and *L. cyclotis* and both occur in Angola (Kingdon et al. 2013a). Despite being the largest, and presumably most conspicuous mammal, records of elephant presence in Angola have declined substantially post-civil war. Their current distribution appears to be restricted to the northern parts of the country. However, elephant signs (dung and tracks but not photographs) were recorded during our 2016 survey in the Okavango catchment.

Rodentia

The 77 Angolan rodent species identified by the GBIF search provided a close match to the 78 Angolan rodent species recorded by Monadjem et al. (2015). Of the 77 species recorded by GBIF, four species were deleted from the final checklist as they could not be validated due to imprecise locality information and/or un-corroborated identifications which were not supported by other studies: the squirrel, *Heliosciurus rufobrachium*, the molerat *Fukomys damarensis*, the cane rat *Thryonomys gregorianus* and the climbing mouse, *Dendromus messorius*. An additional seven species were added based on validated species records from Happold (2013) or Monadjem et al. (2015): the squirrels, *Funisciurus lemniscatus* and *Anomalurus beecrofti* (Happold 2013), the dassie rat *Petromus typicus*, the long-eared mouse *Malacothrix typica*, the striped mouse *Rhabdomys bechuanae*, the giant rat *Cricetomys emini*, and the climbing mouse *D. leucostomus* (Monadjem et al. 2015). Two new species were added by the current survey of the Angolan Highlands: Setzer's mouse *Mus setzeri* and Woosnam's broad-headed mouse *Zelotomys woosnami*. This brings the final total of Angolan rodent species to 82. As indicated below, several additional rodent species are known to occur on or close to the borders of Angola and will probably be shown to occur there in the future.

Sciuridae. Thomas's rope squirrel *Funisciurus anerythrus* has been recorded at the border of Kabinda and could possibly occur there (Monadjem et al. 2015). The ribboned rope squirrel *F. lemniscatus* was recorded from Kabinda by Happold (2013) although not reported there by Monadjem et al. (2015). The red-legged sun squirrel *Heliosciurus rufobrachium* is not known to occur south of the Congo River but a GBIF record from "Raca Camele, north of Quionlungo" was attributed to a specimen from Yale Peabody Museum identified by A. Heinrich. We flagged this as a dubious record. The isolated population of the forest giant squirrel *Protoxerus stangeri* in northern Angola is treated as an endemic subspecies *P. s. loandae* (Happold 2013).

Gliridae. A high diversity of five species of dormice has been recorded in Angola.

Muridae. Muridae comprises by far the largest family of rodents and 42 indigenous murid species occur in Angola. The species list from the GBIF database corresponded closely with that of Monadjem et al. (2015). Our current trapping survey of the Okavango source lakes region added two murid rodent species to the list for Angola, *Mus setzeri* and *Zelotomys woosnami* (Table 1). As both of these rodents are associated with sandy soils in arid savannas it was surprising to record them from mesic miombo woodlands in the Angolan highlands. However, the rivers and source lakes of the upper Okavango catchment are bordered by sandy habitats that form obvious corridors for the dispersal of these species from the lower catchments of the Okavango Basin where they have hitherto been recorded in Botswana and Namibia. Given that two murid species were added to the country after just two weeks of survey effort in a limited area, it is highly likely that more species of murid rodents will be shown to occur in Angola by future collecting. For example tropical species such as *Deomys ferrugineus*, *Lophuromys ansorgei*, *Hybomys univitattus*, *Hylomyscus aeta*, *H. anselli*, *Mylomys dybowski*, *Praomys petteri* and *Stochomys longicaudatus* occur close to the border of Kabinda or north Angola. On the other hand, species from arid savannas associated with the Okavanga River in Botswana and Namibia, such as *Dasymys cabrali*, may also be found to occur in the upper Okavanga catchments. Although the desert pygmy mouse *Mus indutus* was shown to occur in south-east Angola by Happold (2013), identification of this species is difficult and no molecular sequences are available of this species from Angola (Lamb et al. 2014, Monadjem et al. 2015). Hence, this species is included in the checklist as a dubious record. Another semi-arid habitat murid species that extends into extreme south-west Angola is *Rhabdomys bechuanae* (Du Toit et al. 2012).

Nesomyidae. The presence of Emin's giant pouched rat *Cricetomys emini* from Kabinda is vouched by Musser and Carleton (2005) and Monadjem et al. (2015) but the species does not appear to occur as widely in north Angola as indicated in Happold (2013). The banana African climbing mouse *Dendromus messorius* was recorded by three specimens from the Field Museum from Dundo in the extreme north-east Angola collected by A. Barros Machado in 1948, but there are no known records close to this (Monadjem et al. 2015). We suspect this is a misidentification, and it is interesting that Hayman (1963) commented on a series of five *Dendromus* from the Dundo Museum as follows: "This unstriped *Dendromys* of the Lubda District appears to represent *ansorgei* rather than *messorius* Thomas of the Cameroons, under which name Sanborn (1952) listed a series from the Dundo region examined by him". Since *ansorgei* is a synonym of *mystacalis*, it seems very likely that specimens from Dundo refer to the chestnut African climbing mouse *D. mystacalis*. Vernay's African climbing mouse *D. vernayi* is endemic to the central highlands of Angola and only known from the type locality. The monotypic *Dendroprionomys* (velvet climbing mouse) is known only from the type locality Brazzaville which is close to Cabinda and may be shown to occur there. The long-eared mouse *Malacothrix typica* was not recorded in the GBIF database but is known from extreme south-west Angola (Hill and Carter 1941; De Graaff 1981).

Hystricidae. African brush-tailed porcupines *Atherurus africanus* have been recorded on the border region of Cabinda on both Congo and DRC sides, and undoubtedly this species occurs in Cabinda (Monadjem et al. 2015); however without material evidence we do not include it here. The common porcupine *Hystrix africaeaustralis* has been recorded throughout the country.

Petromuridae. Although not recorded in GBIF, several records of the dassie rat *Petromus typicus* are known from south-west Angola (Monadjem et al. 2015).

Thryonomyidae. The GBIF database contains records from three specimens labelled as *Thryonomys gregorianus* from mount Moco collected in 1954, but the closest known records of this species are from central DRC and west Zambia (Happold 2013; Monadjem et al. 2015). Given the difficulty in distinguishing this species from *T. swinderianus*, we treat this record as dubious.

Pedetidae. Only one widespread species is known to occur in Angola.

Anomaluridae. Although not listed in the GBIF database, both Happold (2013) and Monadjem et al. (2015) show Beecroft's scaly-tailed squirrel *Anomalurus beecrofti* occurring in Kabinda. Happold (2013) also indicates additional records from Angola south of the Congo River.

Soricomorpha

Our GBIF search revealed 13 species of shrews occurring in Angola, all from the genus *Crocidura*, but cross-checking against Hill and Carter (1943) and Happold and Happold (2013) resulted in two species being flagged as highly dubious, plus an additional two species known to occur in Angola (Hill and Carter 1943; Happold and Happold 2013), resulting in a final total of 13 species.

The lesser grey-brown shrew *C. silacea* and Dent's shrew *C. denti* are both included in the GBIF database but their known range is nowhere near Angola (Happold and Happold 2013). The *C. denti* record was an undated record from the Natural History Museum with no recorded locality. The *C. silacea* record was from the Field Museum of Natural History, collected in 1954 from near Quela in north Angola by G. H. Heinrich. Given its location many hundreds of kilometers from other known records, and difficulties in identification of this species, we believe this to be a misidentification and this record is regarded as highly dubious. The heather shrew *C. erica* and the blackish shrew *C. nigricans* are both endemic to Angola. Ansell's shrew *C. ansellorum* and the moonshine shrew *C. luna* have both been collected from the border region of Zambia and Angola, and probably occur in Angola.

Both the greater dwarf shrew *Suncus lixus* and the climbing dwarf shrew *Suncus megalura* are known to occur in Angola (Happold and Happold 2013), hence were added to the final checklist (but were not captured in the GBIF database). Our field collections in the Okavango source lakes area in 2016 added an additional species for the country, the lesser dwarf shrew *Suncus varilla* (Table 1). The species has a sparse distribution and was previously known from sout-east DRC so its occurrence in central Angola is not surprising. Although Hill and Carter (1943) described a similar number of species (13) as here recognised (14), some of their names have become synonyms and their list corresponded to nine of the currently recognised species, representing a 44% increase in real diversity since 1943.

Tubulidentata

The aardvark *Orycteropus afer*, is considered to be a common species in suitable habitats across its range which extends through most of sub-Saharan Africa (Taylor and Lehmann 2015), and is strongly associated with ant nests and termitaria. This species was recorded in the Okavango catchment expedition during our camera trap survey in 2016, and probably occurs widely in the country.

Concluding remarks

The current list of 275 species of Angolan mammals represents an increase of 52 species compared to the exhaustive survey of Hill and Carter (1941) that recorded 223 species. Throughout most of the twentieth century, Angola was largely neglected in terms of mammal research, particularly during the period of the civil war in the late twentieth century (1975–2002), but recent decades have seen renewed research efforts. For example, through the National Geographic Okavango Wilderness Project, acoustic, camera trap and trapping surveys carried out between 2015 and 2017 in the upper Okavango catchment, a previously neglected area, has added valuable new data on mammal occurrences. While estimates of bat species richness for the upper Okavango catchment based on combined acoustic and capture data from our study (33 species) correspond closely with predicted bat diversity for the region based on modelling studies (e.g., Herkt et al. 2016), our survey under-estimated non-volant small mammals species richness (13 species compared to 42 species predicted based on GBIF records for the central Angola plateau).

Rodrigues et al. (2015) identified four biogeographical subdivisions in Angola based mostly on ungulate distributions. In the north, the Zaire-Lunda-Cuanza region was mainly associated with Congolian forests. In the south, the Namibe and Cunene-Cuando Cubango regions were mainly characterized by ungulates widespread in south-western and southern Africa. In between these regions, the Central Plateau region was mainly characterized by a few widespread ungulate species that are relatively common in dense miombo woodlands. These patterns were largely determined by a north-south gradient of decreasing humidity, from mesic tropical forests in the north to savannas and then more arid regions in the south. Patterns corresponding to this rainfall gradient are also evident in bats (Monadjem et al. 2010) and rodents (Monadjem et al. 2015). Angolan is known for a fairly high number of endemic or near-endemic species, particularly in the case of rodents and shrews, where our study identified one shrew and six rodents endemic to the central and western Angolan highlands. The western escarpment Afromontane forests of Angola are particularly important as a centre of both species richness and endemism of plants and vertebrates including mammals (Carleton et al. 2015). We suggest that future work should target more intensive surveys of small mammals in the central and western Angolan highlands to verify the presence and conservation status of threatened and/or endemic small mammal taxa.

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Supplementary material 1

Test list of species names

Authors: Peter J. Taylor, Götz Neef, Mark Keith, Sina Weier, Ara Monadjem, Daniel M. Parker

Data type: species data

Link: https://doi.org/10.3897/zookeys.779.25964.suppl1

Supplementary material 2

Labeled images of example sonograms of each bat species identified

Authors: Peter J. Taylor, Götz Neef, Mark Keith, Sina Weier, Ara Monadjem, Daniel M. Parker

Data type: species data

Link: https://doi.org/10.3897/zookeys.779.25964.suppl2

Supplementary material 3

Excel spreadsheet of occurrence data from cleaned GBIG search of Angolan mammal

Authors: Peter J. Taylor, Götz Neef, Mark Keith, Sina Weier, Ara Monadjem, Daniel M. Parker

Data type: occurence

Link: https://doi.org/10.3897/zookeys.779.25964.suppl3

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Phylogeny of hydrothermal vent Iphionidae, with the description of a new species (Aphroditiformia, Annelida)

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Abstract

The scale-worm family Iphionidae consists of four genera. Of these, *Thermiphione* has two accepted species, both native to hydrothermal vents in the Pacific Ocean; *T. fijiensis* Miura, 1994 (West Pacific) and *T. tufari* Hartmann-Schröder, 1992 (East Pacific Rise). *Iphionella* is also known from the Pacific, and has two recognized species; *Iphionella risensis* Pettibone, 1986 (East Pacific Rise, hydrothermal vents) and *I. philippinensis* Pettibone, 1986 (West Pacific, deep sea). In this study, phylogenetic analyses of Iphionidae from various hydrothermal vent systems of the Pacific Ocean were conducted utilizing morphology and mitochondrial (COI and 16S rRNA) and nuclear (18S and 28S rRNA) genes. The results revealed a new iphionid species, described here as *Thermiphione rapanui* **sp. n.** The analyses also demonstrated the paraphyly of *Thermiphione*, requiring *Iphionella risensis* to be referred to the genus, as *Thermiphione risensis* (Pettibone, 1986).

Keywords

East Pacific Rise, Pacific Ocean, polychaete, systematics, scale-worm

Introduction

Annelid scale-worms (Aphroditiformia) are a particularly common and diverse group at hydrothermal vents (Desbruyères et al. 2006). Most of this diversity is within Polynoidae Kinberg, 1856, but there have been several records of another aphroditiform family, Iphionidae Kinberg, 1856, which currently includes four genera and 13 accepted species

(Read and Fauchald 2018). Iphionidae had been regarded as a subfamily of Polynoidae, until Norlinder et al. (2014) gave it family rank, as it appears it is actually most closely related to Acoetidae (Gonzalez et al. 2018). In addition to DNA sequence data, the monophyly of Iphionidae is supported by the presence of feathered notochaetae, areolae on elytra, and the absence of a median antenna (Gonzalez et al. 2018). The majority of the known diversity of iphionids are within *Iphione* Kinberg, 1856, and these are mostly shallow-water taxa. However, three genera of deep-sea hydrothermal vent iphionids have been described: *Iphionella* McIntosh, 1885 and *Thermiphione* Hartmann-Schröder, 1992, each with two species, and *Iphionides* Hartmann-Schröder, 1977, containing only *I. glabra* Hartmann-Schröder, 1977.

With regards to the hydrothermal vent-associated iphionids, *Iphionella risensis* Pettibone, 1986 was erected for specimens collected from the East Pacific Rise at 20°50'N. Similar to *I. philippinensis*, this species has 13 pairs of elytra. *Thermiphione tufari* Hartmann-Schröder, 1992, was described for specimens also collected from the East Pacific Rise at 21°30'S, well to the south of the type locality of *I. risensis*. A new genus, *Thermiphione* Hartmann-Schröder, 1992, was erected for this species. *Thermiphione* was distinguished from *Iphionella* by the presence of 14 pairs of elytra instead of 13, as well as by having a greater number of segments (Hartmann-Schröder 1992). *Thermiphione fijiensis* Miura, 1994 was subsequently described from hydrothermal vents from the western Pacific (North Fiji Basin), also with 14 pairs of elytra (Miura 1994).

This paper focuses on new deep-sea collections of Iphionidae from Pacific Ocean hydrothermal vents. DNA data was previously published for *Thermiphione fijiensis* (as *Thermiphione* sp.) in Norlinder et al. (2012); herein we add additional DNA data for this species and for the other two known hydrothermal vent Iphionidae. Furthermore, we describe a new vent-associated iphionid species from the East Pacific Rise and assess some morphological and taxonomic issues for Iphionidae.

Materials and methods

Sample collection

Sampling was conducted over several years and at multiple localities (Figure 1, Tables 1, 2). *Thermiphione rapanui* sp. n. and *T. tufari* were collected on several dives by the manned submersible *Alvin* in 2005 at hydrothermal vents of the southern East Pacific Rise (Table 2). *Thermiphione fijiensis* was collected from the Lau Back-arc Basin in 2005 utilizing the ROV *Jason II* (Table 2). *Iphionella risensis* was collected in 2012 using the ROV *Doc Ricketts* from the Alarcon Rise in the Gulf of California, just north of its type locality (Table 2). All specimens are deposited in the Scripps Institution of Oceanography Benthic Invertebrate Collection (SIO-BIC), La Jolla, California, USA. Whole specimens were photographed prior to preservation using Leica MZ8 or MZ9.5 stereomicroscopes. Post-preservation, specimens were examined and photographed using Leica S8 APO and DMR HC microscopes.

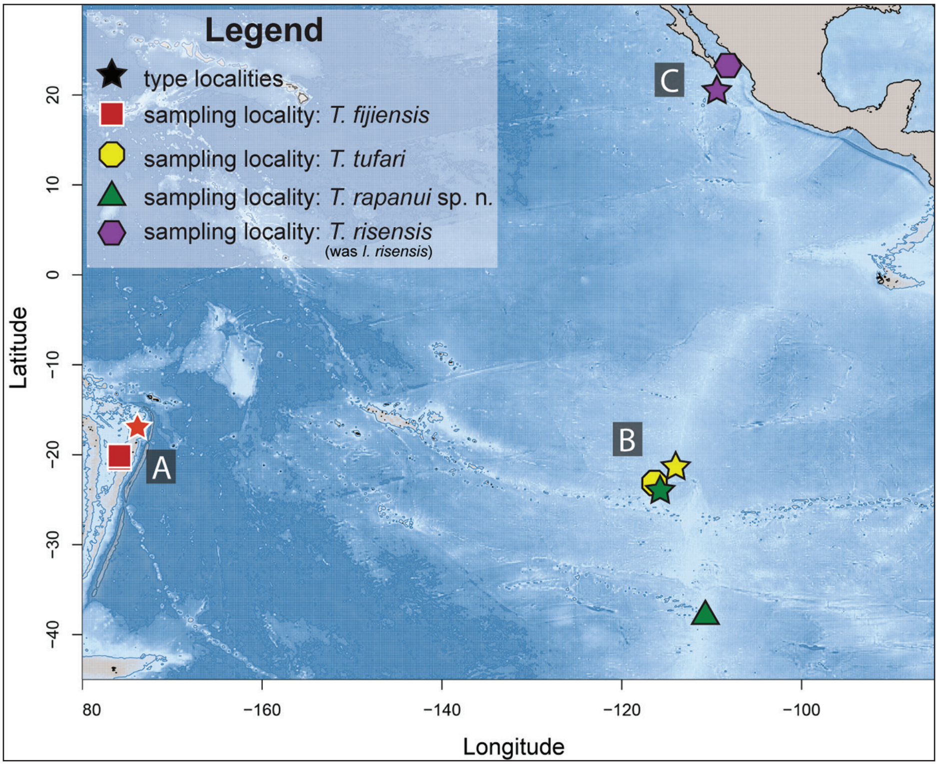

Figure 1. Map of sampling localities for iphionids in this study. Species differentiated by color and shape, type localities represented by stars. **A** *Thermiphione fijiensis* type (star) and sampling (square) localities **B** *Thermiphione tufari* type (star) and sampling (octagon) localities, as well as *Thermiphione rapanui* sp. n. localities (triangle) **C** *Thermiphione risensis* (was *Iphionella risensis*) type (star) and sampling (hexagon) localities.

DNA extraction and amplification

DNA extraction of specimens from the aforementioned collection sites was conducted with the Zymo Research DNA-Tissue Miniprep kit, following the protocol supplied by the manufacturer. Up to 645 bp of mitochondrial cytochrome subunit I (COI) were amplified using the primer set HCO2198 and LCO1490 (Folmer et al. 1994) for multiple specimens in Table 2 and 16S rRNA, 18S rRNA, and 28S rRNA were amplified for a subset of these specimens. Up to 527 bp of 16S rRNA (16S) were amplified using the primer set 16SbrH and 16SarL (Palumbi 1996). 18S rRNA was amplified in three fragments using 18S1F, 18S3F, 18S9R, 18S5R, 18Sbi, and 18Sa2.0 (Giribet et al., 1996; Whiting et al. 1997), resulting in sequence lengths up to 1927 bp. Up to 973 bp of 28S rRNA were amplified using Po28F1 and Po28R4 (Struck et al. 2006). Amplification was carried out with 12.5µl Apex 2.0x Taq RED DNA Polymerase Master Mix (Genesee Scientific), 1µl each of the appropriate forward and reverse primers (10µM), 8.5µl of ddH_2O, and 2µl eluted DNA. The PCR reactions were carried out in a thermal cycler

Table 1. Origin of sequenced terminals, vouchers, and GenBank accession numbers. New sequences in bold. Family assignments follow Zhang et al. (2018).

Scientific name	Origin	Voucher	18S	28S	16S	COI
Panthalis oerstedi	Sweden	SMNH118954	AY839572	JN852845	JN852881	AY839584
Iphione cf. *treadwelli*	Eilat, Israel	–	KY823447	–	KY823478	KY823494
Iphione sp. 1	Hong Kong	–	KY753852	KY753852	KY753835	KY753835
Iphione sp. 2	Papua New Guinea	SMNH118972	JN852819	–	JN852886	JN852921
***Iphione* sp. 3**	Lord Howe Island, Australia	SIO-BIC A8708	–	–	–	**MH389786**
Thermiphione risensis (was *Iphionella risensis)*	Gulf of California	SIO-BIC A6326	**MG994954**	**MH000396**	**MG994947**	**MG981037**
Thermiphione tufari	East Pacific Rise	SIO-BIC A7973	**MG994958**	**MH000401**	**MG994951**	**MG981042**
Thermiphione sp. *(fijiensis)*	Fiji, Lau Basin	SMNH118982	JN852820	JN852849	JN852887	JN852922
Thermiphione fijiensis	Lau back-arc Basin	SIO-BIC A7975	**MG994960**	**MH000402**	**MG994953**	**MG981044**
***Thermiphione rapanui* sp. n.**	East Pacific Rise	SIO-BIC A7969	**MG994955**	**MH000397**	**MG994948**	**MG981038**

Table 2. Sampling localities and GenBank COI accession numbers for all specimens collected and sequenced for this study.

