RESEARCH ARTICLE



Four new species of splanchnotrophid copepods (Poecilostomatoida) parasitic on doridacean nudibranchs (Gastropoda, Opistobranchia) from Japan, with proposition of one new genus

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Abstract

Four new species of splanchnotrophid copepods are described based on specimens collected from 5 species of doridacean nudibranchs from coastal waters of Japan. They belong to 3 genera, one of which, *Majimun* gen. n., is new. The parasites and their hosts are as follows: *Ceratosomicola japonica* **sp. n.** ex *Hypselodoris festiva* (A. Adams); *Splanchnotrophus helianthus* **sp. n.** ex *Thecacera pennigera* (Montagu); *S. imagawai* **sp. n.** ex *Trapania miltabrancha* Gosliner & Fahey; and *Majimun shirakawai* **gen. et sp. n.** ex *Roboastra luteo-lineata* (Baba) and *R. gracilis* (Bergh). *Ceratosomicola japonica* **sp. n.** is the fifth species of *Ceratosomicola* and is characterized by the shape and armature of the prosome in females. Both *S. helianthus* **sp. n.** and *S. imagawai* **sp. n.** are differentiated from 4 known congeners by the absence of posterolateral processes or lobes on the prosome in females, and the females of these 2 new species are separated from each other by the shape and armature of the genera by the segmentation of the antennule as well as the combination of the following characters in females: 2 postgenital somites and the shape of the antenna, the mandible and the swimming legs.

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Keywords

Parasitic Copepoda, Splanchnotrophidae, Ceratosomicola, Splanchnotrophus, Majimun, new genus, sea slugs

Introduction

The Splanchnotrophidae is a bizarre copepod family of Poecilostomatoida. Its members parasitize marine opisthobranch gastropods (Huys 2001). It is often difficult to detect their presence because almost all parts of the parasite's body are usually embedded inside the host, and only the distal part of the urosome and the egg sacs are exposed and visible externally. Since the mid-19th century, splanchnotrophids have been reported or described by many malacologists in the course of studies on opisthobranch gastropods (see Huys 2001). The taxonomic studies have often been inadequate at the generic level because the original descriptions include errors or have omissions. In his revision of Splanchnotrophidae, Huys (2001) clarified the validity of 3 genera, Splanchnotrophus Hancock & Norman, 1863, Ismaila Bergh, 1868, and Lomanoticola Scott T. & A., 1895, and established 2 new genera, Arthurius Huys, 2001 and Ceratosomicola Huys, 2001. Since then, 1 species of Arthurius, 8 of Ismaila, and 3 of Ceratosomicola have been described, and a total of 23 species belonging to 5 genera are recognized in this family at present (Haumayr and Schrödl 2003; Salmen et al. 2008a, b). In this study, 4 new species of splanchnotrophid copepods collected from Japanese waters are described. Based on one new species, a new genus Majimun gen. n. is herein established.

Material and methods

Doridacean nudiblanchs were collected by SCUBA diving in the Seto Inland Sea off Hiroshima, central Japan and in both the North Pacific Ocean and the East China Sea off Okinawa-jima Island, the Central Ryukyu Islands, southern Japan, from April 2008 to December 2010. Collection data including the numbers of copepods found on the nudibranchs examined are shown in Table 1. Copepods were carefully removed from the body cavities of the hosts via dissections and preserved in 80% ethanol. Specimens were soaked in lactophenol for 2 days before dissection. The appendages of the copepods were then dissected and observed using the method of Humes and Gooding (1964). The drawings were made with the aid of a drawing tube. Morphological terminology follows Huys and Boxshall (1991) and Huys (2001). Measurements in millimeters are shown as ranges in parentheses with means and standard deviations. Type specimens are deposited in the crustacean collection of the National Museum of Nature and Science, Tsukuba (NSMT) and the Ryukyu University Museum, Fujukan (RUMF), Okinawa. The scientific names of nudibranchs follow those listed by Debelius and Kuiter (2007), Gosliner et al. (2008), and Gosliner and Fahey (2008).

| Host nudibranch | Number of hosts examined | Locality | Date | Copepod | Number of copepod specimens |
|---------------------------|--------------------------------|---|-----------------|-----------------------|-----------------------------------|
| Hypselodoris | 1 | Off Irukabana, Nohmi-jima Island, | 11 Dec. | Ceratosomicola | 2,48 |
| festiva | | Hiroshima, Seto Inland Sea (34°13'49"N, 132°23'7"E), 5 m | 2010 | japonica | |
| | 2 | Off Irukabana, Nohmi-jima Island, | 12 Dec. | C. japonica | 1♀, 2♂ |
| | | Hiroshima, Seto Inland Sea (34°13'49"N, 132°23'7"E), 3 m | 2010 | | |
| Thecacera | 1 | Off Izaki, Yashiro-jima Island, | 27 Apr. | Splanchnotrophus | 19,18 |
| pennigera | | Yamaguchi, Seto Inland Sea (33°51'49"N, 132°19'29"E), unknown depth | 2008 | helianthus | |
| | 2 | Off Matoba Beach, Takehara, Hiroshima, Seto Inland Sea (34°19'29"N, 132°55'21"E), 15 m | 15 Jan. 2009 | S. helianthus | 1♀ |
| | 3 | Off Matoba Beach, Takehara, Hiroshima, Seto Inland Sea (34°19'29"N, 132°55'21"E), 15 m | 17 Feb. 2009 | S. helianthus | 1♀, 10 ♂ |
| | 4 | Off Matoba Beach, Takehara, Hiroshima, Seto Inland Sea (34°19'29"N, 132°55'21"E), 15 m | 17 Feb. 2009 | S. helianthus | 10 |
| Trapania miltabrancha | 1 | Off Red Beach, Kin, Okinawa-jima Island, North Pacific Ocean (26°26'41"N, 127°54'39"E), 15 m | 29 May 2008 | S. imagawai | 1♀ |
| | 2 | Off Red Beach, Kin, Okinawa-jima Island, North Pacific Ocean (26°26'41"N, 127°54'39"E), 15 m | 23 Apr. 2009 | S. imagawai | 10 |
| Roboastra luteolineata | 1 | Off Miyagi Beach, Chatan, Okinawa- jima Island, East China Sea (26°19'44"N, 127°44'35"E), unknown depth | 14 Oct. 2009 | Majimun shirakawai | 2♀, 1♂ |
| | 2 | Off Miyagi Beach, Chatan, Okinawa- jima Island, East China Sea (26°19'44"N, 127°44'35"E), 6 m | 14 Jun. 2010 | M. shirakawai | 19,18 |
| Roboastra gracilis | 1 | Off Cape Maeda, Onna, Okinawa-jima Island, East China Sea (26°26'41"N, 127°46'20"E). 5 m | Jun. 2010 | M. shirakawai | 1♀,1♂ |

Table 1. Collection data of the nudibranchs infected by splanchnotrophid copepods examined in present study.

Results

Family Splanchnotrophidae Norman & Scott, 1906 *Ceratosomicola* Huys, 2001

Ceratosomicola japonica sp. n.

New Japanese name: umiushi-yadori for the family, the genus, and the species urn:lsid:zoobank.org:act:C4997364-5CBC-4B04-9C1E-8DD13111C5C9 http://species-id.net/wiki/Ceratosomicola_japonica Figures 1A, B, 2–4

Type material. Holotype: female, ex body cavity of *Hypselodoris festiva* (A. Adams) (Nudibranchia: Chromodorididae), off Irukabana, Nohmi-jima Island, Hiroshima,



Figure 1. Live coloration of the host nudibranchs and the splanchnotrophids. **A** *Hypselodoris festiva* infected by an ovigerous specimen of *Certosomicola japonica* sp. n. **B** an egg sac of *C. japonica* sp. n. and the gill circle of *H. festiva* with the mantle malformed into an elongate tube **C** *Thecacera pennigera* infected by an ovigerous specimen of *Splanchnotrophus helianthus* sp. n. **D** *T. pennigera* with the mantle removed to show a female specimen of *S. helianthus* on the visceral sac **E** *Trapania miltabrancha* infected by an ovigerous specimen of *S. helianthus* on the visceral sac **E** *Trapania miltabrancha* infected by an ovigerous specimen of *S. helianthus* on the visceral sac **E** *Trapania miltabrancha* infected by an ovigerous specimen of *S. imagawai* sp. n (photo by K. Imagawa) **F** gill circle of *T. miltabrancha* with egg sacs of *S. imagawai* sp. n. (photo by K. Imagawa) **G** *Roboastra luteolineata* infected by an ovigerous specimen of *Majimun shirakawai* gen. et sp. n. (photo by N. Shirakawa) **H** female *M. shirakawai* gen. et sp. n. with dwarf male attached to the posterior part of the body. Scale bars = 5 mm in **A**; 1 mm in **B**, **D**, **H**.