Specimen	Voucher	Locality	Latitude	Longitude	Depth (m)	COI Accession No.
Iphionella risensis	SIO-BIC A6326	Alarcon Rise, Gulf of California	23°22'37"N	108°31'52"W	2,309	MG981037
Thermiphione rapanui sp. n.	SIO-BIC A7969	Pacific Antarctic Ridge	37°47'60"S	110°55'0"W	2,216	MG981038
Thermiphione rapanui sp. n.	SIO-BIC A7970	Pacific Antarctic Ridge	37°47'60"S	110°55'0"W	2,216	MG981039
Thermiphione rapanui sp. n.	SIO-BIC A8557	Pacific Antarctic Ridge	37°47'60"S	110°55'0"W	2,216	–
Thermiphione rapanui sp. n.	SIO-BIC A7971	East Pacific Rise	23°32'47"S	115°34'11"W	2,595	MG981040
Thermiphione rapanui sp. n.	SIO-BIC A7972	East Pacific Rise	23°32'47"S	115°34'11"W	2,595	MG981041
Thermiphione tufari	SIO-BIC A7973	East Pacific Rise	23°32'47"S	115°34'11"W	2.595	MG981042
Thermiphione tufari	SIO-BIC A7974	East Pacific Rise	23°32'47"S	115°34'11"W	2.595	MG981043
Thermiphione fijiensis	SIO-BIC A7975	Lau Back-Arc Basin	20°19'0"S	176°9'0"W	2,719	MG981044
Thermiphione fijiensis	SIO-BIC A8510	Kilo Moana, Lau Back-Arc Basin	20°3'0"S	176°9'0"W	2,657	MG981045
Iphione sp. 3	SIO-BIC A8708	Lord Howe Island, Australia	31°31.603'S	159°4.518'E	5	MH389786

(Eppendorf). The COI temperature profile was as follows: 94 °C/180 s – (94 °C/30 s – 47 °C/45 s – 72 °C/60 s) * 5 cycles – (94 °C/30 s – 52 °C/45 s – 72 °C/60 s) * 30 cycles – 72 °C/300 s. The 16S temperature profile was as follows: 95 °C/180 s – (95 °C/40 s – 50 °C/40 s – 72 °C/50 s) * 35 cycles – 72 °C/300 s. The 18S1F/18S5R temperature profile was as follows: 95 °C/180 s – (95 °C/30 s – 50 °C/30 s – 72 °C/90 s) * 40 cycles – 72 °C/480 s. The 28S temperature profile was as follows: 95 °C/180 s – (95 °C/30 s – 55 °C/40 s – 72 °C/75 s) * 40 cycles – 72 °C/300 s. The PCR product was purified with the ExoSap-it protocol (USB, Affimetrix) and sequencing was performed by Eurofins Genomics (Louisville, KY).

Phylogenetic analyses

Alignments of the newly generated sequences, along with sequence data from GenBank for the four genes presented in Table 1 and published in the most recent aphroditiform phylogeny (Zhang et al. 2018) were performed using MAFFT (Katoh and Standley 2013). Poorly-aligned regions of the three rDNA genes were removed using Gblocks v.0.91b (Catresana 2000), with least stringent settings. This resulted in two concatenated alignments, referred to here as complete and Gblocked. Maximum likelihood (ML) analyses were conducted on the two datasets using RaXML v.8.2.10 (Stamatakis 2014) with each partition assigned the GTR+G model. Node support was assessed via thorough bootstrapping (1000 replicates). Bayesian Inference (BI) analyses were also conducted using MrBayes v.3.2.6 (Rohnquist et al. 2012). Best-fit models for these partitions were selected using the Akaike information criterion (AIC) in jModelTest 2 (Darriba et al. 2012; Guindon and Gascuel 2003). Maximum parsimony (MP) analyses were conducted using PAUP* v.4.0a161 (Swofford 2002), using heuristic searches with the tree-bisection-reconnection branch-swapping algorithm and 100 random addition replicates. Support values were determined using 100 bootstrap replicates. The acoetid *Panthalis oerstedi* Kinberg, 1856, was selected as the outgroup based on recent phylogenomic analyses that place Acoetidae as the sister clade to Iphionidae (Zhang et al., 2018). Uncorrected pairwise distances were calculated for the COI dataset with PAUP* v.4.0a161 (Swofford 2002). Median-joining haplotype networks (Bandelt et al. 1999) for *Thermiphione rapanui* sp. n. and *T. fijiensis* were created with PopART v.1.7 (Leigh and Bryant 2015).

Morphology

Most parsimonious reconstructions for a few relevant characters were mapped onto the molecular phylogeny of Iphionidae using Mesquite v.3.4 (Maddison and Maddison 2018). No DNA data is presently available for *Iphionella philippinensis*, or *Iphionides glabra*, and they are not included in this study. Their eventual phylogenetic placement in Iphionidae will influence the inferred transformations found in this study. Morphological characters used were:

1. Elytra. Thirteen pairs of elytra are found in *Iphionella* (Pettibone, 1986), while *Thermiphione* has 14 pairs (Hartmann-Schröder 1992). Members of *Iphione* have 13 pairs of elytra (Pettibone 1986). The monotypic *Iphionides* has up to 20 pairs (Hartmann-Schröder 1977). Other Aphroditiformia, including the outgroup Acoetidae, normally have many elytral pairs. States, **0**. Many pairs; **1**. 13 pairs; **2**. 14 pairs.
2. Palps. Within Iphionidae, *Iphione* have papillate palps, while all other Iphionidae and the outgroup have smooth palps (Pettibone 1986, Gonzalez et al. 2018). States, **0**. Smooth; **1**. Papillate.
3. Eyes. Within Iphionidae, *Thermiphione* and *Iphionella risensis* lack obvious eyes, while all other Iphionidae and the outgroup have them (Pettibone 1986, Gonzalez et al. 2018). States, **0**. Present; **1**. Absent.
4. Antennae. In general, Aphroditiformia have a median antenna, while most have lateral antennae (Gonzalez et al. 2018). Acoetidae have lateral and median antennae. A median antenna is absent in all Iphionidae, while the presence of lateral antennae varies. In *Iphione*, lateral antennae are present, while they are absent in *Iphionella*, *Iphionides* and *Thermiphione* (Pettibone 1986, Hartmann-Schröder 1992, Miura 1994). States, **0**. Present; **1**. Absent.

Taxonomic note

Iphionella was erected by McIntosh (1885) as a new genus of Polynoidae for a specimen collected from ~900 meters depth from off Philippines, identified as *Iphione cimex* Quatrefages, 1866. This species was therefore the type species for *Iphionella* by monotypy. Pettibone (1986) determined that this identification by McIntosh as *Iphione cimex* was incorrect as the type of *Iphione cimex*, described from the Malacca Strait, actually belonged to Polynoidae and should be placed in a new genus, *Gaudichaudius* Pettibone, 1986, and so it was referred to as *G. cimex* (Quatrefages, 1866). Pettibone (1986) then redescribed the specimen McIntosh (1885) had used to erect *Iphionella* as a new species, *Iphionella philippinensis* Pettibone, 1986. This was not in accordance with the International Code on Zoological Nomenclature at the time (see Art. 70.3; ICZN, 1999). According to 70.3.1, the correct type species name for *Iphionella* was *Iphione cimex* Quatrefages, which should have become *Iphionella cimex* (Quatrefages, 1866). Furthermore, since *Iphione cimex* is the type species of *Gaudichaudia*, then *Gaudichaudia* should become a junior synonym of *Iphionella*. As a result of this, *Iphionella* should be referred to Polynoidae, and the two currently accepted species of *Iphionella*, *I. philippinensis* and *I. risensis* Pettibone, 1986 are in the incorrect genus and require new names. While technically correct, we regard this as not being in accordance of a goal of taxonomic nomenclature to provide stability of names. We therefore endorse Pettibone's (1986) non-ICZN-compliant actions. In order to preserve stability, the type species of *Iphionella* is now fixed here (under Art. 70.3.2 of the ICZN) as *Iphionella philippinensis* Pettibone, 1986, misidentified as *Iphione cimex* in the original designation by McIntosh (1885).

Results

The complete and Gblocked ML, BI and MP analyses (Figure 2) were congruent, showing the same topology for relationships and generally similar high support values within Iphionidae (Figure 2), except for relationships within *Iphione*. The *Iphione* terminals formed a sister clade to a well-supported clade comprised of all the iphionids from hydrothermal vents.

The two known *Thermiphione* species, *T. fijiensis* and *T. tufari*, formed a grade with respect to *Iphionella risensis* (Figure 2). The new species, *Thermiphione rapanui* sp. n., was the well-supported sister group to the sympatric *T. tufari*. The three East Pacific Rise taxa, *I. risensis*, *T. tufari* and *T. rapanui* sp. n. were recovered as the sister group to the western Pacific *T. fijiensis*. The taxonomic implications of the paraphyly of *Thermiphione* and our rationale for the generic placement of the new species are discussed below. The analysis of uncorrected pairwise COI distances (Table 3) showed that *T. rapanui* sp. n. was ~10.5% divergent from its sister taxon, *T. tufari*, and 13–15% divergent from *I. risensis* and *T. fijiensis* (Table 3). For the four specimens of *T. rapanui* sp. n. that we obtained COI sequences for there were three haplotypes that varied from each other by only two base pairs (Figure 4B).

The parsimony reconstruction of ancestral states revealed an unambiguous convergent appearance of 14 pairs of elytra in *Thermiphione fijiensis* and *Thermiphione tufari* and that an elytral number of 13 represents the plesiomorphic state for Iphionidae. The absences of eyes and lateral antennae may be apomorphies for *Thermiphione* (but see below) (Figs 2, 3). The presence of papillate palps was apomorphic for *Iphione* (Figure 3).

Taxonomy

Iphionidae Kinberg, 1856

Thermiphione Hartmann-Schröder, 1992, emended

http://zoobank.org/7BC3CE3F-4C9B-476A-A263-B8B77B961467

Type species. *Thermiphione tufari* Hartmann-Schröder, 1992

Diagnosis (emended). Ventrally flattened, short, oval-shaped body. Between 28 and 32 segments in adults, with 13 or 14 pairs of elytra on segments 2, 4, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 26 (and 27, if 14 pairs) that cover dorsal side. Elytra rounded, covered with polygonal and/or hexagonal areas with lattice-like areolae; may exhibit papillae along elytral margins and on elytral surface near margins. Bilobed prostomium square to oval, merged with segment 1, with short, smooth, bulbous palps. Lateral and median antennae absent. Eyes absent. Segment 1 with paired enlarged anterior cirri (*sensu* Rouse and Pleijel 2001; = tentacular cirri), bearing each pair on a tentaculophore with an acicula and capillary chaetae. Mouth anterior, not ventral. Eversible pharynx with papillae and two pairs of jaws. Segment 2 bears first pair of elytra and parapodia,

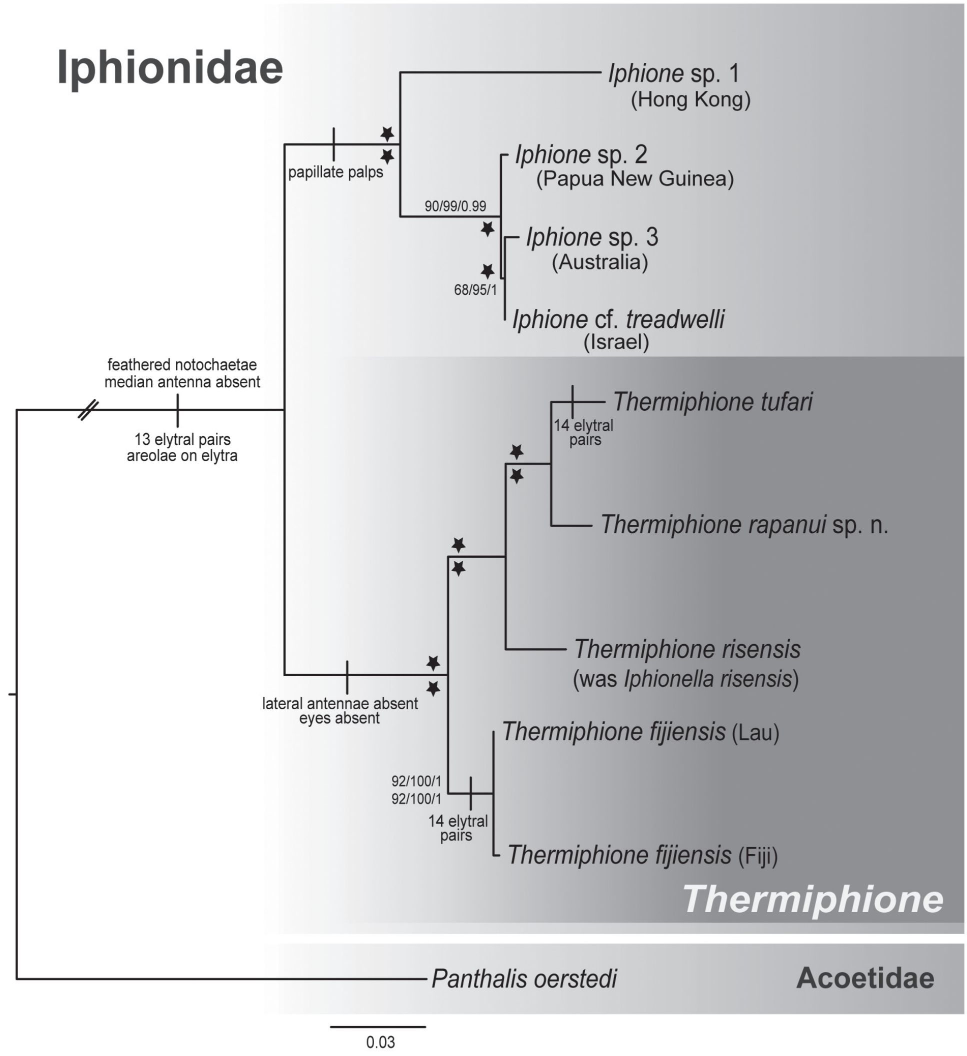

Figure 2. Maximum likelihood tree of the combined analysis from four genes (28S, 18S, 16S, COI) aligned with MAFFT and then concatenated (No Gblocks). Numbers above nodes are bootstrap support percentages from RAxML and Maximum Parsimony analyses (separated by slashes), followed by Bayesian posterior probabilities from the complete dataset alignment (no Gblocks) and below nodes from Gblocks. Support values of 95% or greater for all analyses are indicated by stars.

spherical papillae. Segment 3 barely visible dorsally, with parapodia wedged between segments 2 and 4. Segments 4 and 7 bear spherical ventral papillae. All parapodia biramous: notopodia rounded and much smaller than neuropodia, with bundles of thin, feathered notochaetae; neuropodia large with thicker, single-tipped neurochaetae. Dorsal cirri with short papillae and cylindrical cirrophores. Ventral cirri much smaller than dorsal cirri, short and cirriform. Pygidium inconspicuous, lacking anal cirri.

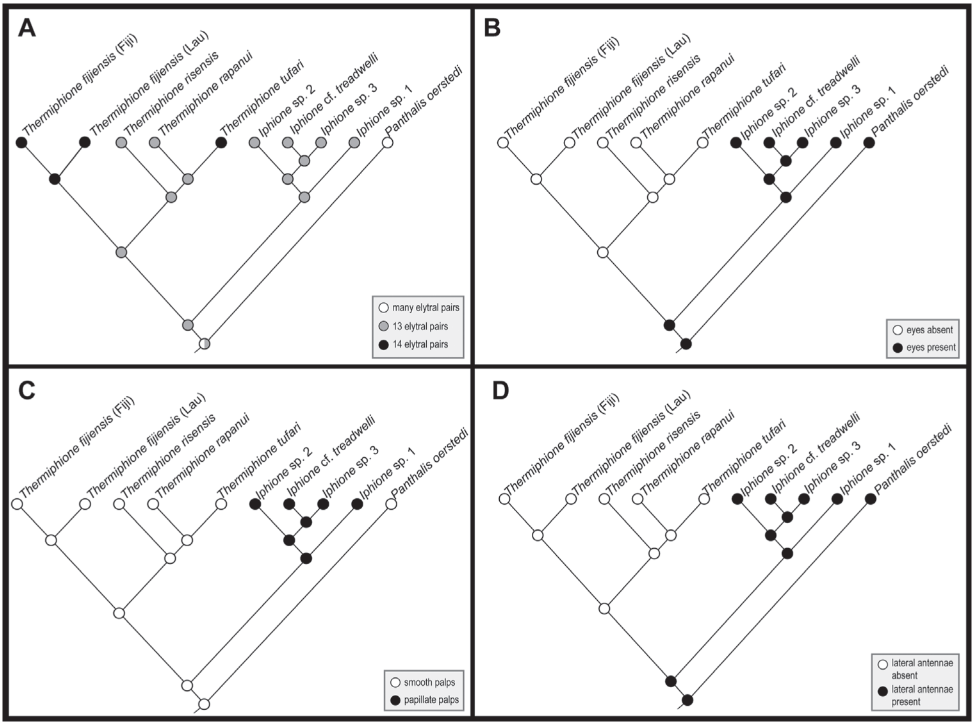

Figure 3. Most parsimonious reconstructions of four traits mapped onto the molecular phylogeny (complete dataset). **A** Elytral pairs **B** Eyes **C** Palps **D** Lateral antennae.

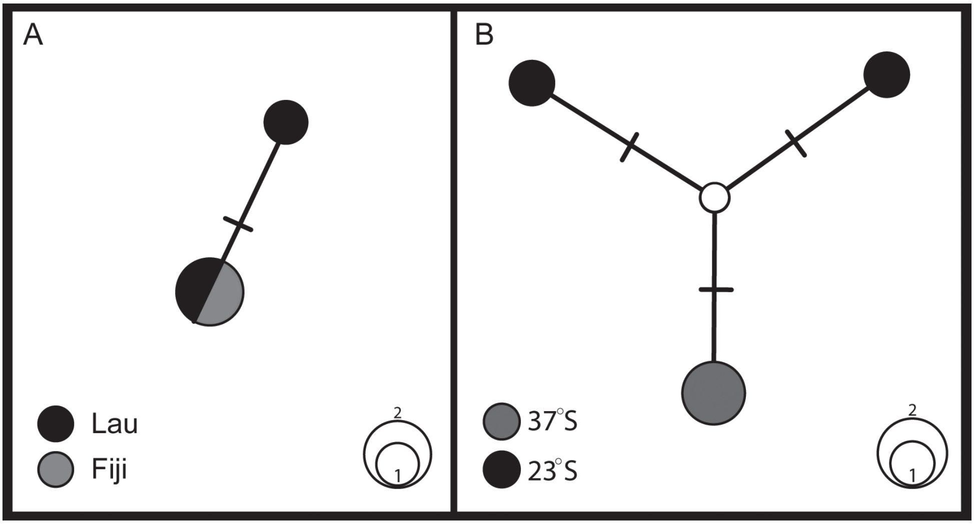

Figure 4. Haplotype networks from COI data: **A** *Thermiphione fijiensis* network includes two sequences from specimens from the Lau Back-Arc Basin (black), and one from the type locality in Fiji (grey) **B** *Thermiphione rapanui* sp. n. network includes two sequences from 23°S (black) and two from 37°S (grey).

Table 3. Uncorrected pairwise distances for COI data, generated with PAUP*.

	***Thermiphione rapanui* sp. n.**	***Thermiphione tufari***	***Thermiphione fijiensis***	***Thermiphione (Iphionella) risensis***	***Iphione* cf. *treadwelli***	***Iphione* sp. 1**	***Iphione* sp. 2**
Thermiphione tufari	10.48%	–	–	–	–	–	–
Thermiphione fijiensis	15.39%	16.67%	–	–	–	–	–
Thermiphione (*Iphionella*) *risensis*	13.39%	14.25%	14.79%	–	–	–	–
Iphione cf. *treadwelli*	18.14%	19.88%	17.27%	19.23%	–	–	–
Iphione sp. 1	21.75%	19.73%	20.39%	21.52%	18.78%	–	–
Iphione sp. 2	23.81%	24.01%	21.66%	24.00%	23.35%	24.73%	–
Iphione sp. 3	18.49%	19.92%	17.42%	19.06%	0.76%	19.75%	23.14%

Remarks. Hartmann-Schröder's (1992) diagnosis of *Thermiphione* has been amended to accommodate the inclusion of *Iphionella risensis* and *Thermiphione rapanui* sp. n. The genus now comprises *Thermiphione fijiensis* (Figure 5A, D), *T. risensis* (Figure 5B, E), *T. tufari* (Figure 5C), and *T. rapanui* sp. n (Figs 6–9). The morphology of these taxa and phylogenetic evidence suggests that segment and elytral numbers are more variable than in the previous diagnosis. *Thermiphione* all have smooth palps, but this is plesiomorphic for Iphionidae. The absence of eyes may be an apomorphic state, depending on the eventual placement of *Iphionella philippinensis*, which was not included here owing to the lack of material for DNA sequencing. Similarly, the loss of lateral antennae may also be an apomorphy for *Thermiphione* once the position of *Iphionella philippinensis* and *Iphionides glabra*, which also lack them, is resolved.

Thermiphione rapanui sp. n.

http://zoobank.org/D201192A-0569-4C3E-8B22-4C3C3C6A27D7
Figures 6–9

Type-locality. German Flats, hydrothermal vents of Pacific Antarctic Ridge, 110°55'W, 37°48'S.

Material Examined. *Type specimens.* Holotype (SIO-BIC A8557) from German Flats, hydrothermal vents of Pacific Antarctic Ridge, (type locality above), HOV *Alvin* Dive 4088, 2216m depth, 22 March 2005; fixed in 10% SW formalin, preserved in 50% ethanol. The holotype was not sequenced directly to avoid damage but was morphologically identical to sequenced specimens from the same locality. Post-preservation, holotype 10 mm long, 8.5 mm wide including parapodia, 31 segments.