Seto Inland Sea, Japan (34°13'49"N, 132°23'7"E), 5 m depth, 11 December 2010 (NSMT–Cr 22240). Allotype: male (NSMT–Cr 22241), collection data same as that

of holotype. Paratypes: 1 female and 3 males (NSMT–Cr 22242), collection data same as that of holotype; 1 female and 2 males ex body cavity of *H. festiva*, off Irukabana, Nohmi-jima Island, Hiroshima, Seto Inland Sea, Japan (34°13'49"N, 132°23'7"E), 3 m depth, 12 December 2010 (NSMT–Cr 22243).

Type locality. Off Irukabana, Nohmi-jima Island, Hiroshima, Seto Inland Sea, Japan (34°13'49"N, 132°23'7"E).

Description of holotype female. Body length from rostrum to posterior margin of anal somite: 4.27. Body (Figure 2A) composed of large prosome with 3 pairs of ventrolateral processes and small 3-segmented urosome. Prosome indistinctly 3-segmented, composed of anterior region, cephalosome, middle region comprising first to second pedigerous somites, and posterior region as third and fourth pedigerous somites. Cephalosome (Figures 2A, B, 3A) ellipsoid bearing rostrum with round margin, wider than long, bearing single apical lobe and 1 paired lateral lobes. Middle region large, bearing two transverse dorsal bulges and 5 ventral protrusions; anterodorsal bulge ornamented by 2 paired anterior and 1 paired lateral protrusions; posterior dorsal bulge carrying 2 pairs of lateral protrusions. Posterior region (Figure 2A-C) bearing two ventral protrusions on third pedigerous somite and constriction at border between third and fourth pedigerous somites. Ventrolateral processes (Figure 2A) long and slender, distinctly longer than body. Urosome (Figure 2D) onion-like shaped, comprising genital double somite and two free postgenital somites ornamented with pattern of small scales on ventral surface. Genital double somite bearing paired ventral genital apertures. Caudal rami (Figure 2E) globular bearing two and three spiniform elements on outer margin and tip, respectively; one element on tip serrated.

Antennule (Figure 3A, B) 4-segmented; proximal segment rectangular bearing 4 spines on anterior margin; second segment with 3 anterior spiniform and 1 posterior setiform elements; third segment bearing 2 anterior and 1 posterior elements; terminal segment bearing 6 spiniform and 1 setiform elements. Antenna (Figure 3A, C) 3-segmented, conical with large sclerite at base, comprising coxobasis and 2-segmented endopod; coxobasis unarmed; proximal endopodal segment bearing 1 seta; terminal endopodal segment claw-like bearing 7 small elements. Labrum (Figure 3A) bilobate, unarmed. Labium (Figure 3A) bearing two paired spinulose lobes. Mandible (Figure 3A, D) represented by single recurved blade covered with numerous spinules along both anterior and posterior margin. Maxillule absent. Maxilla (Figure 3A, E) weakly sclerotized globular tapering into lanceolate tip. Maxilliped absent.

Swimming legs rudimentary; protopod largely incorporated into ventral wall of prosome. Leg 1 (Figures 2B, 3F) represented by outer basal seta, small exopodal lobe with seta and conical process along outer margin and 2 processes on tip, and spiniform endopodal element. Leg 2 (Figures 2B, 3G) bearing basal seta, elongate exopod indistinctly 2-segmented, tapering into apical process with 4 elements and single process, and endopodal lobe elongate, unarmed with intermedial constriction. Leg 3 on holotype indistinct.

Egg sacs (Figure 2F) curved, semicircle; color in life crimson.



Figure 2. *Ceratosomicola japonica* sp. n., female, holotype NSMT–Cr 22240 (**A–C**), female, paratype NSMT–Cr 22243 (**D–F**). **A** habitus dorsal **B** habitus, ventral, p1 = leg 1, p2 = leg 2 **C** posterior portion of body, ventral **D** urosome, ventral **E** caudal ramus, ventral **F** egg sac. Scale bars = 1 mm in **A**, **B**; 300 μ m in **C**; 100 μ m in **D**; 10 μ m in **E**; 500 μ m in **F**.