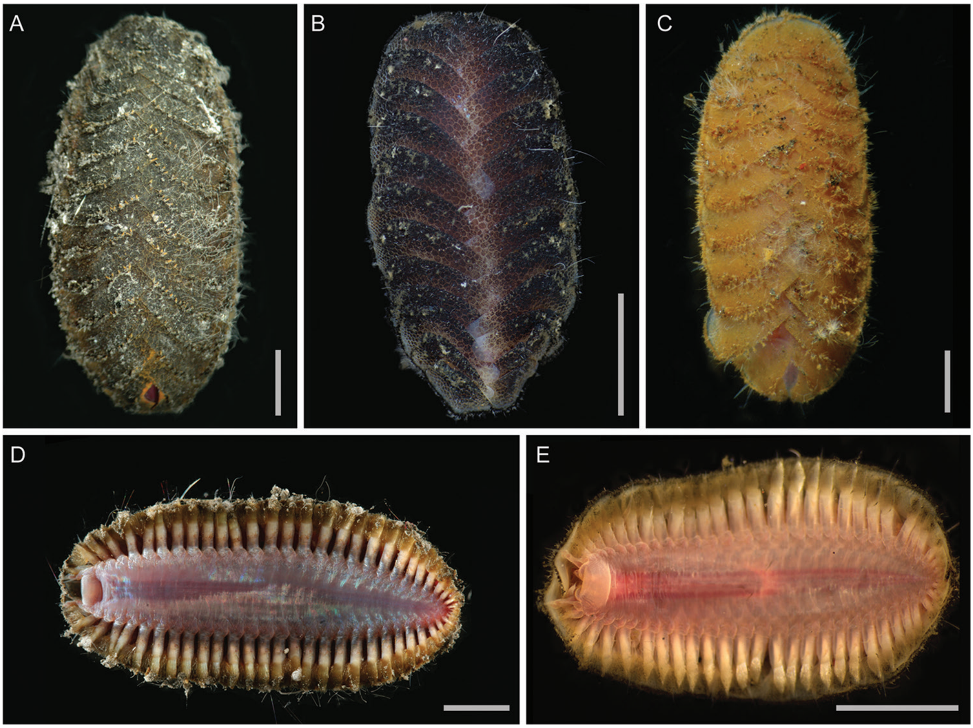

Figure 5. Dorsal and ventral micrographs of species in *Thermiphione*. Scale bars represent 5 mm. **A** *Thermiphione fijiensis* (SIO-BIC A7975), dorsal **B** *Thermiphione risensis* (SIO-BIC A6326, was *Iphionella risensis*), dorsal **C** *Thermiphione tufari* (SIO-BIC A7973), dorsal **D** *Thermiphione fijiensis* (SIO-BIC A7975), ventral **E** *Thermiphione risensis* (SIO-BIC A6326), ventral.

Paratypes: 1 specimen (SIO-BIC A7969) fixed and preserved in 95% ethanol, same location as holotype, post-preservation 9 mm long, 7 mm wide, 29 segments; 1 specimen (SIO-BIC A7970) from same location as holotype: anterior of specimen (approximately 14 segments) fixed in 10% SW formalin and preserved in 50% ethanol and posterior (approximately 14 segments) fixed and preserved in 95% ethanol; 2 specimens (SIO-BIC A7971, juvenile; SIO-BIC A7972) from the western flank of the Easter Microplate, East Pacific Rise, 115°34'W, 23°32'S, HOV *Alvin* Dive 4096, 2595m depth, 6 April 2005. SIO-BIC A7971 fixed and preserved in 95% ethanol, post-preservation 7 mm long, 4 mm wide, 19 segments; SIO-BIC A7972: anterior of specimen (approximately 20 segments) fixed in 10% SW formalin and preserved in 50% ethanol and posterior (approximately 9 segments) fixed and preserved in 95% ethanol.

Diagnosis. Ventrally flattened, oval-shaped body. Between 29 and 31 segments, with 13 pairs of elytra on segments covering dorsum. Elytra covered completely by polygonal areas enclosing areolae, with marginal papillae covering edges. Prostomium bilobed and slightly rounded. Eyes absent. Lateral and median antennae absent. Segment 1 with

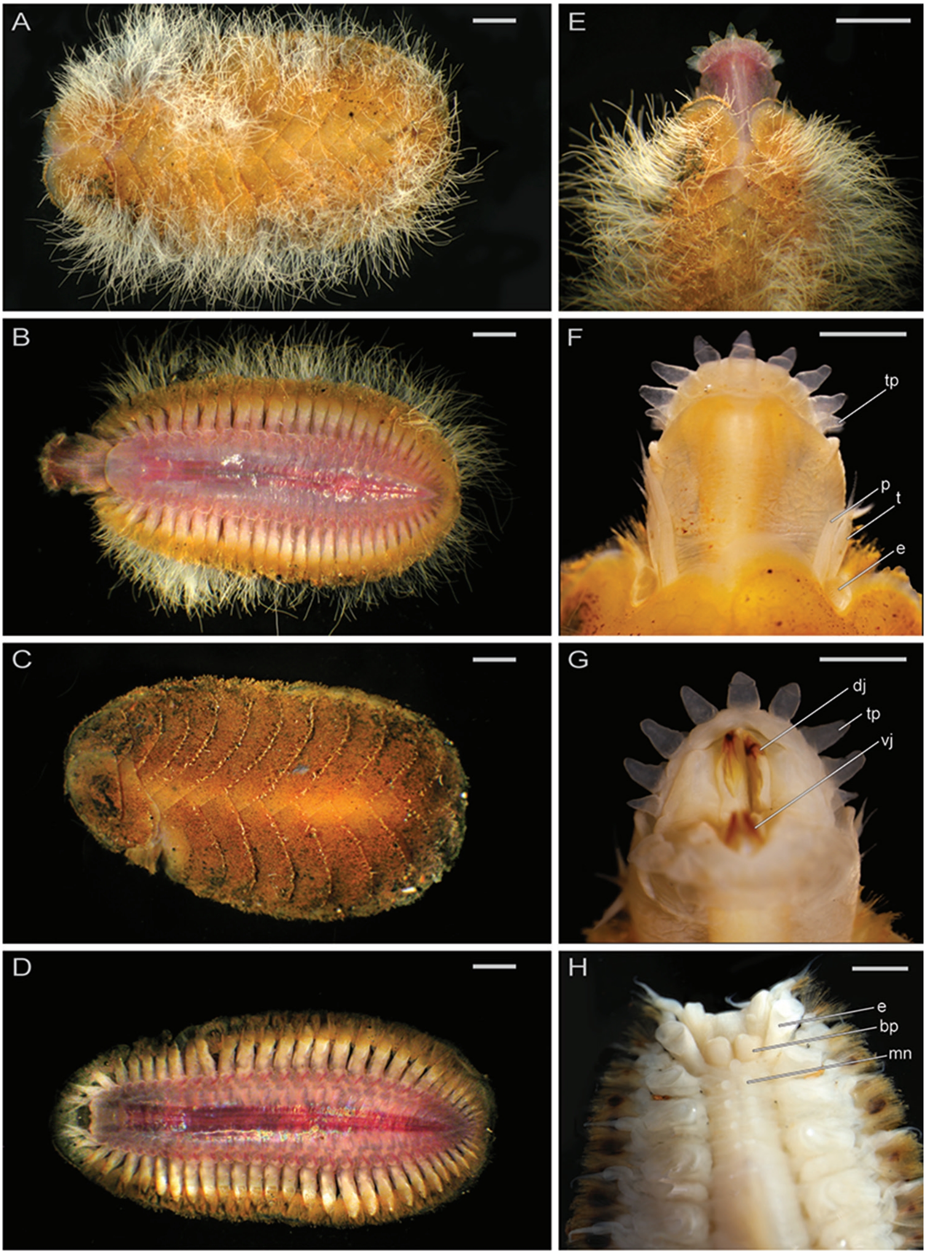

Figure 6. Micrographs of live *Thermiphione rapanui*, sp. n., holotype (SIO-BIC A8557) and paratype (SIO-BIC A7969). Scale bars in A–E represent 1 mm, and scale bars in F–H represent 0.5 mm. **A** Dorsal view of whole body, holotype **B** Ventral view of whole body with pharynx everted, holotype **C** Dorsal view of whole body, paratype **D** Ventral view of whole body, paratype **E** Dorsal view of anterior region with scales, holotype **F** Dorsal view of anterior region with 2 pairs of scales removed, holotype. Abbreviations as follows: *tp*, terminal papilla; *p*, palp; *t*, tentaculophore; *e*, elytrophore **G** Ventral view of anterior region with pharynx and jaws everted/visible, holotype. Abbreviations: *dj*, dorsal jaw; *tp*, terminal papilla; *vj*, ventral jaw **H** Dorsal view of anterior region, paratype. *e*, elytrophore; *bp*, prostomium (bilobed); *mn*, medial nodule.

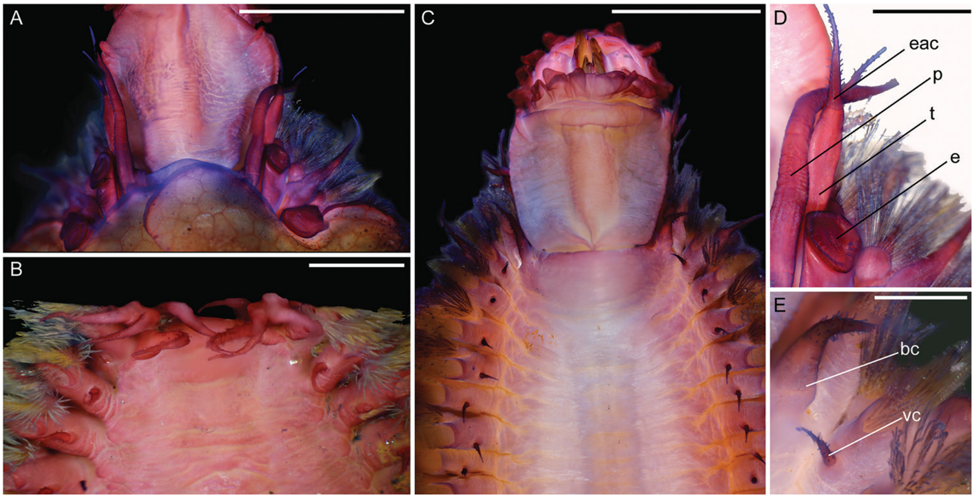

Figure 7. Micrographs of *Thermiphione rapanui* sp. n. holotype (SIO-BIC A8557) and paratype (SIO-BIC A7971), stained with Shirlastain-A. Scale bars in A–C represent 1 mm, and scale bars in D–E represent 0.25 mm. **A** Dorsal view of anterior with 2 pairs of scales removed, holotype **B** Ventral view of anterior showing palps tentaculophore and cirri, paratype. **C** Ventral view of anterior with pharynx everted and jaws visible, holotype **D** Magnified dorsal view of anterior right side, holotype. Abbreviations as follows: *e,* elytrophore; *p,* palp; *t,* tentaculophore; *eac,* enlarged anterior cirrus **E** Magnified ventral view of left anterior parapodia and ventral cirri on segments 2 and 3, holotype. Abbreviations: *bc*, buccal cirrus; *vc*, ventral cirrus.

pair of smooth palps and pair of tentaculophores plus enlarged anterior cirri (tentacular cirri). Mouth anterior with eversible pharynx. Segment 2 with buccal cirri. Segment 3 with dorsal tubercles. Dorsal cirri long with short styles. Ventral cirri short. Anus dorsal. Parapodia biramous with dense bundles of feathered notochaetae and less dense hooked neurochaetae.

Description. In life, elytra pale brown with yellow tinge, becoming slightly paler after preservation. Body ventrally flattened, slightly tapered at anterior and posterior ends (Figure 6A–D). Holotype with 31 segments, 13 pairs of elytra, bacterial filaments on elytra (Figure 6A, B). One mature paratype SIO-BIC A7969, 29 segments, 13 pairs of elytra (Figure 6C, D). One juvenile paratype (SIO-BIC A7971), 19 segments, eight pairs of elytra (identified by scars; elytra lost in sampling).

Pharynx everted anteriorly in holotype, with 9 pairs terminal papillae, and dorsal and ventral pairs of hook-shaped jaws (Figs 6E–G, 7A–C). Prostomium bilobed, slightly rounded; eyes lacking (Figure 6H). Dorsal small circular medial nodules on segments 4 (1), and 5–8 (2 per segment) (Figure 6H). Lateral and median antennae lacking (Figs 6F–H, 7A–C). Pair of smooth palps, longer than pair of tentaculophores plus enlarged anterior cirri (tentacular cirri) (Figs 6F, 7A–B, D). Tentaculophores extending laterally to prostomium (Figs 6F, 7A–B, D), each with single acicula and very thin, short capillary chaetae on inner side. Enlarged anterior cirri, dorsal cirri, and ventral cirri with papillae (Figure 7). Buccal cirri on segment 2, also papillate, appearing larger than

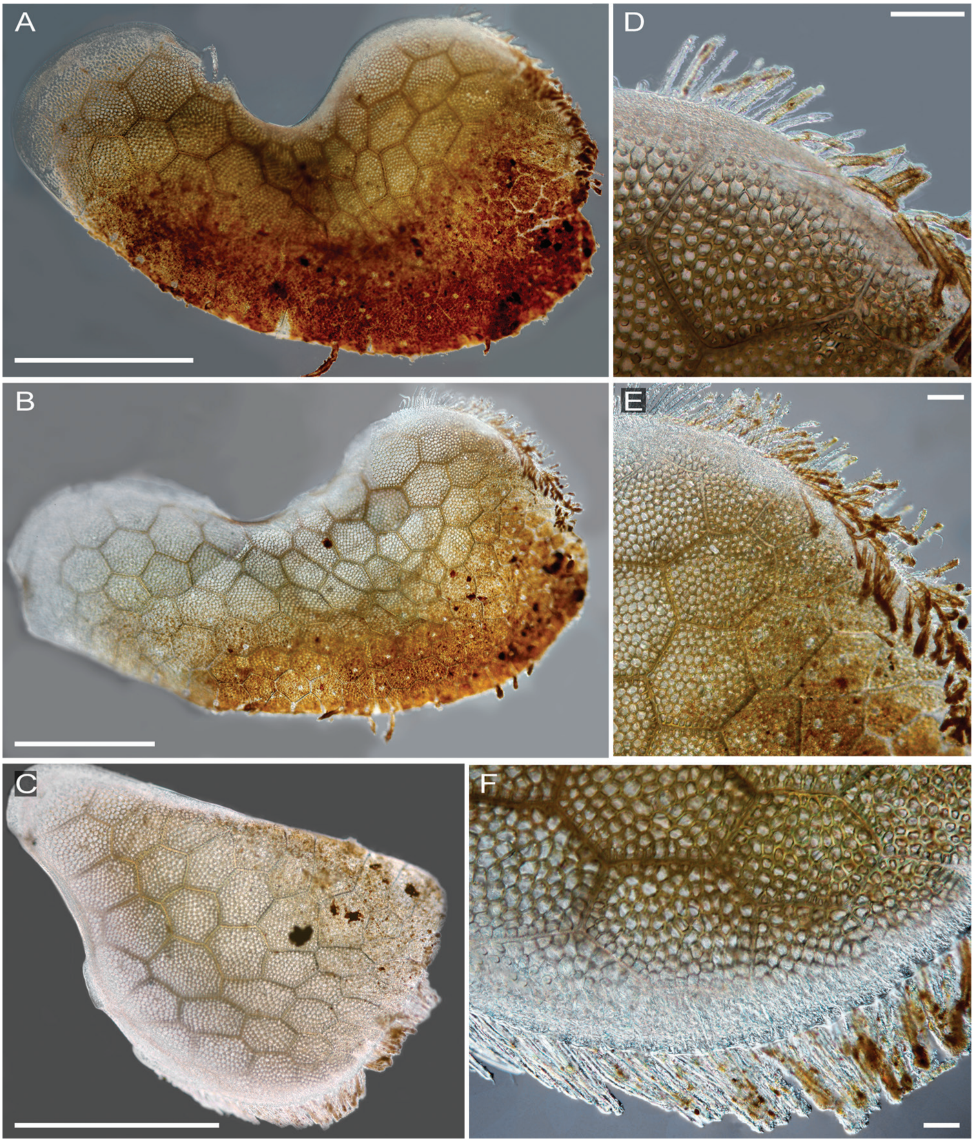

Figure 8. Interference contrast micrographs of *Thermiphione rapanui* sp. n elytra, paratype (SIO-BIC A7969). Scale bars in A–C represent 1mm, and scale bars in D–F represent 0.1 mm. **A** Right elytron 1 **B** Right elytron 3 **C** Left elytron 13 **D** Right elytron 1 margin **E** Right elytron 3 margin **F** Left elytron 13 margin.

remaining ventral cirri (Figure 7C, E). Thirteen pairs of elytra covering dorsum and oval in shape, on segments 2, 4, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 26 (Figure 8). First pair of elytra slightly compressed (Figure 8A); last pair much smaller in size and tapered at one end compared to other elytra (Figure 8B–C). Elytra covered completely by polygonal (generally hexagonal) areas enclosing areolae (Figure 8D–F). Thin, rounded marginal papillae covering lateral edges of elytra, sometimes sparsely extending towards posterior edges of elytra (Figure 8D-F). Remaining segments cirrigerous. Dorsal tubercles and dorsal cirri on segment 3, alternating on segments 6–29, with short, clavate papillae; anal cirri on segments 30, 31 (Figure 6B, D). Dorsal cirri long with short styles, usually extending to near tips of neurochaetae. Ventral cirri much shorter and smaller than dorsal cirri, present on segments 2–29 (Figure 7B–C, E). Anus dorsal; short ventral anal cirri similar to posterior dorsal cirri. Parapodia biramous (Figure 9), with short, subconical notopodia anterodorsal to larger neuropodia (Figure 9). Dense bundles of slender feathered notochaetae, shorter than neurochaetae (Figure 9F, H, J, L). Longer, simple, or slightly hooked neurochaetae, less dense but more numerous than notochaetae (Figure 9G, I, K). Upper neurochaetae generally longer than lower neurochaetae, with length of neurochaetae gradually decreasing towards dorsal and ventral edges (Figure 9).

Variation. Paratypes vary in segment number from holotype and were observed with fewer bacterial filaments on elytra.

Genetic distance. Paratype specimens from the 23°S sampling locality varied by two nucleotide bases from the holotype specimen, 37°S (Figure 4B). This genetic distance is so small that they are certainly all the same species. Unfortunately, our sampling was too limited for any analyses of connectivity.

Etymology. *Thermiphione rapanui* sp. n. is named after the traditional Polynesian name for Easter Island (Rapa Nui), which lies near one of the paratype localities. Neither of the specimens from near Easter Island were chosen as the holotype as they were in poor condition.

Remarks. *Thermiphione rapanui* sp. n. was collected from hydrothermal vents across 15 degrees of latitude, with the northernmost samples collected from the western flank of the Easter Microplate region at 23°S latitude, and the samples from further south collected on the East Pacific Rise at 37°S. The northernmost samples of *Thermiphione rapanui* sp. n. were collected from the same locality as samples of its sister taxon, *T. tufari*, which previously has only been recorded from slightly further north at 21°30'S (Hartmann-Schröder 1992).

Thermiphione rapanui sp. n. differs from its sister taxon *T. tufari* in that it has 13 pairs of elytra instead of 14 pairs of elytra and the last pair of elytra are on segment 26 instead of segment 27 (compare dorsal photos of each in Figs 6A and 5C, respectively). Like *T. tufari*, the new species also has up to 31 segments (Hartmann-Schröder 1992). Both *T. tufari* and *T. fijiensis* (Figure 5A) have 14 pairs of elytra and 30–31 segments (Pettibone, 1986), so elytral number may be convergent (Figure 3). *Thermiphione* was erected by Hartmann-Schröder (1992) and distinguished from other Iphionidae largely based on the presence of 14 pairs of elytra and 30–31 segments, but *Iphionella risensis* (Figure 5B), which nests within the *Thermiphione* (Figure 2), and *Thermiphione rapanui* sp. n. have 13 elytral pairs (Pettibone 1986). However, the two latter species differ in that *I. risensis* has 28–29 segments (Pettibone 1986) and *T. rapanui* sp. n. has 29–31 segments. *T. rapanui* sp. n. also differs from *I. risensis* in the presence of medial nodules on segments 6–8 in *T. rapanui* sp. n., which are absent on these segments in *I. risensis* (Pettibone 1986).

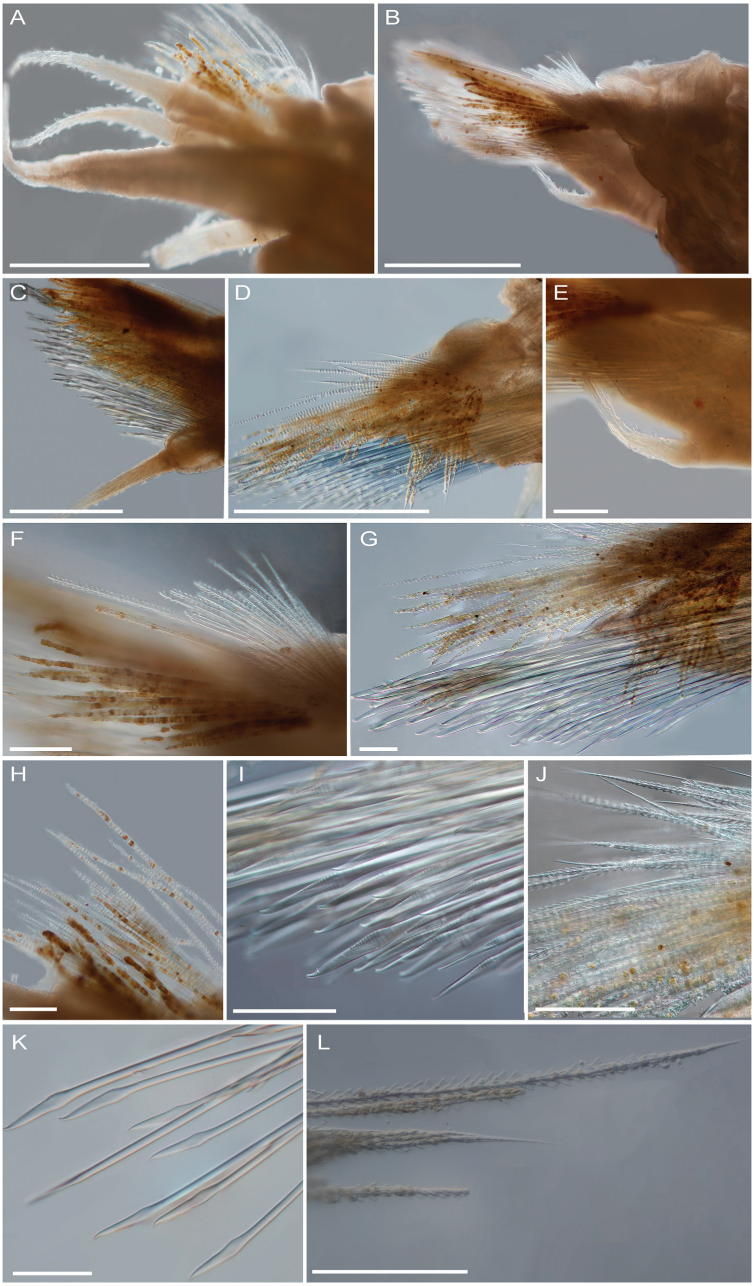

Figure 9. Interference contrast micrographs of *Thermiphione rapanui* sp. n. parapodia, (paratype SIO-BIC A7969). Scale bars in A–D represent 0.5 mm, and scale bars in E–L represent 0.1 mm. **A** Right parapodium 1 **B** Right parapodium 2 **C** Right parapodium 13 **D** Right parapodium 25 **E** Enlarged view of ventral cirrus (parapodium 2) **F** Feathered notochaetae (parapodium 2) **G** Chaetae of parapodium 25 **H** Notochaetae of right parapodium 2. **I** Slightly hooked neurochaetae (right parapodium 25) **J** Feathered notochaetae of parapodium 25 **K** Simple neurochaetae (some slightly hooked) from right parapodium 13. **L** Feathered notochaetae from right parapodium 13.

Discussion

The topologies of the likelihood and parsimony phylogenies are similar to those recovered in the recent analyses of Norlinder et al. (2012), Gonzalez et al. (2018), and Zhang et al. (2018) and support the maintenance of Iphionidae as a family distinct from Polynoidae.

The phylogeny demonstrates that our newly generated sequences for *Thermiphione fijiensis* represent the same species as the *Thermiphione* sp. published in Norlinder et al. (2012). These specimens were collected on the same cruise as the Norlinder et al. (2012) specimen. The *Thermiphione* sp. (Norlinder) specimen was collected at the White Lady hydrothermal vent, near the type locality for *Thermiphione fijiensis*. It is therefore identified here as *T. fijiensis*. The two specimens of *Thermiphione fijiensis* collected from the Lau Back-Arc basin, varied at most by a single base pair from the Norlinder et al. (2012) sequences (Figure 4A).

The distribution of the three East Pacific Rise iphionids sampled in this study (Table 2) and the phylogenetic results (Figure 2) indicate that *Iphionella risensis* forms a northern sister clade to the more southern *Thermiphione rapanui* sp. n. and *T. tufari* clades. This combined eastern Pacific clade is then sister group to *Thermiphione fijiensis* (Figure 2). The placement of *Iphionella risensis* makes *Thermiphione*, as currently formulated, paraphyletic. To resolve the paraphyly of *Thermiphione, Iphionella risensis* should be placed within *Thermiphione* and we do so here by amending the diagnosis for *Thermiphione* to allow for the presence of 13 or 14 pairs of elytra and 28–31 segments (see below). No DNA data currently exists for the type species of *Iphionella*, *I. philippinensis*.

Acknowledgements

Many thanks to Robert Vrijenhoek (MBARI) for inviting Greg Rouse on cruises to various Pacific hydrothermal vent localities. Thanks also to Nerida Wilson for help with sorting the samples that included *T. rapanui* n. sp., to Geoff Read who gave valuable advice on resolving *Iphionella* nomenclature, and to Charlotte Seid for her collections support. The crews of the R/V *Melville*, R/V *Western Flyer*, and the pilots of the ROVs *Jason II* and *Doc Ricketts* provided essential expertise, for which we are grateful. We would also like to thank an anonymous reviewer and Christina Piotrowski, as well as Zookeys editor Chris Glasby. Analysis of the specimens for this project was funded by the US National Science Foundation (NSF OCE-1634172).

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DATA PAPER

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Monitoring data of marine turtles on the Togolese coast during 2012–2013

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Abstract

This dataset contains information on the presence and distribution of sea turtles in Togo. Observations were carried out through a network of ten ecoguards (local guides), facilitated by five fishermen, and coordinated by a field technician, all under the supervision of a scientific coordinator. Data on the occurrence or direct observation of sea turtles on the Togolese coast from September 2012 to August 2013 is presented based on 740 occurrences.

Keywords

Chelonia mydas, Cheloniidae, coast, Dermochelyidae, *Dermochelys coriacea*, ecoguards, *Lepidochelys olivacea*, Togo, West Africa

Introduction

Sea turtles emerged approximately 130 million years ago and are the only marine forms of the reptilian class (Márquez 1990, Frazier 2003). They are migratory species whose populations are essentially distributed in the intertropical zone (Pritchard 1997). Because of their phylogeny, physiology, and behaviour, these species represent an ancient and important component in marine and coastal ecosystems (Ferraroli et al. 2003). They occupy all ecological niches available in the marine ecosystems. They are herbivorous, carnivorous, or omnivorous and are preyed upon by large marine predators such as sharks and orcas (Bjorndal 1997). Frazier (1999) and Bjorndal and Jackson (2003) have demonstrated that these animals play an important role in their habitats, and their vitality depends on the exploitable resources (fish, molluscs, and mangroves). According to Segniagbeto et al. (2017), fishing nets represent the main conservation problem for the various Togolese sea turtle species, and cause demographic strain of turtle populations.

As sea turtles migrate over thousands of kilometres, and the fact that they take tens of years to reach maturity, sea turtles serve as health indicators of coastal and marine environments, both locally and globally (Meylan and Donnelly 1999, Frazier 1999, Fretey 2001). In West Africa and particularly in Togo, a number of studies have focused on marine turtles (Fretey 2001, Segniagbeto et al. 2013, 2014, 2016, 2017). Of the six species known worldwide, five are present in Togo. These are the Green turtle (*Chelonia mydas* (Linnaeus, 1758)), Olive ridley (*Lepidochelys olivacea* (Eschscholtz, 1829)), Loggerhead turtle (*Caretta caretta* (Linnaeus, 1758)), Hawksbill turtle (*Eretmochelys imbricata* (Linnaeus, 1766)), and Leatherback turtle (*Dermochelys coriacea* (Vandelli, 1761)). According to the above-mentioned works, feeding, and reproduction are the main reasons explaining the presence of these species on the Togolese coast.

As part of the implementation of the Environmental and Social Management Plan (ESMP), linking to the container terminal construction at the Lome Autonomous Port (by Lome Containers Terminal - LCT), a follow-up program for marine turtles was developed between September 2012 and August 2013, to determine the dynamics of their attendance on the Togolese coast. For this purpose, a monitoring protocol has been developed to collect data on the presence of marine turtles at the construction site of the terminal and its area of influence. The objective of this monitoring program was to verify the assumptions made in the ESIA report, which asserts the presence of marine turtles in the project construction zone, and to propose measures to reduce risks of disturbance and accidents of these animals caused by the construction works. The data collected also made possible to analyse the ecological parameters connected to the use of Togolese beaches by marine turtle species. In the following paragraphs, we present the data collection method used in this monitoring program.

Previous knowledge available in digital form from the GBIF data network is summarized in Table 1 and compared with the data contributed by the dataset described herein, which almost doubles the number of records known for the three species of sea turtles from the region (Figure 1).

Table 1. Sea turtle records from the region per species and datasets as available from www.gbif.org (March 2018).

Datasets contributing sea turtle records for the region (Coastal areas of Ghana, Togo and Benin)	**Number of records per species**			**Source**
	L. olivacea	***C. mydas***	***D. coriacea***	
Tortue Olivâtre. Données publiées dans le cadre du projet JRS Bénin	161	12	25	Dossou-Bodjrènou 2016
Census of the threatened species of Benin.	1	1	1	Kiki and Ganglo 2017
SMNS Herpetologie	1	–	–	Schlüter 2015
iNaturalist Research-grade Observations	1	1	–	iNaturalist.org 2018
National census of *Lepidochelys olivacea* (Benin)	163	12	25	Dossou-Bodjrenon and Dossa Gbo 2015
Census of the animals of Benin	–	1	1	Kingbo and Kiki 2016
Sizing Ocean Giants	–	–	1	McClain and Mackay 2017
Dataset described herein	409	309	19	

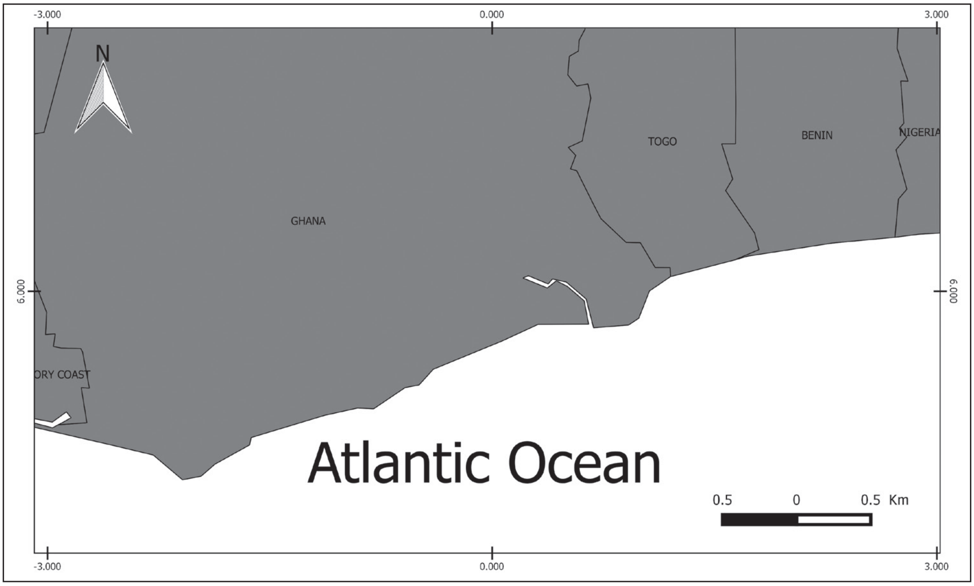

Figure 1. Region of the record Datasets of sea turtles (coastal areas of Ghana, Togo and Benin).

Project details

Project title: BID-AF2015-0004-NAC Monitoring data of marine turtles on the Togolese coast over 2012-2013 years.

Personnel: Délagnon Assou, Gabriel H. Segniagbeto

Data published through GBIF: http://ipt-togo.gbif.fr/resource?r=marine_turtles

Funding

This marine turtle monitoring program was funded by the LCT (Lome Container Terminal). This company, established in Togo, carried out a project including the design, financing, construction, management, and operation of a private containers terminal at the port of Lomé. In compliance with the setting of the framework environmental law in Togo, an Environmental and Social Impact Assessment (ESIA) with an Environmental and Social Management Plan (ESMP) was carried out before the implementation of the project. Recommendations of this study required a sea turtle monitoring program entrusted to the NGO named AGBO-ZEGUE. Indeed, this NGO is specialized in monitoring populations of endangered marine and coastal species in Togo and West Africa.

Thus, to carry out this monitoring of marine turtles along the Togolese coast, AGBO-ZEGUE has initiated and installed a network of ecoguards whose mission was to collect data on sea turtle attendance and egg-laying.

Design description

As part of this monitoring program, five observation sites have been defined to cover the Togolese littoral zone where the construction work of the container terminal is likely to impact, in particular on the access of sea turtles to the coast to lay eggs (Figure 2). The description of these sites includes their geographical limits, their physical, topographical and biological characteristics, justifying the motivations that led to their choice.

Site 1: Length = 6 km; Going from the Ghana-Togo border to the Rond-Point of “Hotel de la Paix”. This site presents a sandstone favourable to the nesting of marine turtles. The site is under heavy light disturbance: the lights emitted at night by ships added to the lights of streetlights and buildings can influence the behaviour of egg-laying females.

Site 2: Length = 5.5 km. It extends from “Hotel de la Paix” to the main pier of the autonomous port of Lome. It corresponds to the construction site of the LCT container terminal and is one of the most affected areas by the port activities. The monitoring program took into account this site in order to evaluate the impact of construction activities on its attendance by sea turtles.

Site 3: Length 10 km, from Gbetsogbe village to Kpogan. Like site 2, site 3 is also part of the zone of influence of the construction of LCT. It is the longest site of the monitoring program. It is characterized by a beach-rock bench visible at low tide. This natural building is sometimes a serious handicap for female sea turtles coming to lay at low tide.

Site 4: Length = 8 km, from Kpogan to Adissen village. It is part of the area of influence of the LCT as the site 3. It has the distinction of having a dark beach back at night and therefore conducive to nesting behaviour of sea turtles. The beach-rock bench is present but of low amplitude. This supralittoral consists of sand grain favour-

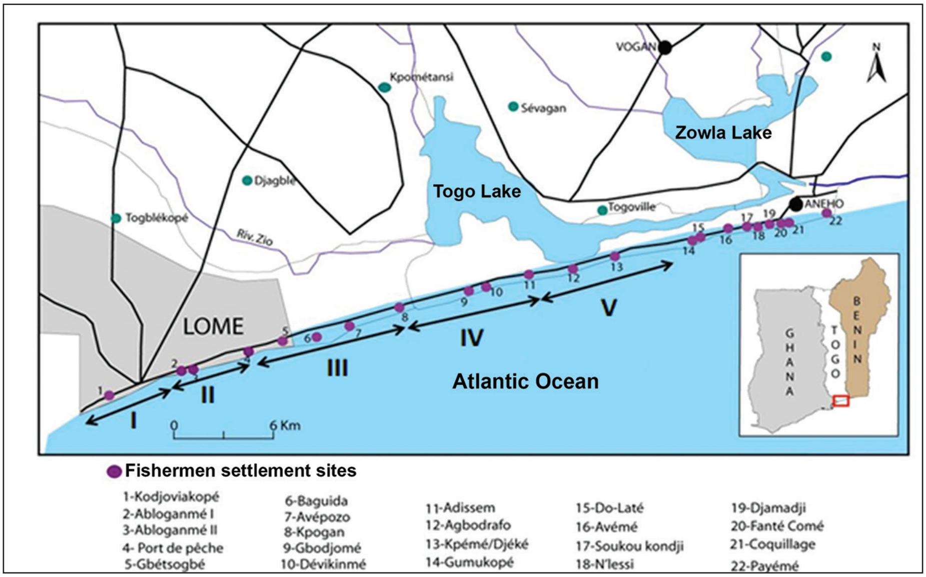

Figure 2. Monitoring program sites (Segniagbeto et al. 2017).

able to the incubation of sea turtle eggs. This site is the second most frequented by marine turtles in Togo (Segniagbeto 2004).

Site 5: Length = 8 km, from Adissen to the ore port of Kpémé. It is one of the most frequented sites for sea turtle nesting in Togo (Segniagbeto 2004 and Segniagbeto et al. 2013). Referring to the data on migratory behaviour and local movements of these animals, site 5 is part of the influence zone of construction activities of the container terminal. Indeed, these local displacements are favoured by marine currents that appear on the Togolese coast from West to East. Site 5 offers a dark backside beach at night. Ship lights and human settlements reported on Site 1, would divert these animals to these dark areas. The beach-rock bench is virtually non-existent and thus favours the access of sea turtles to the beach even at low tide. The granulometry of the sand is also favourable to the incubation of sea turtles. Hatchery trials had already been successfully conducted on this site between 2002 and 2005 (Segniagbeto et al. 2013).

Taxonomic coverage

General taxonomic coverage description

During the 2012–2013 period and the monitoring campaign, three species of marine turtles were observed. 740 occurrences were recorded and distributed as follows: 409 individuals of Olive ridley turtle (*Lepidochelys olivacea* (Eschscholtz, 1829)) (Figure 5a), 309 occurrences of Green turtle (*Chelonia mydas* (Linnaeus, 1758)) (Figure 5b), and

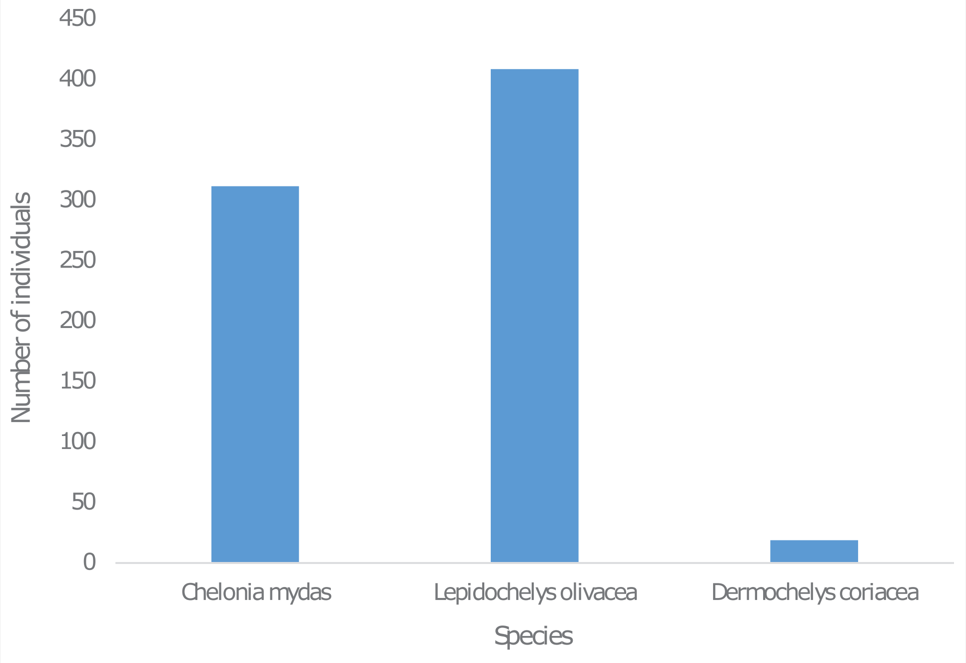

Figure 3. Distribution of marine turtle occurrences by species on the Togolese coast.

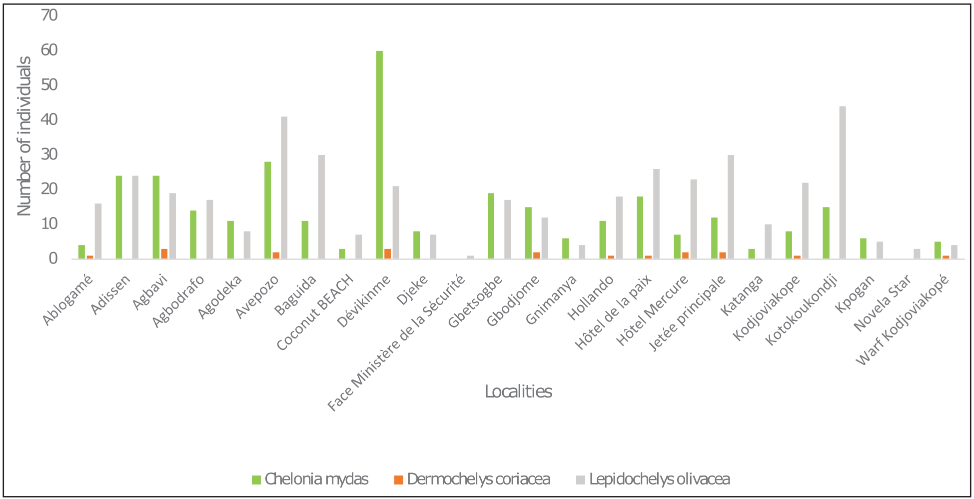

Figure 4. Distribution of marine turtle occurrences by species and location on the Togolese coast.

19 of Leatherback turtle (*Dermochelys coriacea* (Vandelli, 1761)) (Figure 5c). Figure 3 shows the distribution of these turtle on the Togolese coast. This distribution is more or less extensive depending on the collection locations (Figure 4).

Figure 5. Sea turtle species considered in the survey: **a** *Lepidochelys olivacea* **b** *Chelonia mydas* **c** *Dermochelys coriacea.*

Common names

Lepidochelys olivacea (Eschscholtz, 1829) (Figure 5a): Olive ridley turtle (English); Eklo (Ewe).

Chelonia mydas (Linnaeus, 1758) (Figure 5b): Green turtle (English); Eklo (Ewe).

Dermochelys coriacea (Vandelli, 1761) (Figure 5c): Leatherback turtle (English), Agbozegue (Ewe).

Spatial coverage

General spatial coverage: This monitoring program was carried out all over the Togolese coast. This coast stretches from Kodjoviakopé, the last Togo district before Togo-Ghana border to Aného, a city in Togo which borders Benin (Togo-Benin border). For the study, this area was subdivided into five sites.

Coordinates: The study area is located between 6°6'32.4"N and 6°17'31.2"N Latitude; and 1°8'13.2"E and 1°49'1.2"E Longitude (DMS).

Methods

Temporal coverage: The monitoring program was implemented from September 2012 to August 2013. During this period, the local guides do the patrols every day from 04:00 to 06:00 am.