Variation of female morphology. The morphology of female paratypes is as in the holotype, except leg 2 shows variability. Leg 3 is distinctly visible on the paratype females. Paratype female (NSMT-Cr 22243) has the exopod of leg 2 (Figure 3H) tapering into apical process with constriction and 2 elements. Paratype female (NSMT-Cr 22242) possesses a vestigial leg 3 (Figure 3I), represented by a blunt element on a protrusion. The specimens from type series (n = 3) range from 3.11-4.27 (3.76 ± 0.59) in body length (BL).

Description of allotype male. Sexual dimorphism present in body form, and swimming legs. Body (Figure 4A–C) 2.81 long, composed of cephalothorax and 5 cylindrical somites. Cephalothorax large, bulbous, incorporating first and second pedigerous somites, bearing transverse constriction and paired lateral and single dorsal protrusions posterior to mouthparts, paired posterolateral outgrowth, and paired and single ventral protrusions. Genital somite (Figure 4D) incompletely segmented, bearing transverse dorsal folding and paired apertures; opercula unarmed. Caudal rami (Figure 4E) globular, 2 and 3 elements along outer margin and on tip, respectively. No marked sexual dimorphism in antennule, antenna, and mouthparts. Tip of maxilla (Figure 4F) slightly sharper than that of female.

Leg 1 (Figure 4B, G) represented by outer basal seta, lobate exdopod with 2 elements, and spiniform endopodal element. Leg 2 (Figure 4B, H) represented by outer basal, serrated seta, elongate exopodal lobe with single element, and elongate endopodal lobe with single blunt element on tip. Leg 3 (Figure 4D, I) represented by spiniform element.

Variation of male morphology. The morphology of male paratypes is as in the allotype. The specimens from type series (n = 6) range from 2.16-2.81 (2.42 ± 0.43) in BL.

Site. Female and male specimens were found in the body cavity of the host nudibranchs. Only the posterior tip of the urosome and the egg sacs were exposed from the host's gill circle (Figure 1A, B). The mantle around the gill circle of the infected nudibranch was malformed into elongate tubes which obscured the host's gills and the egg sacs of the copepod (Figure 1B).

Etymology. The specific name of the new species "*japonica*" refers to Japan, where it was collected. *Hypselodoris festiva*, the type host of this new species, is widely distributed around the Japanese archipelago and is one of the common nudibranchs of Japan. *Ceratosomicola japonica* sp. n. is the first species of parasitic copepods to have been described from Japan (Fujita 1895).

Remarks. Ceratosomicola sacculata (O'Donoghue, 1924) was originally described as Splanchnotrophus sacculatus. Huys (2001) redescribed this species based on a female and established a new genus Ceratosomicola. Three species, C. coia Salmen, Wilson & Schrödl, 2008; C. delicata Salmen, Wilson & Schrödl, 2008; C. mammilata Salmen, Wilson & Schrödl, 2008, were subsequently described based on specimens of both sexes, and this genus is now composed of four species (Salmen et al. 2008b). The new species clearly differs from C. coia and C. delicata in having 7 ventral protrusions on the prosome of the female (vs. without ventral protorusions on C. coia and C. delicata; Salmen et al. 2008b). Although the female of C. mammilata shares 7 protrusions, this species can be differentiated from the new species by having 2 pairs of lateral lobes



Figure 3. *Ceratosomicola japonica* sp. n., female, holotype NSMT–Cr 22240 (**A–G**), female, paratype NSMT–Cr 22243 (**H**), female, paratype NSMT–Cr 22242 (**I**). **A** cephalosome, anterior portion. ventral **B** antennule, anterior **C** antenna **D** mandible **E** maxilla **F** leg 1 **G** leg 2 **H**, leg 2 (drawn from a paratype, NSMT–Cr 22243) **I** leg 3. Scale bars = 100 μm in **A**; 20 μm in **B**; 30 μm in **C**, **G**, **H**; 10 μm in **D**, **I**; 50 μm in **E**, **F**.