Method step description

Study extent description

Monitoring was carried out by a network of ten ecoguards and five facilitator fishers. All activities of ecoguards and facilitators are coordinated by a technical facilitator coming from the NGO. The latter retrieves weekly all data recorded by ecoguards and strips them. In addition to this coordination, he participates in night patrols, in the surveillance of fishermen's camps during the day and, when necessary, participates in the facilitation of the release of captured sea turtles. Data collected are then transmitted to the NGO scientific coordinator who analyses the data recorded and outputs summary results every semester.

Sampling description

The method of data collection is based on regular monitoring of nesting beaches and the different camps of fishermen. Surveillance is organized day and night. For security reasons, patrols at night were organized in the early morning between 04:00 and 06:00 am. Visits were carried out every day from September 2012 to August 2013. During the day, fishing camps are monitored between 10:00 am and 4:00 pm in order to recover and release the animals captured alive by these fishermen. This work is carried out according to the characteristics of the sites in particular the parameters related to the safety of the places and the work period of the coastal fishermen. The average observation effort per ecogard and per site varies between 60 and 90 hours per month. Two ecoguards positioned per site are chosen from the villages located at the ends of their site, and specifically educated for this study patrolled the sections on a daily basis collecting data. During patrols, ecoguards per site meet every day at a point on their site. This technique allows to cover all the highlights of their site. Thus, all information is collected systematically. Each turtle was identified to species and sex, and its carapace was measured with a tape following the technique by Bolten (1999). Data for females coming to lay on these beaches were recorded on a form where details such as species, date and time of laying, the locality, the biometric measurements, the return to sea or the death of the individual were recorded. The form also allowed recording of tracks on beaches, accidental capture and killing by fishermen, killing and other information. Besides this collection form, field notebooks were used to register other observations on each individual encountered on the beaches. Ecoguards used the identification sheets developed by Fretey and Prichard (2000) to recognize the different species present on the Togolese coast.

Dataset description

Object name: Darwin Core Archive BID-AF2015-0004-NAC Monitoring data of marine turtles on the Togolese coast over 2012–2013 years.

Character encoding: UTF-8
Format name: Darwin Core Archive format
Format version: 1.0
Distribution http://ipt-togo.gbif.fr/resource?r=marine_turtles
Publication date of data: 2018-04-17
Language: English
Licences of use: Creative Commons Attribution (CC-BY) 4.0 License

Metadata language: English
Date of metadata creation: 2018-04-17
Hierarchy level: Dataset

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RESEARCH ARTICLE

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Araneus bonali sp. n., a novel lichen-patterned species found on oak trunks (Araneae, Araneidae)

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Abstract

The new species *Araneus bonali* Morano, **sp. n.** (Araneae, Araneidae) collected in central and western Spain is described and illustrated. Its novel status is confirmed after a thorough revision of the literature and museum material from the Mediterranean Basin. The taxonomy of *Araneus* is complicated, but both morphological and molecular data supported the genus membership of *Araneus bonali* Morano, **sp. n.** Additionally, the species uniqueness was confirmed by sequencing the barcode gene cytochrome oxidase I from the new species and comparing it with the barcodes available for species of *Araneus*. A molecular phylogeny, based on nuclear and mitochondrial genes, retrieved a clade with a moderate support that grouped *Araneus diadematus* Clerck, 1757 with another eleven species, but neither included *Araneus bonali* sp. n. nor *Araneus angulatus* Clerck, 1757, although definitive conclusions about the relationships among *Araneus* species need more markers examined and a broader taxonomic coverage. The new species was collected on isolated holm oaks and forest patches within agricultural landscapes. Adults were mostly trapped on tree trunks, where their lichen-like colours favour mimicry, while juveniles were collected on tree branches. Specimens were never found either in ground traps or grass samples. This species overwinters as egg, juveniles appear in early spring, but reproduction does not take place until late summer-early autumn. *Araneus bonali* Morano, **sp. n.** was found in the same locality from where another new spider species was described. Nature management policies should thus preserve isolated trees as key refuges for forest arthropods in agricultural landscapes, as they may be hosting more unnoticed new species. After including *Araneus bonali* Morano, **sp. n.** and removing doubtful records and synonymies, the list of *Araneus* species in the Iberian Peninsula numbers eight.

Keywords

DNA barcoding, Iberian Peninsula, isolated trees, mimicry, molecular phylogeny, *Quercus ilex*

Introduction

The genus *Araneus* Clerck, 1757 includes 641 species of orb weaver spiders distributed worldwide (World Spider Catalog 2018), of which ten species have been cited in the Iberian Peninsula (Morano et al. 2014; Nentwig et al. 2018). It initially comprised species now included in different genera such as *Araniella* Chamberlin & Ivie, 1942, *Larinioides* Chamberlin & Ivie, 1942 or *Aculepeira* Caporiacco, 1934. While copulatory organs of the species in the younger genus are quite uniform, those of the species remaining in *Araneus* are highly heterogeneous (Dondale et al. 2003), suggesting that the genus is still far to form a natural grouping. The genus *Araneus* has never been revised and currently constitutes a melting pot of species that superficially resemble the type species *Araneus angulatus* Clerck, 1757. For example, some species described from Australia most likely do not really belong to *Araneus* (see Framenau 2012). Moreover, recent studies based on analyses of species from North America, Europe, and Australia have shown that *Araneus* is polyphyletic (Gregoric et al. 2015), which has been further corroborated by recent phylogenomic studies (Kallal et al. 2018).

Here, a new species, *Araneus bonali* sp. n. (Araneae, Araneidae), collected at several localities in western and central Spain is described. The comparison of *Araneus bonali* with reference material from the Iberian Peninsula and the south-western Mediterranean Basin available at the National Museum of Natural Sciences (Madrid) confirmed that the specimens represent a new species. In addition, a bibliographic review of the descriptions of Holarctic *Araneus* species was performed (Levi 1971, 1973, 1991; Dondale et al. 2003, Simon 1929; Nentwig et al. 2017; Grasshoff 1968; Šestáková et al. 2009, Yin et al. 1997, Tanikawa 2007, 2009). A special focus was paid to the species catalogues from northern Africa, where the spider fauna is still insufficiently known. In total seven species of *Araneus*, three of which are doubtful, have been cited in five countries: Egypt (Audouin 1826; Cambridge 1876; Denis 1945), Tunisia (Pavesi 1880; Simon 1885), Lybia (Cambridge 1872; Simon 1908; Caporiacco 1934), Algeria (Lucas 1846; Simon 1899) and Morocco (Thorell 1875; Simon 1909; Denis 1945; Jocqué 1997).

Due to the challenging taxonomy of *Araneus*, the generic delimitation of the new species was queried by using morphological and molecular data. For the morphological analyses, the criteria exposed in the cladistic analysis of the family Araneidae performed by Scharff and Coddington (1997) were followed. For the molecular analyses a nuclear gene (28SrRNA) was sequenced and blasted in GenBank to assess the closer genera in terms of raw sequence similarity. Additionally, a fragment of the barcoding mitochondrial gene cytochrome oxidase I was sequenced and compared with the sequences of *Araneus* available in GenBank and BOLD (Barcoding of Life Datasystem). All the evidence pointed to the same conclusion: a new species of the genus *Araneus* had been collected.

Most of the specimens of the new species were collected during an extensive sampling campaign carried out in central Spain, in the same site where a new Eutichuridae spider was recently described, namely *Cheiracanthium ilicis* (Morano & Bonal, 2016). As in the case of *C. ilicis*, *Araneus bonali* sp. n. was found on the branches and trunks of holm oaks *Quercus ilex* interspersed within agricultural fields. Based on a one-year-long systematic sampling, the habitat selection and the phenology of the new species were analysed, testing whether juveniles and adults showed different patterns. Finally, the literature was consulted and all the *Araneus* species cited from the Iberian Peninsula listed. Their taxonomic status is discussed and the number of species updated after removing dubious species records and including *Araneus bonali* sp. n.

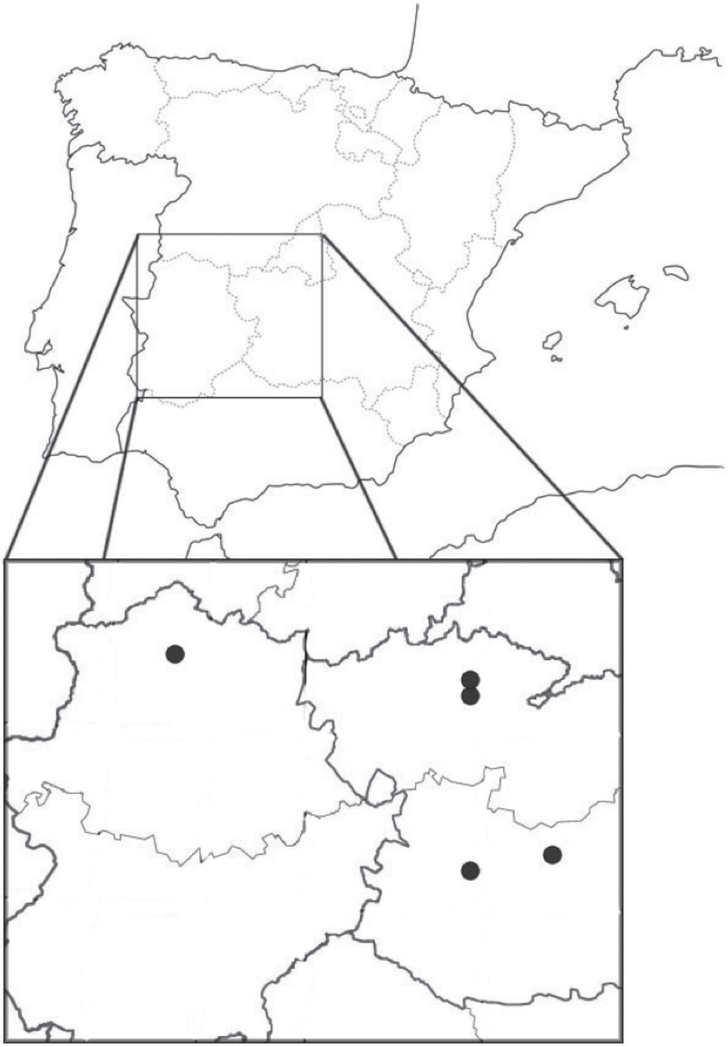
Materials and methods

Study area

Intensive spider sampling (see Morano and Bonal 2016 for details) was carried out in the locality of Huecas, province of Toledo, central Spain (coordinates lat. 39.994°N, long. -4.216°W; elevation 581 m a.s.l.), from September 2012 to September 2013 and collecting once a month. The climate in Huecas is dry Mediterranean, with a marked summer drought in which temperatures may reach 40°C and a precipitation of 365 mm per year. The study area extends over 900 Ha of flat agricultural landscape, with isolated oak trees and oak forest plots interspersed within a matrix of grasslands and cereal fields (see Bonal et al. 2012 for a detailed description). Tree density in the forest patches ranges from 20 to 50 trees per hectare, whereas the distance between isolated holm oaks ranges from 40 metres to more than 2 kilometres. Besides this systematic sampling, small numbers of spiders in other localities of central and western Spain were occasionally collected: Piedrabuena, province of Ciudad Real (lat. 39.041°N long. -4.230°W), Parque Nacional de Las Tablas de Daimiel, province of Ciudad Real (lat. 39.167°N long. -3.661°W) and Dehesa Casablanca, Guijo de Granadilla, province of Cáceres (lat. 40.077°N long. -6.097°W) (see Map 1).

Sampling procedure

In Huecas, four different microhabitats were sampled: tree branches, tree trunks, grasses, and soil, at 23 holm oaks randomly selected (both isolated and within forest patches). The canopy of each tree was split into four parts based on the four cardinal points, a beating tray was placed under the canopy, and the branches of the corresponding canopy quarter beaten six times with a wooden stick. All the spiders falling on the beating tray were collected. Trunk traps consisted of a mosquito net attached to the tree trunk that trapped the spiders climbing up and eventually marching into the net. The net partially covered the trunks and ended in an inverted cone with a dry



Map 1. Geographic location of the sampling localities in the Iberian Peninsula.

bottle on the top in which the spiders were collected. Soil spiders were caught in pitfall traps placed in pairs, one pair below the oak canopy and another pair at open grassland 10–15 metres far from the tree. These traps consisted of a cone through which ground-living spiders fell into a bottle filled with 90% ethanol and 10% glycerine to preserve the specimens. A small plastic roof was placed on each pitfall trap to protect them from direct sunlight and prevent alcohol evaporation. Lastly, in the grassland close to each study tree, the grass was swept for spiders with a sweep net along two 10 metres long transects on both sides of the straight line joining the two pitfall traps. Each specimen was placed in an Eppendorff tube tagged with the collection data and filled with alcohol 96% to preserve them for further morphological and molecular analyses.

Morphological analyses

The epigynes of the females were extracted, cleaned, and mounted on slides for further analysis of the internal genitalia. In males one palp was extracted and illustrated; the palpal organ was expanded after maceration in lactic acid. Genitalia were preserved in microvials together with the specimen. The spiders were inspected with a Meiji EMZ-5 stereomicroscope, equipped with a Canon EOS 350D camera to take photos that were further used as templates to draw pictures of the specimens and their copulatory organs. The holotype and paratypes were deposited in the collection of the National Museum of Natural Sciences (**CSIC**), Madrid, Spain (**MNCN** collection of non-insect arthropods).

Abbreviations

Eyes:

ME	median eyes;
LE	lateral eyes;
ALE	Anterior lateral eye(s).
AME	Anterior median eye(s).
PLE	Posterior lateral eye(s).
PME	Posterior median eye(s).

Female epygine:

bs	basal epigynal plate;
co	copulatory openings;
ct	copulatory ducts;
sc	scape;
spt	spermathecae.

Male palp:

c	conductor;
cy	cymbium;
dh	distal hematodocha;
e	embolus;
fe	femur;
ma	median apophysis;
p	patella;
r	radix;
s	stipes,
sta	subterminal apophysis;
t	tegulum;
ta	terminal apophysis,
ti	tibia.

All the measurements are given in millimetres.

Molecular analyses

To corroborate that *Araneus bonali* sp. n. is not any of the recorded species, the DNA of three specimens (one male, one female and a juvenile) was extracted following the Aljanabi and Martínez (1997) salt extraction protocol. Due to the challenging taxonomy of *Araneus* species, the genus membership of the new species had to be confirmed in the first place. To do so a fragment (311 bp) of the nuclear ribosomal gene 28SrRNA was amplified using the primer pair (28Sa: GACCTGCCTTGAAACACGGA; 28Sb: TCGGAAGGAACCAGCTTACTA) (Whiting et al. 1997). This gene was chosen because of its low mutation rate, which makes it more appropriate for assessing deep phylogenetic relationships, for example among genera. Sequence chromatograms were assembled and edited using Sequencher 4.6 (Gene Codes Corp., Ann Arbor, MI, USA). The 28S sequences of the new species were blasted in GenBank to identify which were the closest species in terms of raw sequence similarity (i. e. proportion of identical nucleotides divided by the total number of nucleotides compared). In addition, a 28S gene tree was built to assess the position of *Araneus bonali* sp. n. with respect to the species of *Araneus* and other araneid genera (41 species) within the first 100 records retrieved after blasting the 28S of the new species in GenBank. All the sequences were aligned using MUSCLE (Edgard 2004) and the gene tree inferred using Bayesian inference analyses defining GTR + Invariants + Gamma evolutionary model, as implemented in Mr Bayes 3.2 software (Ronquist et al. 2012). The non-araneid orb-weaver *Tetragnatha extensa* (Tetragnathidae) was used as out-group.

Additionally, the barcoding fragment of the mitochondrial gene cytochrome oxidase I (cox1) was amplified using the primer pair HCO/LCO (Folmer et al. 1994). Sequence chromatograms were assembled and edited using Sequencher 4.6 (Gene Codes Corp., Ann Arbor, MI, USA). The cox1 sequences available for species of *Araneus* were downloaded from either GenBank or BOLD (Barcoding of Life Datasystem) (a total of 41 species; Accession Codes in Table 3), then combined with those of *Araneus bonali* sp. n. finally all of them aligned using MUSCLE (Edgard 2004) as implemented in MEGA7 software (Kumar et al. 2016). The uncorrected intra-specific genetic divergence was calculated within the three specimens of *Araneus bonali* sp. n. and the uncorrected inter-specific divergence between *Araneus bonali* sp. n. and the available *Araneus* species. (Table 3).

Phylogenetic relationships of the new species and the Holartic *Araneus* species were inferred by concatenating the available nuclear 28SrRNA and mitochondrial cox1 sequences (Accession Codes in Table 3). The focus was placed on the Holartic species because previous studies have shown that *Araneus* species from the southern hemisphere (e.g., Australia), most likely do not belong to the genus (Framenau 2012). The aligned concatenated matrix had a length of 944 base pairs (633 bp mitochondrial cox1, 311bp nuclear 28SrRNA). The best tree was inferred in a Bayesian framework as implemented in Mr Bayes 3.2 software (Ronquist et al. 2012). The species *Argiope aemula* (Walckenaer, 1841) (Araneidae) was used as out-group. The best nucleotide substitution model for each gene was determined with jModelTest 0.1.1 (Posada 2008). Two parallel runs of ten million generations each were conducted using one cold and two incrementally heated Markov chains ($\Lambda = 0.2$), sampling every 1,000 steps. The standard convergence diagnostics implemented in MrBayes and the average standard deviation of the split frequencies were checked to assess whether the Markov chain had reached stationary. After 500,000 generations, the average standard deviation of the split frequencies stabilized in values close to zero (0.001). The trees sampled were summarized using the all compatible consensus command with 25% burn-in.

Statistical analyses

Habitat preferences (tree branches, trunks, grass, and soil) of *Araneus bonali* sp. n. were analysed by comparing the proportion of individuals captured at each habitat. Because of the different sampling methods employed, the number of *Araneus bonali* sp. n. individuals captured at each habitat at random is not expected to be the same. Therefore, the proportion of the whole sample of spiders (all species) collected at each habitat was used as expected frequencies.

To assess habitat distribution and phenological differences between adults and juveniles Chi-square tests were used. In the case of phenology, the year was divided in four trimesters starting from January 1st to assess whether the proportion of juveniles and adults differed among these periods. The correlation between the number of individuals and canopy surface (m^2) was tested using a GLM (Generalized Linear Model, Poisson distribution, Logistic link function). Finally, a Mantel test was used to assess whether the spatial distance (in metres) between trees was correlated with the differences in the number of individuals collected.

Taxonomy

Araneus bonali Morano, sp. n.

http://zoobank.org/EC47CE37-07D2-4957-A28E-F989CCBFBCF6
Figures 1–5

Material. ***Holotype.*** Female holotype collected by E. Morano in Spain (Map 1): Huecas (Toledo), 581 metres above sea level (lat. 39.994°long. -4.216°). 27 Sep 2013 (collected by branch beating) (voucher number MNCN 20.02/17497, National Museum of Natural Sciences (CSIC), Madrid, Spain).

Paratypes. Collected in the same locality than the holotype but on different dates, the following 4 males & 3 females. Coordinates and voucher numbers are shown: 1♂, 25 Sep 2012 (trunk traps), 570 m.a.s.l. (lat. 40.013°long. -4.213°) (MNCN 20.02/17499); 1♀, 15 Oct 2012 (beating), 548 m.a.s.l. (lat. 39.994°long. -4.216°) (MNCN 20.02/17504); 1♂, 22 Oct 2012 (trunk traps), 548 m.a.s.l. (lat. 39.994°long. -4.216°) (MNCN 20.02/17500); 1♂, 30 Oct 2012 (trunk traps), 548 m.a.s.l. (lat. 39.994°long. -4.216°) (MNCN 20.02/17501); 1♀, 31 Oct 2012 (trunk traps), 564 m.a.s.l. (lat. 40.013°long. -4.213°) (MNCN 20.02/17502); 1♂, 20 Aug 2013 (beating), 570 m.a.s.l. (lat. 40.013°long. -4.213°) (MNCN 20.02/17498); 1♀, 27 Sep 2013 (trunk traps), 570 m.a.s.l. (lat. 40.013°long. -4.213°) (MNCN 20.02/17503). All these individuals were deposited in the collection of the National Museum of Natural Sciences (CSIC), Madrid, Spain (MNCN).

Additional specimens studied (Map 1). Spain. Ciudad Real: Piedrabuena, Bullaque river, "Tabla de la Yedra", 551 m.a.s.l., (lat. 39.041°long. -4.230°), 07 August 97,

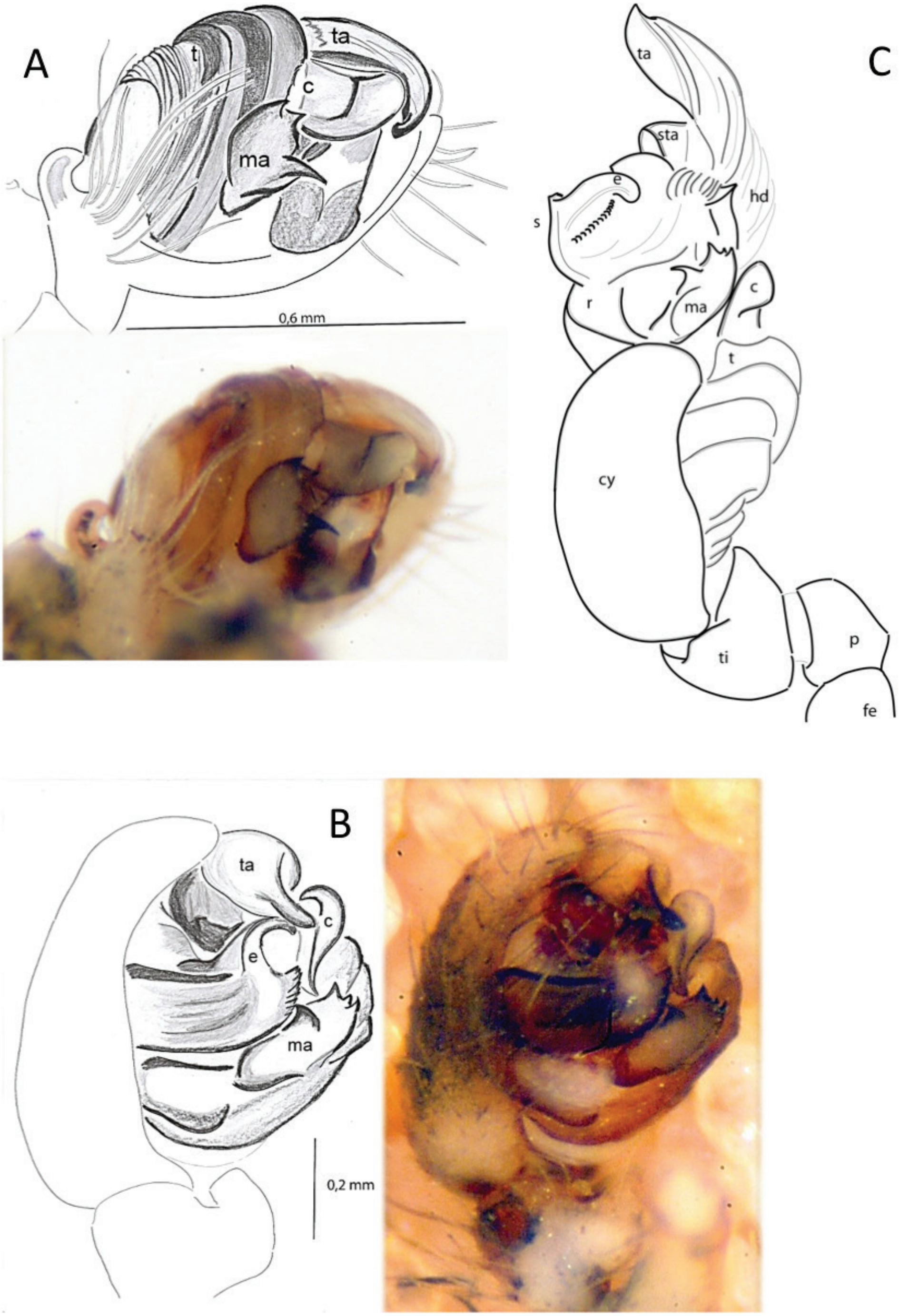


Figure 1. Right palp of *Araneus bonali* sp. n. **A** ventral **B** mesal **C** expanded pedipalp. Abbreviations: c – conductor; cy – cymbium; dh – distal hematodocha; e – embolus; fe – femur; ma – median apophysis; p – patella; r- radix; s – stipes, sta – subterminal apophysis; t – tegulum; ta – terminal apophysis, ti – tibia.

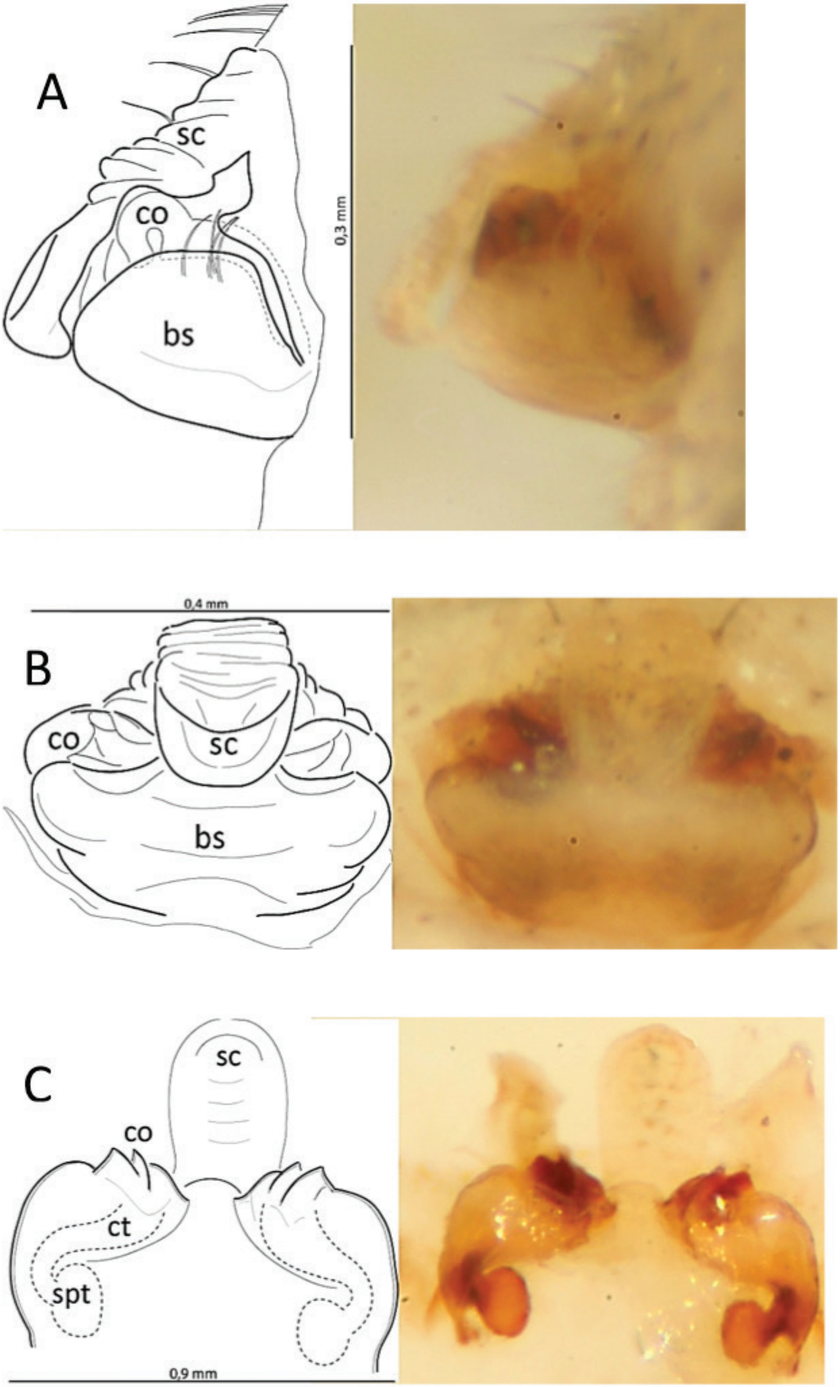


Figure 2. Epigyne and vulva of *Araneus bonali* sp. n. **A** epygine lateral **B** idem, ventral **C** vulva, posterior. Abbreviations: bs – basal plate; co – copulatory opening; ct – copulatory tube; sc – scape; spt – spermatheca.

1♂ (beating) E. Morano leg (EMH-0899); Isla de Algeciras, P.N. Las Tablas de Daimiel, 617 m.a.s.l, (lat. 39.167°long. -3.661°), 15 July 2015, 2 imm (beating) & 15 October 2015, 1♀ (beating), E. Morano leg (vials n°1522 & 1936). Cáceres: Dehesa Casablanca, Guijo de Granadilla, 405 m.a.s.l. (lat. 40.077°long. -6.097°), 02 November 2016, 1♀, (beating) Morano et al. leg (vial n°1489). Collected by E. Morano in the same year (2013) and locality (Huecas) than the holotype and paratypes but on different months the following specimens have been studied and deposited in the personal collection of Eduardo Morano: February, 1 imm (beating), (lat. 40.013°long. -4.213°); May, 1 imm (beating) (lat. 39.994°long. -4.216°); June, 7 imm (beating) (lat. 39.994°long. -4.216°) & 8 imm (beating) (lat. 40.013°long. -4.213°); July, 17 imm (beating) (lat. 39.994°long. -4.216°) & 7 imm (beating) (lat. 40.013°long. -4.213°); August, 1 imm (beating) (lat. 39.994°long. -4.216°) & 1 imm (beating) (lat. 40.013°long. -4.213°) ; September, 1 ♀ (beating) (lat. 39.994°long. -4.216°) & 1 ♀, 2♂ (trunk traps) (lat. 40.013°long. -4.213°); October, 1 ♂, 1 imm (beating) & 1♀, 1♂ (trunk traps) (lat. 39.994°long. -4.216°).

Etymology. The specific name is dedicated to Dr. Raul Bonal.

Diagnosis. Within the European fauna *Araneus bonali* sp. n. resembles *Gibbaranea gibbosa* (Walckenaer, 1802) due to its colouration (Figure 3) but does not have its characteristic humps on the opisthosoma. The design and greenish coloration of the opisthosoma and the lack of modifications in the male tibias II differentiates the new species from the small sized, and also usually collected in tree canopies, *A. sturmi* (Hahn, 1831) and *A. triguttatus* (Fabricius, 1775). The structure and morphology of the median apophysis of the male palp and the scape and basal plate of the female epigyne of *Araneus bonali* distinguishes it from any similar *Araneus* species.

Description. Female (holotype). Measurements of the holotype are shown (ranges for paratypes in parentheses). Total length: 6.0 (5.1–7.2); Prosoma length: 2.4 (2.0–2.5); Prosoma width: 2.5 (1.8–2.5); Opisthosoma length: 4.4 (3.6–4.4); Opisthosoma width: 4.0 (3.2–4.1). Eye diameter: AME: 0.125; ALE: 0.10; PME: 0.10; PLE: 0.075. Distance between eyes: AME – AME: 0.150; AME – ALE: 0.325; PME – PME: 0.125; PME – PLE: 0.375; AME – PME: 0.10; ALE – PLE: 0.05; Height from clypeus to AME: 0.05; Height from clypeus to ALE: 0.05.

Carapace covered by white hairs (Figs 3, 4). Greyish green cephalic area, with a pair of black side bands going from the ocular area to the fovea. Clypeus and sides of the cephalic area dark brown. Glabrous and cream-coloured posterior thoracic region, usually covered by the opisthosoma. Eight eyes in two transverse rows, the four ME arranged in a trapezoid widely separated from two LE. AME distance wider than PME, ME protrude frontally and AME slightly larger than PME (which have a narrow tapetum). Chilum absent. Chelicerae with a proximal boss, their base of the same dark colour than the ocular region and the clypeus. Chelicerae with three teeth in their margins, the median tooth smaller in both cases. Greyish green sternum with dark radial and central bands in the ventral side of the prosoma. Wider than longer labium with a distal white margin. Endites swollen, rebordered, and square, with white internal area, their length only slightly larger than their width. Both have the same colour than the sternum.

Figure 3. Images of an *Araneus bonali* sp. n. female and the surface of an oak trunk showing the lichen mimicry of this spider species.

Short and relatively stout legs. The first pair the longest and the third the shortest; the second slightly longer than the fourth (Table 1). Dark green coxae and trochanters, rest of segments pale green. Brownish apical third of the femur, base dotted. Patellae usually dark brown, the rest of the segments with dark brown rings at the middle and end. Tarsi without trichobothria.

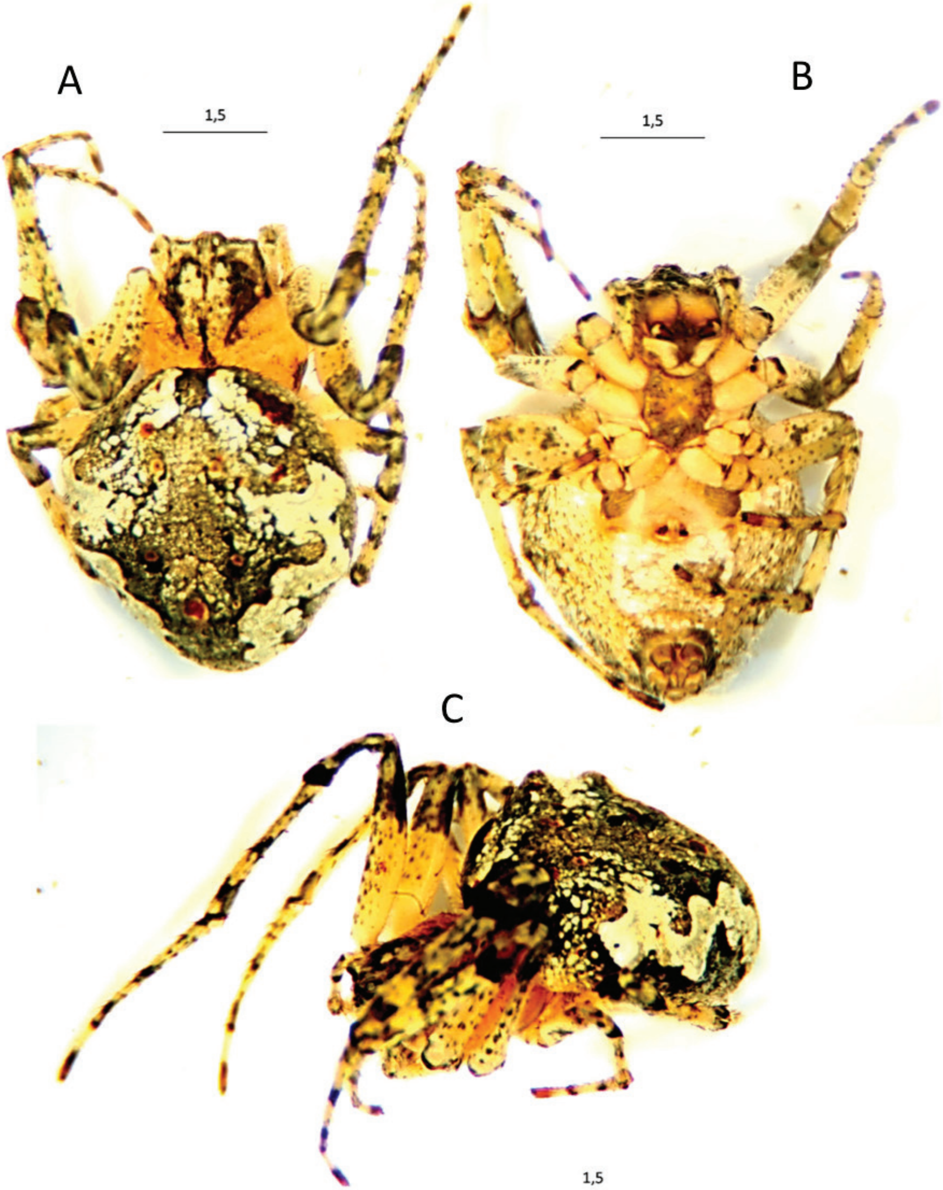


Figure 4. *Araneus bonali* sp. n., female under stereoscopic binocular microscope. **A** dorsal view **B** ventral view **C** lateral view (dimensions in mm).

In females, tibia I and II with 3–4 pairs of lateral spines and 5–6 pairs of ventral spines. Metatarsus I and II with 5–6 spines on the inner side and three basal spines on the outer side. However, the spines are an extremely variable character because they can be lost and may appear in unusual positions or vary with the dimensions of the segments (Grasshoff 1968; Berman and Levi 1971; Carmichael 1973).

Table 1. Morphological measurements of *Araneus bonali* sp. n. holotype. All measurements are given in millimetres. Leg formula: I>II>IV>III.

Holotype (♀)					
leg	**segment**	**length**	**leg**	**segment**	**length**
I	**Femur**	3.3	**III**	**Femur**	1.3
	Patella	1.2		**Patella**	0.6
	Tibia	2,5		**Tibia**	0.7
	Metatarsus	2.4		**Metatarsus**	0.7
	Tarsus	1.0		**Tarsus**	0.5
	total	**10.4**		**total**	**3.8**
II	**Femur**	2.1	**IV**	**Femur**	1.7
	Patella	0,9		**Patella**	0.7
	Tibia	1.4		**Tibia**	1.2
	Metatarsus	1.5		**Metatarsus**	1.2
	Tarsus	0.6		**Tarsus**	0.6
	total	**6.5**		**total**	**5.4**

Triangular opisthosoma slightly longer than wide, with brown setae. Folium with a black band and a narrow white line that marks the limit between the anterior spots, the posterior humps, and the greenish folium sides. Three pairs of sigillae, the two anterior ones larger. In females, two pairs of anterior humps, much smaller in males. General colouration mimetic with lichens and mosses, difficult to tell the spiders apart when on the oak branches and trunks. White ventral background with two dark lateral spots; two book lungs and an inconspicuous spiracle before the colulus, behind the colulus six spinnerets.

Female genitalia. The scape of the epigyne short and wrinkled (Fig. 2A, B), with setae directed backwards on the surface. Straight scape ending in a spoon-shaped tip and attached to the basal epigynal plate by lateral sclerites. The basal plate or posterior piece as long as the epigyne, with a light tonality and rectangular-shaped, without paired basal lamellae. Ventral genital openings continued internally with the copulatory ducts that connect with the small elliptic spermathecae (Figure 2C).

Male (Paratypes). Ranges are shown (and mean values within parentheses) (Table 2). Total length: 3.2–4.3 (3.84); Prosoma length: 1.8–2.3 (2.10); Prosoma width: 1.3–2.1 (1.73); Opisthosoma length: 1.7–2.8 (2.28); Opisthosoma width: 1.2–2.0 (1.76). Eye diameter (average): AME: 0.125; ALE: 0.10; PME: 0.10; PLE: 0.075. Distance between eyes: AME – AME: 0.150; AME – ALE: 0.250; PME – PME: 0.125; PME – PLE: 0.325; AME – PME: 0.10; ALE – PLE: 0.05; Height from clypeus to AME: 0.05; Height from clypeus to ALE: 0.05.

Male general appearance and colouration similar to females (Figure 5) but, according with the large sexual size dimorphism typical of araneids (Hormiga et al. 2000), males are 1.5 times smaller; males slender than females and with a smaller triangular opisthosoma. Light greenish humps, delimited by white lines that continue into the posterior folium. One tooth on the side of the endites. Leg colour identical between

Table 2. Morphological measurements of *Araneus bonali* sp. n. paratypes. The values are in millimetres, indicating the minimum, the maximum and, in brackets, the average of each measure. Leg formula: I>II>IV>III.

Dimensions		**Paratypes** (4 ♂)	**Paratypes** (3 ♀)	**Dimensions**		**Paratypes** (4 ♂)	**Paratypes** (3 ♀)
total length		3.2–4.3 (3.84)	5.1–7.2 (5.74)	**leg**	**segment**	**length**	**length**
prosoma	**wide**	1.3–2.1 (1.73)	1.8–2.5 (2.11)	**I**	**Femur**	2.5–3.4 (2.8)	2.5–3.3 (2.8)
	long	1.8–2.3 (2.10)	2.0–2.5 (2.23)		**Patella**	0.8–1.4 (1.2)	1.1–1.5 (1.3)
opisthosoma	**wide**	1.2–2.0 (1.76)	3.2–4.1 (3.71)		**Tibia**	2.0–2.7 (2.4)	1.8–2.7 (2.3)
	long	1.7–2.8 (2.28)	3.6–4.4 (4.10)		**Metatarsus**	2.0–2.5 (2.3)	1.8–2.4 (2.1)
					Tarsus	0.8–1.1 (1.0)	0.8–1.0 (0.9)
					total	**8.3–10.3 (9.61)**	**8.0–10.7 (9.36)**
				II	**Femur**	1.9–2.5 (2.3)	2.0–3.0 (2.4)
					Patella	0,7–1.2 (1.0)	0.9–1.3 (1.1)
					Tibia	1.2–2.0 (1.7)	1.4–2.2 (1.7)
					Metatarsus	1.5–2.0 (1.8)	1.5–1.8 (1.6)
					Tarsus	0.7–1.0 (0.8)	0.6–0.9 (0.8)
					total	**6.0–8.5 (7.56)**	**6.5–9.0 (7.56)**
				III	**Femur**	1.1–1.6 (1,5)	1.3–2.0 (1.6)
					Patella	0.4–0.6 (0.5)	0.5–0.8 (0.6)
					Tibia	0.7–0.9 (0.8)	0.7–1.0 (0.8)
					Metatarsus	0.7–1.0 (0.9)	0.7–1.2 (0.9)
					Tarsus	0.5–0.6 (0.6)	0.5–0.7 (0.6)
					total	**3.4–4.6 (4.20)**	**3.8–5.7 (4.63)**
				IV	**Femur**	1.6–2.5 (2.1)	1.7–2.8 (2.1)
					Patella	0.6–0.9 (0.8)	0.6–1.2 (0.9)
					Tibia	1.1–1.6 (1.4)	1.2–1.9 (1.5)
					Metatarsus	1.0–1.5 (1.4)	1.2–1.9 (1.5)
					Tarsus	0.5–0.7 (0.6)	0.6–0.7 (0.6)
					total	**5.0–7.2 (6.23)**	**5.4–8.5 (6.67)**

sexes, but not their morphology. In males, coxa I has an apical curved hook distally that fits in into the corresponding groove of femur II during copulation. Tibias II curved and armed with four pairs of lateral spines. Metatarsus II with strong spines.

Male genitalia (Figure 1). Palpus with two dorsal macrosetae (on the patella and on the tibia), palpal femur with a small ventral tubercle. Compact genital bulb attached at the base of the cymbium, reduced paracymbium with the standard araneid shape (hook or knob); tegulum covering the base of the palpus. Median apophysis comparatively short on the right side of the radix, with a spur and three distal teeth easy to observe in mesal view (Figure 1B). In this view, the stipe margin is serrated in the area close to the median apophysis. Embolus short, stout and curved towards the conductor and partially covered by the terminal apophysis. Conductor with a swollen distal margin and a large terminal apophysis ending in a fine and blunt tip. The expanded bulb (Figure 1C) shows the presence of radix, stipes, distal haematodocha, and subterminal apophysis.