Figure 4. *Ceratosomicola japonica* sp. n., male, allotype NSMT–Cr 22241. **A** habitus, dorsal **B** habitus, ventral, p1 = leg 1, p2 = leg 2 **C** habitus, lateral **D** free thoracic somites and abdomen, ventral, p3 = leg 3 **E** caudal ramus, dorsal **F** maxilla **G** leg 1 **H** leg 2 **I** leg 3. Scale bars = 500 μ m in **A**, **B**, **C**; 200 μ m in **D**; 10 μ m in **E**, **I**; 20 μ m in **F**, **G**; 30 μ m in **H**.

on the anterior region of the prosome and a posterior pair of ventral protrusions located anterior to the base of third ventrolateral processes, i.e. on the second pedigerous somite (vs. 1 pair of lateral lobes on the anterior region of the prosome and a posterior pair of ventral lobes located posterior to the base of third ventrolateral processes, i.e. on the third pedigerous somite). In Huys' (2001) redescription of *C. sacculata*, the ventral protrusions on the prosome was not described, while in the original description, O'Donoghue (1924) referred to the presence of at least 2 paired ventral lobes on the prosome. However, *C. sacculata* is distinguishable from the new species by the following characters in females: the anterior region of the prosome is trilobate (vs. ellipsoidal and bearing a pair of lateral lobes in the new species) and the middle region of the prosome bears 3 transverse dorsal bulges (vs. 2 transverse dorsal bulges in the new species).

In the course of dissection to describe Hypselodoris festiva (as Chromodoris marenzalleri) (Nudibranchia: Chromodorididae) from the western North Pacific Ocean off Misaki, Kanagawa Japan (Fujita 1893), one female specimen of splanchnotrophid was discovered by Fujita (1895) from the body cavity of the host. Although Fujita (1895) recognized some differences between this copepod and other splanchnotrophids, he did not describe it as a new species nor deposit it in any museum because of the incomplete specimen. The species was subsequently recognized as a member of *Ceratosomicola* by Huys (2001). Ceratosomicola japonica sp. n. was collected from the same host species (H. festiva) in the Seto Inland Sea off Nohmi-jima Island, Hiroshima, Japan and shares important characters as follows: the anterior region of the prosome is wider than long, bearing a pair of lateral lobes (Fujita 1895, figure 2) and middle region of the prosome bears a paired anterior and single posterior ventral protrusions with the latter being larger than the others (Fujita 1895, figure 1). Fujita (1895, figure 2) also described the middle region of the prosome as bearing a cross-shaped concavity. This corresponds to the transverse dorsal bulges with 4 bulbous protrusions on each corner of C. japonica sp. n. Therefore, the Fujita's splanchnotrophid is apparently conspecific with *C. japonica* sp. n.

Splanchnotrophus Hancock & Norman, 1863

Splanchnotrophus helianthus sp. n.

New Japanese name: himawari-umiushi-yadori for both the genus and the species urn:lsid:zoobank.org:act:744FEC5A-C377-442C-94CC-3F56132053E0 http://species-id.net/wiki/Splanchnotrophus_helianthus Figures 1C, D, 5–7

Type material. Holotype: female, ex body cavity of *Thecacera pennigera* (Montagu) (Nudibranchia: Polyceridae), off Matoba Beach, Takehara, Hiroshima, Seto Inland Sea, Japan (34°19'29"N, 132°55'21"E), 15 m depth, 17 February 2009 (NSMT–Cr 22244). Allotype: male (NSMT–Cr 22245), collection data same as that of holotype. Paratypes: 1 female and 9 males (NSMT–Cr 22246), collection data same as that of holotype; 1 female ex body cavity of *T. pennigera*, off Matoba Beach, Takehara, Hiro-



Figure 5. *Splanchnotrophus helianthus* sp. n., female, holotype NSMT–Cr 22244. **A** habitus, dorsal **A'** enlarged view of egg sac **B** habitus, ventral, p1 = leg 1, p2 = leg 2 **C** posterior portion of body, ventral, p3 = leg 3 **D** fourth pedigerous somite and genito-abdomen, ventral **E** caudal ramus, dorsal **F** cephalosome, ventral. Scale bars = 1 mm in **A**; 100 μ m in **A**'; 500 μ m in **B**; 200 μ m in **C**, **F**; 100 μ m in **D**; 20 μ m in **E**.

shima, Seto Inland Sea, Japan (34°19'29"N, 132°55'21"E), 15 m depth, 15 January 2009 (NSMT–Cr 22247); 1 female and 1 male ex body cavity of *T. pennigera*, off Izaki, Yashiro-jima Island, Yamaguchi, Seto Inland Sea, Japan (33°51'49"N, 132°19'29"E), unknown water depth, 27 April 2008 (NSMT–Cr 22248).