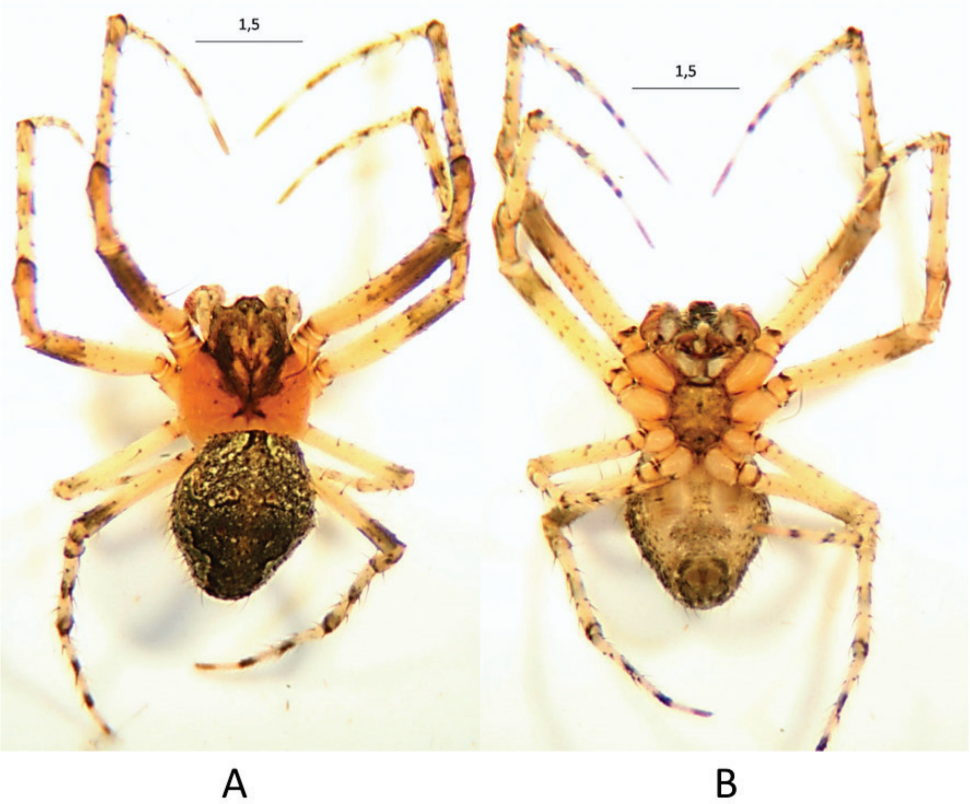


Figure 5. *Araneus bonali* sp. n. male under stereoscopic binocular microscope. **A** dorsal view **B** ventral view (dimensions in mm).

Phylogenetic analyses. The blast of the nuclear 28SrRNA sequence of the new species recovered *Araneus angulatus* and *Araneus diadematus* as the most closely related species (sequence similarity 99%). The 28S gene tree (Figure 6) showed that, except *Araneus dimidiatus* (L. Koch, 1871), *Araneus* spp. formed a nonexclusive clade, albeit with low support (PP = 0.64), that also include species from closely related genera such as *Neoscona* Simon, 1864 and *Larinioides*.

The three specimens of the new species analysed yielded the same cox1 haplotype. The lowest uncorrected intraspecific genetic distance was 11.7%, to *Araneus alsine* (Walckenaer, 1802). The average genetic distance among *Araneus* species was 14.9%. The node support values of the concatenated tree were low and most relationships were unresolved. *A. bonali* formed a small clade with *A. iviei* (Archer, 1951) and *A. alsine*, but with a very low support (PP = 0.55) (Figure 7). A clade including *A. diadematus* and eleven additional species, was recovered albeit with low support (PP = 0.82) (Figure 7). The results show that *Araneus angulatus* and the new species are excluded in the *diadematus* group.

Habitat distribution and phenology. *Araneus bonali* is linked to trees, as not a single individual was collected in ground traps or in grasslands. The distribution

Figure 6. Gene tree of the nuclear gene 28SrRNA. *Araneus bonali* sp. n. underlined in red. Sequences for all species except *Araneus bonali* sp. n. were downloaded from GenBank (accession codes shown besides the species name). Tree topology was inferred using Bayesian inference analyses (GTR + I + Gamma substitution model).

patterns differed significantly between juveniles and adults though, as all juveniles (44 individuals) were collected in oak branches whereas most adults (66% of a total of 15 specimens) were captured in trunk traps (Figure 8; Chi = 6.66; df = 1; $P < 0.01$). Phenology also differed between age classes (Chi = 135; df = 3; $P < 0.001$). With the exception of one capture in February, the bulk of juveniles appeared in May and matured throughout spring and summer. Adult presence and reproduction was concentrated in the months of September and October and none were caught in winter (Figure 9).

Araneus bonali was collected in 18 of the 24 holm oaks sampled. The number of juveniles and adults caught at each oak were significantly related ($F_{1, 22} = 31.41$; $P < 0.001$). The total number of individuals trapped at each tree was unrelated with canopy surface (GLM Estimate = 0.001; Z = 0.35; P = 0.72) or the pairwise spatial distance between holm oaks (Mantel test R = -0.04; P = 0.62).

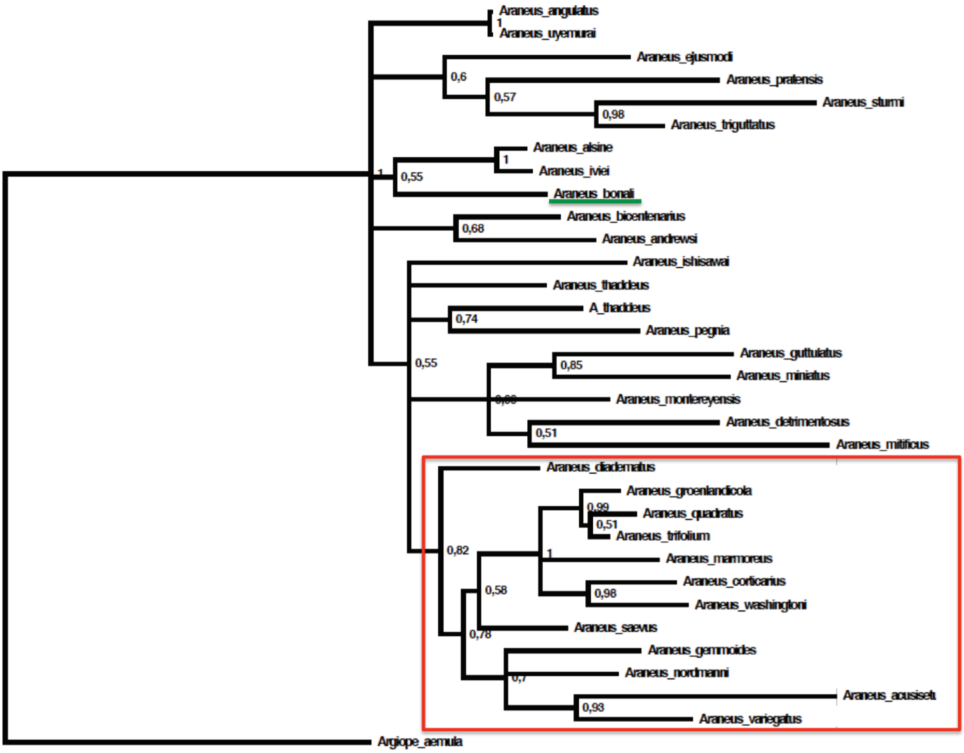


Figure 7. Concatenated mitochondrial cox1 and nuclear 28S genes phylogeny. *Araneus bonali* sp. n. underlined in green within the Holartic *Araneus* sequences available at GenBank or BOLD (accession codes shown in Table 3). The red-coloured frame shows the clade corresponding to the *Araneus diadematus* group. Tree topology was inferred using Bayesian inference analysis (GTR + I + Gamma substitution model).

Discussion

Araneus bonali sp. n. exhibits a combination of somatic and genital characters typical of *Araneus* according to the cladistic analyses performed by Scharff and Coddington (1997), which established the intergeneric relationships within the family Araneidae. These traits include a hairy carapace in both sexes and in males the presence of an endite tooth, coxa I with hook and curved tibia II with strong macrosetae. Male genitalia were thoroughly analysed (including the expanded bulb) and has two patellar setae, a median apophysis with spines and hooks, stipes and two apophysis (subterminal and terminal) separated from distal hematodocha. The cap on the tip of the embolus present in virgin males was not found in the individuals analysed. Females showed the pockets near the tip of the epigynal scape characteristic of the genus (Levi 1991; Scharff and Coddington 1997). The molecular analyses supported genus membership of the new species by identifying *Araneus angulatus* (the type species of the genus) and the common *Araneus diadematus* as the two closest species, and the 28S gene tree showed the new species within the *Araneus* group along with other species of the genus.

Table 3. Accession codes for the mitochondrial cytochrome Oxidase I (cox1) and nuclear (28S) gene sequences downloaded from GenBank and BOLD (Barcoding of Life Datasystems) (BOLD sequences in bold characters).

	Cytochrome Oxidase I	28 S
Araneus acusisetus	JN817144.1	JN816939.1
Araneus alsine	KY268481.1	
Araneus andrewsi	**BBUSE10611.COI5P**	
Araneus angulatus	JN817138.1	KC848983.1
Araneus bicentenarius	**CNSLH311-12.COI-5P**	
Araneus bonali	MH517392	MH493065
Araneus bogotensis	KR058594.1	
Araneus corticarius	KF367835.1	
Araneus detrimentosus	**BBUSE122211.COI5P**	
Araneus diadematus	KY017584.1	KY016926.1
Araneus dimidiatus	KC849065.1	KC848985.1
Araneus ejusmodi	JN817143.1	JN816938.1
Araneus gemmoides	DQ146861.1	
Araneus groenlandicola	GU682824.1	
Araneus guttulatus	**CNKOK095-14.COI-5P**	
Araneus ishisawai	JN817140.1	JN816935.1
Araneus iviei	KM837836.1	
Araneus marmoreus	JN817141.1	FJ525384.1
Araneus miniatus	**BBUSE179312.COI5P**	
Araneus mitificus	KY467247.1	
Araneus monteryensis	**BBUSE01411.COI5P**	
Araneus nordmanii	GU684587.1	
Araneus omnicolor	KP031493.1	
Araneus pegnia	**BBUSE144212.COI5P**	
Araneus poltyoides	**VAQTB01711.COI5P**	
Araneus pratensis	KP653307.1	
Araneus psittaccinus	**VAQTB01111.COI5P**	
Araneus quadratus	FR775772.1	
Araneus saevus	JN309620.1	
Araneus stella	JN817142.1	JN816937.1
Araneus sturmi	KY269282.1	
Araneus thaddeus	HQ924458.1	
Araneus tijuca	KT945066.1	
Araneus trifolium	GU682571.1	FJ525384.1
Araneus triguttatus	KY269635.1	
Araneus uyemurai	JN817137.1	JN816933.1
Araneus variegatus	JN817139.1	JN816934.1
Araneus venatrix	KR058592.1	
Araneus vincibilis	KR058596.1	
Araneus washingtoni	**ARSO191-08.COI-5P**	
Araneus workmani	KR058597.1	
Argiope aemula	JX307083.1	DQ018845

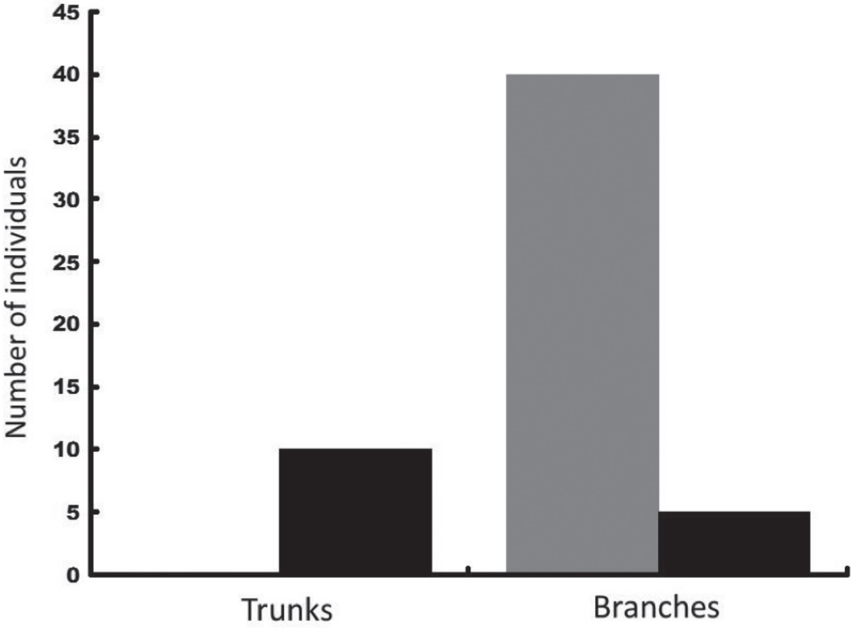


Figure 8. Number of juveniles (grey bars) and adults (black bars) of *Araneus bonali* sp. n. collected on the tree trunks and branches.

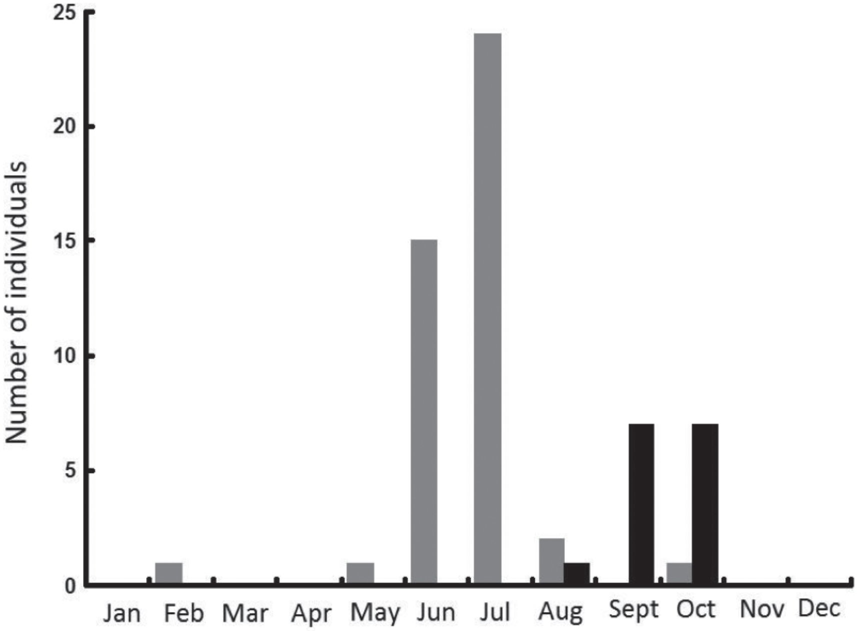


Figure 9. Number of juveniles (grey bars) and adults (black bars) of *Araneus bonali* sp. n. collected throughout the year.

The morphology of the male and female genitalia clearly distinguished the new species from any other *Araneus* species. Moreover, large uncorrected genetic distance to the closest relative (11.7%), further confirms its species status (Ratnasingham and Herbert 2007). *Araneus bonali* colouration is similar to that of *Araneus circe* (Audouin, 1826), however, the presence of the latter species in the Iberian Peninsula has not been confirmed (see discussion about the taxonomic status of Iberian *Araneus* spp. below). Moreover, *A. circe* is a large orbweaver (female length more than 20 mm), about three times bigger than *A. bonali*. The new species resembles another three small European araneids, namely *Gibbaranea gibbosa*, *Araneus sturmi* (Hahn, 1831) and *Araneus triguttatus* (Fabricius, 1775) and, like them, inhabits trees. It could be confused with the first species due to the greenish body (Figure 1). However, the new species lacks the prominent humps on the opisthosoma typical of *G. gibbosa*. Their genitalia are also different, in *G. gibbosa* male median apophysis has a broad base and a tapering, curved spur and the scape of female epigyne finishes in a characteristic spoon-shaped tip. Regarding the other two species, the lichen-like and greenish grey opisthosoma of *Araneus bonali* distinguishes it from the orangish red colour of *A. sturmi* and *A. triguttatus*. In addition, the structure and morphology of the copulatory organs (especially the straight scape of female epigyne and the three small teeth of the median apophysis of male palps in *A. bonali*) allow their differentiation.

A thorough revision of the literature was carried out including reports and descriptions of *Araneus* in Northern Africa (Audouin 1826; Cambridge 1872; Thorell 1875; Cambridge 1876; Pavesi 1880; Simon 1885; Lucas 1846; Simon 1899; Simon 1908; Simon 1909; Caporiacco 1934; Denis, 1945; Jocqué 1997) and three species not cited in Europe were found, namely *Araneus arganicola* (Simon, 1909), *Araneus klaptoczi* (Simon, 1908) and *Araneus v-notatus* (Thorell, 1875). The specimens of *A. klapotczi* and *A. v-notatus* could not be examined but, based on the original descriptions, they cannot be mistaken with *A. bonali*. In the first case, the description matches that of the modern genera *Pararaneus* Caporiacco, 1940, *Larinia* Simon, 1874 or *Siwa* Grasshoff, 1970. In the case *A. v-notatus*, the scape of the female genitalia has the characteristic "S" shape of *Araneus sturmi*. In spite of the different body colour, Simon (1929) included this species as a variety of *A. sturmi* when it was collected in France. The syntypes of *A. arganicola* deposited at the collection of the National Museum of Natural Sciences (Madrid) (voucher MNCN 20.02/12093) could be examined. They were collected by Martínez Escalera in Mogador (Morocco) in the nineteenth century. After a detailed inspection it was concluded that they were two subadults (♀ and ♂), as can be appreciated in Figure 10 where the outlines of female epigyne and swollen male palps can be observed. Both specimens probably belong to the species *Neoscona subfusca* (CL Koch, 1837). Besides these specimens, there were other two collected by the same person in Morocco (vouchers and collecting sites: MNCN 20.02/12120 -Mogador- and MNCN 20.12/12115 -Tanger-). They were labelled as *A. circe* but, after a detailed morphological analysis, we concluded that they were two females de *A. angulatus*.

The 28S gene tree confirmed that the only non-Holartic species that was in the analysis (28S sequence available), namely the Australian *Araneus dimidiatus*, does not

Figure 10. *Araneus arganicola* syntype specimens. **A** dorsal view **B** ventral view **C** data labels.

belong in the *Araneus* spp. group (fig. 28S tree) (Framenau 2012) and supports our decision of not including them in further phylogenetic analyses. The *Araneus* clade included species of other genera such as *Larinioides* and *Neoscona*, probably due to the lack of informative characters in this short fragment. The concatenated phylogenetic tree of the Holartic *Araneus* spp. recovered a low supported clade (PP = 0.82) that grouped *Araneus diadematus* with another eleven species. Neither *Araneus bonali* nor *Araneus angulatus* or *Araneus sturmi* seem to belong to this group. Nonetheless, the poor resolution of our tree caution against drawing any major conclusions. New phylogenetic analyses based on a more thorough taxonomic sampling and a larger combination of molecular markers would be required to confirm some of our results and provide a fully resolved phylogenetic hypothesis for the genus and its close allies.

The new species is a tree-specialist: in addition to the more intensively studied area, it was found in holm oaks in the other localities of central and western Spain sampled. However, microhabitat selection did differ among life stages. While juveniles were in all cases collected in the branches, adults were more often caught in trunk traps. The differences in colouration might explain such a contrast in some extent. Juveniles are greenish, similar to oak new shoots, whereas adults show a greyish green colour remarkably similar to the lichens that cover the oak trunks (Figure 3). This mimicry might help them to remain undetected by prey and/or avoid predators, as has been found in other species (Théry and Casas 2002). This lichen-like appearance is not unique to the new species but also shared by another closely related species, namely the American *Araneus bicentenarius* (McCook, 1888) (Figure 6), so called `giant lichen orbweaver´, which was collected on trees among lichens (Levi 1971). The new small Iberian species would thus have a "giant" counterpart on the other side of the Atlantic, as the females of *A. bicentenarius* (total length 28 mm) are more than four times larger than those of *A. bonali* (total length 6 mm).

The number of individuals of the new species collected at each tree was not spatially correlated, what suggests that *A. bonali* is able to disperse even in a landscape in which trees are isolated. Its small size may favour ballooning dispersal, a behaviour previously described in different araneids (Bell et al. 2005). Nonetheless, adult and juvenile numbers were significantly related at the tree level, what also points to the relative importance of local reproduction. With the exception of one individual in February, juveniles were present from mid-spring onwards and the first adults did not appear until late summer. Based on Schaeffer (1977) and Ysnel and Canard (1986) classification, *A. bonali* would be an autumn stenocorus species, characterized by a short biological cycle with a brief adult presence between late summer and early autumn and a wintering period as egg. This life-cycle phenology is the most common in European *Araneus* spp. (Nentwig et al. 2017), although in the new species adult presence extends a little more into early autumn, probably due to the warmer temperatures in the Mediterranean climate.

The status of *Araneus* spp. in the Iberian Peninsula: updating the species list

The bibliographical review and the revision of the material from several collections allowed evaluation of the taxonomic status and update the list of the Iberian *Araneus* spp. So far, there were several records of the following seven species in the Iberian Peninsula: *A. angulatus, A. diadematus*, *A. marmoreus* Clerck, 1757, *A. pallidus* (Olivier, 1789), *A. quadratus* Clerck, 1757, *A. sturmi,* and *A. triguttatus* (Morano, Carrillo & Cardoso, 2014). Another three species, namely *Araneus circe*, *A. grossus* (C. L. Koch, 1844) and *Epeira spinivulva* (Dufour, 1835), have been cited in the past, but either the records need to be confirmed or their taxonomic status carefully reviewed.

The presence of *Araneus circe* (Pozuelo de Calatrava & Fuente, 1898) and *A. grossus* (Coimbra & Bacelar, 1928), species well known in other European regions, needs confirmation as only two records from old bibliographical references are available for the Iberian Peninsula. The taxonomic status of *Epeira spinivulva* is doubtful, and very probably corresponds to a synonymy. Léon Dufour describes a female of *Epeira spinivulva* in Sagunto (Valencia) (Dufour 1835), a species also cited in the Portuguese locality of Povoa de Varzim (Bacelar 1927). The type material of this species was insufficiently described and, unfortunately, it could not be found after contacting the museum where it was deposited. Simon (1874) considers it valid, including among the synonyms *Epeira vulpina* (Hahn, 1835). In 1929, Simon mentions it in the third footnote (p 756): "est absolument impossible de savoir ce que peut être l'*Epeira spinivulva* of Léon Dufour", including it as a synonym of the species *Araneus angulatus*. Bonnet

(1955) separates them as valid species and on footnote (p. 631) indicates that "Cette espece a été homologuée avec doute, avec l'*Epeira spinivulva* de Dufour". Nonetheless, the current World Spider Catalog considers *Epeira spinivulva* a synonym of *A. vulpinus* (Hahn, 1834). After these doubts, it should be considered as "*nomen nudum*", because Dufour only vaguely describes the scape of the epigyne and the colour of the specimen. With so little information it is not possible to differentiate it from species like *A. angulatus*, *A. circe*, *A. grossus* or even *A. diadematus*.

Finally, in his articles from the beginning of the 20th century, Franganillo described a series of varieties of different species, and even new species of *Araneus*, using ambiguous descriptions lacking illustrations (Franganillo 1909, 1910, 1913, 1918a, b). Recent reviews of bibliographical references and collection materials have concluded that those species are really synonymies of known species (Breitling et al. 2016). It was also Franganillo (1910) who, following Emerton (1884), determined a specimen collected in Spain as "*E. thadeus* Hent" in his article entitled "Arañas de la desembocadura del Miño" (Franganillo 1909). He briefly describes the epigyne of the specimen as "a very short and wide hook, quite convex, like a lid over the genital openings; rounded in its distal extreme and with a small flap in the middle", the colouration of the individual is described as "greenish". Neither the morphology of the epygine nor the body colour match those of *Araneus thaddeus* Hentz (Levi 1973, Dondale et al. 2003). Unfortunately, the specimen is probably lost, as it could not be found in the most recent revision of Franganillo´s collection (Breitling et al. 2016). In our view, Franganillo´s description could correspond to a specimen of *Araniella cucurbitina* (Clerck, 1757) or *Araniella opistographa* (Kulczyński, 1905), both present in the Iberian Peninsula.

Conclusions

The morphological and genetic analyses confirm that *Araneus bonali* is a new species, hence, the list of *Araneus* in the Iberian Peninsula now numbers eight species: *A. angulatus*, *A. bonali*, *A. diadematus*, *A. marmoreus*, *A. pallidus*, *A. quadratus*, *A. sturmi*, and *A. triguttatus*. The inclusion of *A. circe* and *A. grossus* remains to be confirmed.

The geographical distribution of the new species remains to be fully delimited to confirm whether it is widespread or, similar to other species (e.g., *Araneus pallidus*), its distribution is restricted to the western Mediterranean Basin. The fact that it has not been collected elsewhere in Europe before suggests a potential small geographical range, however, it is true that due to its habitat preferences it may have gone unnoticed, as tree trunks are not so frequently sampled. In the case of Spain, this is the second new species collected in isolated holm oaks within croplands (the first, *Cheiracanthium ilicis* was sampled in the same study site; see Morano and Bonal 2016). Hence, sampling efforts on tree branches and trunks in further spider field surveys are encouraged. From a conservation perspective, the preservation of isolated trees and forest patches in croplands should be high in the agenda of nature management policies (Guevara et al. 2005, Manning et al. 2006, Bonal et al. 2012).

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A new species of *Parlatoria* from China (Hemiptera, Coccomorpha, Diaspididae)

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Abstract

A new species of armoured scale insect, *Parlatoria menglaensis* **sp. n.** is described and illustrated, which infests leaves of *Cinnamomum camphora* in China. A key to the *Parlatoria* species occurring in China is provided.

Keywords

China, Diaspididae, Hemiptera, new species, *Parlatoria*

Introduction

Armoured scale insects (Hemiptera: Coccomorpha: Diaspididae) are ubiquitous sap-sucking parasites that have a worldwide distribution (Andersen 2009). Compared with other family groups of Coccoidea, the armoured scale insects have several unique characteristics. For example, they have a cecum, which has no direct connection between the stomach and the anal opening, so they do not produce sweet secretions called honeydew (Henderson 2011). Females of the armoured scale insects are immobile and firmly attached to plant leaves, stems, fruits, or roots (Chou 1982). They have an extremely simplified morphology with several fused segments (Andersen 2010). They have no wings, or legs, and the eyes and antennae are reduced (Balachowsky 1948). The Diaspididae is the largest family of the Coccoidea with 2595 species currently identified (García Morales et al. 2018).

The genus, *Parlatoria* Targioni Tozzetti, 1868, is a large group of Diaspididae that are members of the tribe Parlatoriini. This genus was originally established by Curtis (1843) and *Aspidiotus proteus* Curtis, 1843, has been subsequently designated as the type species by Leonardi. The genus currently is made up of 73 species (García Morales et al. 2018). Some species of *Parlatoria*, such as *P. oleae* (Colvée) and *P. ziziphi* (Lucas) are considered to be serious pests of economic plants (McKenzie 1945).

Parlatoria is distributed in both tropical and subtropical regions (García Morales et al. 2018). This genus is found predominately in southeastern Asia but has also extended its range into Australia and Africa (McKenzie 1945). Some species have been introduced in the tropics and other warm parts of the world (Takagi 1969). About 34 species of this genus have been reported from China (García Morales et al. 2018).

Recently, a new species of *Parlatoria* was discovered in China and is described and illustrated in this study. This discovery raises the number of species recorded in this genus to 74, of which 35 are recorded from China. A key to the Chinese species of *Parlatoria* is presented in this study.

Materials and methods

Plant samples infested by the new species were collected from Mengla city, Yunnan Province. Permanent slide mounts of adult females from the samples were prepared using the method described by Henderson (2011).

The illustrations of the adult female shown in Figs. 1–9 were drawn from slide-mounted specimens. Fig. 1 shows an overview of the dorsal body surface on the left side and the ventral body surface on the right side, and an enlarged detail of the significant features of the body, which are not drawn in direct proportions to each other.

Slide-mounted type specimens of the new species have been deposited in the Entomological Museum, Northwest A&F University, Yangling, Shaanxi, China (NWAFU).

Taxonomy

Parlatoria Targioni Tozzetti

Parlatoria Targioni Tozzetti, 1868: 735.

Type species. *Parlatoria orbicularis* Targioni Tozzetti, subsequently designated by Leonardi (1899: 208).

Generic diagnosis. Female scale. Various colors, oval to elongate, with exuviae terminal, often occupying greater part of scale.

Male scale. Elongate and smaller than the female.

Adult female. Female body nearly round or broadly ovate, and the widest at metathorax or abdominal segment I. The body free segments distinct but not strongly produced, and membranous except for the pygidium. Antennae with one or two setae. Prosomatic tubercles present or absent. Anterior spiracles with disc pores, the posterior spiracles without disc pores. Derm pocket present or absent between the posterior spiracle and body margin. Peribuccal granulations present or absent. The pygidium rounded or rather triangular. Three pairs of lobes well developed, unilobate, usually notched, median lobes not yoked; marginal macroducts of pygidium, one present or absent between the median lobes, one between the median and second lobes, and also between the second and third lobes. Two or three fimbriate plates present between lobes, then extend to the whole lateral margin of the pygidium, and each with one microduct. The fourth and fifth lobes often present, sclerotized or replaced by a membraneous fimbriate plates. Submarginal dorsal ducts usually present, scattered in a broad, continuous, irregular row on each side of the abdomen. Submedian dorsal ducts present or absent. Anal opening positioned about centre of pygidium. Four or five groups of perivulvar pores present (Adapted from McKenzie 1945, Takagi 1969, Williams and Watson 1988, Henderson 2011).

Remarks. This genus *Parlatoria*, like other groups of the subfamily Aspidiotinae has an ovate body, and the second lobes are not divided into two lobules. Fringed plates are present between the lobes. *Parlatoria* is distinguished from other genera, especially *Parlagena* McKenzie, 1945, and *Parlatoreopsis* Lindinger, 1912, by having fringed plates across two sides of the prepygidial abdominal segments.

Parlatoria menglaensis sp. n.

http://zoobank.org/5F9869BC-25A3-4633-B445-6C6B76453DD8

Figures 1–9

Material examined. Holotype and 30 paratypes, adult female. China: Yunnan Province. Mengla city, longitude 101.57, latitude: 21.48, on *Cinnamomum camphora* (L.) Presl., 30.vii.2017, coll. Minmin Niu.

Description. Female scale. Adult female cover convex, circular dark green/grey; exuvia on front end. Male scale. Not recorded.

Adult female. Body elongate-ovate, the broadest in first abdominal segment or thoracic region; segmentation distinct.

Antenna composed of two stout tubercles and a seta, located midway between frontal margin and mouthparts, interantennal distance being equal to width of mouthparts. Prosomatic tubercles absent. Anterior spiracle with 1–2 parastigmatic pores; posterior spiracle without pores. Derm pocket absent between the posterior spiracle and body margin. Submarginal duct tubercules arranged as follows: 1–2 on cephaloprothoracic sternum, 3–5 on the mesothorax, and 4–8 on the metathorax.

Pygidium rounded, with 3 pairs of well-developed lobes. The median lobes distinctly notched once on each side, the second and third usually notched once distinctly on outer side. Fourth lobes replaced by a fimbriate plate, similar to adjacent fimbriate plates in form. Fimbriate plates present throughout pygidium, mostly as long as lobes, and two between median lobes, two between the median and second, three between

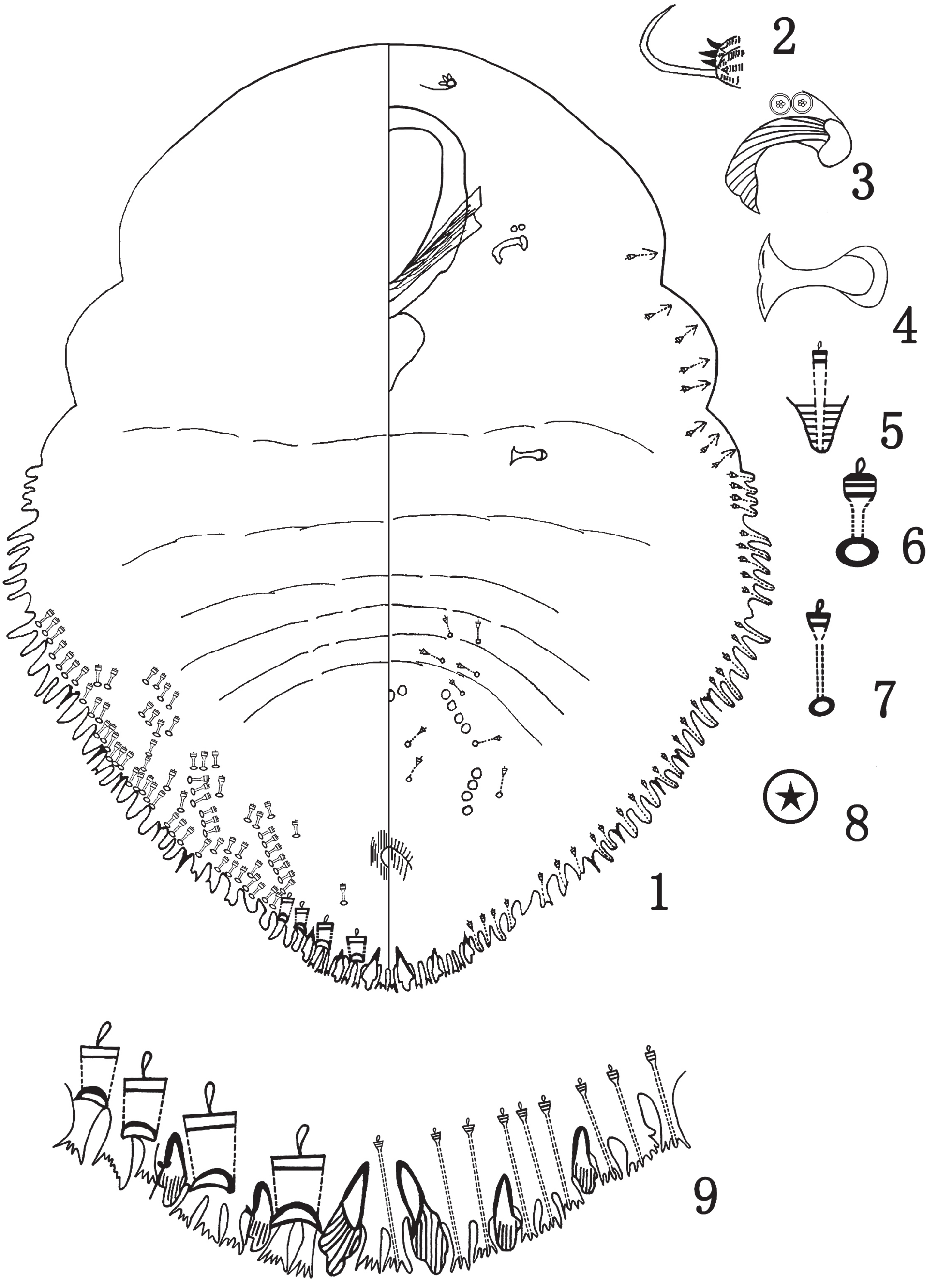

Figure 1–9. *Parlatoria menglaensis* sp. n., adult female. **1** body **2** antenna **3** anterior spiracle **4** posterior spiracle **5** gland tubercle **6** detail of dorsal gland duct **7** ventral microduct **8** perivulvar pore **9** pygidium.

the second and third; pygidial marginal macroducts largest closer to median lobes, arranged as follows: absent between the median lobes, one in each interlobar space, two outside the third lobe. Submarginal dorsal ducts on prepygidium numerous, 60–70 ducts on each side. Submedian ducts absent. Ventral microducts few, scattered on pygidium. Anal opening small, positioned about centre of pygidium. Perivulvar pores present in five groups; 3–4 in the median group, laterocephalic group with four pores, and the laterocaudal group with four pores.

Remarks. This species is very similar to *P. machilicola* (Takahashi 1933) in body shape, but differs in having (character-states on *P. machilicola* in brackets): (i) perivulvar pores present in five groups (perivulvar pores present in four groups); (ii) dorsal macroducts absent (dorsal macroducts present); (iii) marginal macroducts only four on each side (marginal macroducts more than four on each side).

The new species also resembles *P. tsujii* Tanaka, 2010, in the shape and spiracles. However, it differs from *P. tsujii* by the following characters (character-states on *P. machilicola* in brackets): (i) perivulvar pores present in five groups (perivulvar pores present in four groups); (ii) derm pocket absent (derm pocket present); (iii) marginal macroducts absent between the median lobes (marginal macroducts present between the median lobes).

Host plant. *Cinnamomum camphora* (L.) Presl (Lauraceae).

Etymology. The specific epithet is named after Mengla, the type locality.

Distribution. China (Yunnan).

Key to the adult females of *Parlatoria* Targioni Tozzetti from China

(There are records of *P. ligustri* Wu from China (Wu Chenfu 1935), but the information is inadequate for this key, thus it has not been included).

1 Perivulvar pores absent................................ ***P. pseudaspidiotus* (Lindinger)**
– Perivulvar pores present in 4 or 5 groups.. **2**
2 A marginal macroduct absent between the median lobes **3**
– A marginal macroduct present between the median lobes **4**
3 Perivulvar pores absent in the median group ...... ***P. machilicola* (Takahashi)**
– Perivulvar pores present in the median group .... ***P. menglaensis* sp. n.**
4 Puparium of adult female black; with 1 peculiar, large and ear-like lobe on each side margin of head region about opposite anterior spiracles .... ***P. ziziphi* (Lucas)**
– Puparium of adult female variable in color, but not black; on margin without ear-like lobes or lobes very small on each side of head region about opposite of anterior spiracles **5**
5 Pygidium with 2 pairs of well-developed lobes, the 3rd quite small, but sclerotized .... ***P. cupressi* (Ferris)**
– Pygidium with 3 or more pairs of well-developed lobes................................ **6**

6 Anterior spiracles without disc pores .... ***P. mytilaspiformis*** **Green**

– Anterior spiracles with disc pores **7**

7 Derm pockets present between each posterior spiracle and body margin..... **8**

– Derm pockets absent between each posterior spiracle and body margin **13**

8 Fourth lobes definitely present, not closely resembling adjacent plates **9**

– Fourth lobes absent, replaced by a membranous, plate-like process, smaller than the adjacent plates... ***P. proteus*** **(Curtis)**

9 Four plates present between third lobe and fourth lobe....... ***P. pinicola*** **Tang**

– Three plates present between third lobe and fourth lobe **10**

10 Eyespots modified to form a stout spur .... ***P. crotonis*** **(Douglas)**

– Eyespots various, flat, irregular or absent.. **11**

11 Peribuccal granulations absent .... ***P. camelliae*** **(Comstock)**

– Peribuccal granulations present **12**

12 Pygidial lobes almost equal in size; perivulvar pores always in 4 groups......... .... ***P. pini*** **Tang**

– Pygidial lobes of different sizes, the third pair smallest; perivulvar pores sometimes present in 5 groups .... ***P. theae*** **(Cockerell)**

13 Pygidium normally with four plates present between third lobe and fourth lobe.. **14**

– Pygidium with three plates present between third lobe and fourth lobe **17**

14 Peribuccal granulations present **15**

– Peribuccal granulations absent .... ***P. multipora*** **McKenzie**

15 Dorsal ducts present on submedian area on abdominal segments I-III .... ***P. yanyuanensis*** **Tang**

– Dorsal ducts absent from submedian area on abdominal segments I-III.... **16**

16 Median lobes usually with a deep lateral notch and no medial notch (rarely with a small medial notch).. ***P. oleae*** **(Colvée)**

– Median lobes with deep lateral and medial notches.......... ***P. bambusae*** **Tang**

17 Fourth lobes definitely present, not closely resembling adjacent plates **18**

– Fourth lobes replaced by a membranous, plate-like process, smaller than the adjacent plates **27**

18 Lobes with outer margins normally notched **19**

– Lobes with outer margins minutely toothed................ ***P. yunnanensis*** **Ferris**

19 Lobes with outer margins with more than one notch **20**

– Lobes with outer margins with a single notch **22**

20 Plates reduced.. **21**

– Plates well developed, fimbriate .... ***P. desolator*** **McKenzie**

21 Dorsal median macroducts present on pygidium within frame formed by perivulvar pores .... ***P. cinerea*** **(Doane & Hadden)**

– Dorsal median macroducts absent on pygidium within frame formed by perivulvar pores .... ***P. fluggeae*** **Hall**

22 Peribuccal granulations present **23**

– Peribuccal granulations absent **24**

23 Submedian dorsal macroducts absent; Eyespot modified to form rounded elevation .... ***P. machili* Takahashi**

– Submedian dorsal macroducts present on abdominal segments IV–V; Eyespot absent or inconspicuous .... ***P. cinnamomicola* Tang**

24 Median pygidial lobes deeply notched on outer and inner margins **25**

– Median pygidial lobes deeply notched only on outer margins **26**

25 Perivulvar pores absent in the median group, antero- and posterolateral groups confluent on each side; submedian dorsal macroducts present on abdominal segments I-III .... ***P. emeiensis* Tang**

– Perivulvar pores absent or a single one present in the median group, 6 to 8 in the anterolateral group, and 6 to 8 in the posterolateral group; submedian dorsal macroducts absent .... ***P. pergandii* (Comstock)**

26 Fourth lobes represented by small sclerotized spurs ... ***P. ghanii* Hall & Williams**

– Fourth lobes well developed .... ***P. reedia* Zhang, Feng & Liu**

27 Peribuccal granulations absent **28**

– Peribuccal granulations present **30**

28 Anterior spiracle with 1–3 disc pores **29**

– Anterior spiracle with 6–15 disc pores .... ***P. stigmadisculosa* Bellio**

29 Plates tapering apically to a point .... ***P. lithocarpi* Takahashi**

– Plates well fimbriated .... ***P. acalcarata* McKenzie**

30 Perivulvar pores present in the median group, with 5 groups ... ***P. piniphila* Tang**

– Perivulvar pores absent in the median group **31**

31 Prosomatic tubercles rounded .... ***P. arengae* Takagi**

– Prosomatic tubercles pointed apically, inconspicuous or absent **32**

32 Posterolateral groups of perivulvar pores with 2–3 pores each .... ***P. hydnocarpus* Hu**

– Posterolateral groups of perivulvar pores with more than 6 pores each **33**

33 Gland tubercles: 1 to 3 prespiraculars, 3 to 4 anterior spiraculars, 3 to 5 mesothoracics, 4 metathoracics, and 4 first abdominals; submarginal dorsal ducts containing 18 to 22 on each side .... ***P. liriopicola* Tang**

– Gland tubercles: 1 to 7 prespiraculars, 4 to 10 anterior spiraculars, 14 to 16 mesothoracics, 8 to 16 metathoracics, and 5 to 8 first abdominals; submarginal dorsal ducts containing 35 to 47 on each side ...... ***P. keteleericola* Tang & Chu**

Discussion

Parlatoria menglaensis sp. n. is a pest in urban areas of China. Its primary host plant is *Cinnamomum camphora* (L.) Presl (Lauraceae), which grows south of the Yangtze River in China. This plant is also found in southern Japan, Korea, and Vietnam, and has been introduced to many other countries, including Australia and the United States. Currently, *P. menglaensis* sp. n. has only been identified in China, but it could eventually spread to the previously mentioned countries where its potential host plants occur.

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