Type locality. Off Matoba Beach, Takehara, Hiroshima, Seto Inland Sea, Japan (34°19'29"N, 132°55'21"E).

Description of holotype female. Body (Figure 5A) 3.44 long, composed of elongate, slender prosome with 3 pairs of long lateral processes and small 2-segmented urosome. Prosome (Figure 5A, B) composed of anterior region, cephalosome, middle region comprising first to second pedigerous somites, and posterior region as third pedigerous somite. Cephalosome (Figure 5B, F) elongate, bent ventrally, with projecting rostral area. Middle region (Figure 5A, B) large, constricted posterior to base of anterior lateral processes with paired and single dorsal protrusions. Posterior region (Figure 5A–C) broad, without armature. Lateral processes (Figure 5A) long and slender, distinctly longer than body length. Urosome (Figure 5C, D) small; genito-abdomen narrower posteriorly with paired posterolateral lobes; unarmed opercula and genital aperture located on ventral. Caudal rami (Figure 5E) small, about twice as long as wide, bearing 6 setae and 1 dorsal spiniform process; apical seta long, styliform.

Antennule (Figures 5F, 6A) 2-segmented; terminal segment bearing 2 constrictions making it appearing as original segmentation; proximal segment bearing 2 blunt spines; terminal segment bearing 2 blunt spines and 1 seta in proximal part, 3 setae and 1 aesthetasc in middle part, and 9 setae and 2 aesthetascs in distal part. Antenna (Figures 5F, 6B) 3-segmented; coxo-basis broad, bearing 1 inner spine with spiniform tip; proximal segment of endopod bearing 1 inner spine; terminal segment of endopod tapering into strong apical claw, with 2 spines and 2 setal elements. Labrum (Figure 6B) bilobate, bearing flat surface. Mandible (Figure 6C) spatulate, tapering into single curved blade with 2 dentiform processes giving trifid appearance. Labium (Figure 6B) developed with paired spinulose patches. Maxillule not observed. Paragnath (Figure 6B) developed, represented by pinnate lobe. Maxilla (Figure 6B, D) 2-segmented; syncoxa unarmed; allobasis tapering into spiniform element, with seta. Maxilliped absent.

Leg 1(Figures 5B, 6E) unsegmented, weakly sclerotized and drawn out into elongate exopod and small endopod; protopod bearing outer basal seta; exopod drawn out into spiniform lobe bearing multiple constrictions, wrinkly surface, 3 outer and 1 inner setal elements; endopod a small lobe tipped with seta. Leg 2 (Figures 5B, 6F) unsegmented, weakly sclerotized; protopod drawn out into long exopod and small, cylindrical endopod; protopod bearing outer basal seta; exopod tapering into a pointed process with three outer and 1 inner small element; endopodal lobe bearing small apical seta. Leg 3 (Figures 5C, 6G) represented by conical process with apical seta, located near posterolateral corner on ventral side of prosome.

Egg sacs (Figure 5A) bilobate, bearing curved side and swollen side; color in life cream (Figure 1C, D).

Variation of female morphology. The morphology of female paratypes is as in the holotype. The specimens from type series (n = 3) range from 2.81–4.47 (3.57 ± 0.83) BL.



Figure 6. *Splanchnotrophus helianthus* sp. n., female, holotype NSMT–Cr 22244. **A** antennule, anterior **B** oral area **C** mandible, posterior **D** maxilla **E** leg 1 **F** leg 2 **G** leg 3. Scale bars = 20 μm in **A**, **E**, **F**; 30 μm in **B**; 10 μm in **C**, **D**, **G**.

Description of allotype male. Sexual dimorphism prominent in body form. Body (Figure 7A–C) cyclopiform, 0.63 long, composed of cephalothorax and 5 cylindrical somites. Cephalothorax (Figure 7A–C) large, incorporating first and second pedigerous somites, with constriction posterior to mouthparts. Urosome 3-segmented (Figure 7D); genital somite scarcely discernible in dorsal view, bearing paired apertures; opercula carrying 2 processes along posterior margin. Anal somite (Figure 7D) nearly completely withdrawn into genital somite. Caudal rami (Figure 7E) cylindrical, about three times as long as wide, bearing 5 setae, styliform terminal seta bipinnate toward tip, and 2 dorsal spiniform spines.



Figure 7. *Splanchnotrophus helianthus* sp. n., male, allotype NSMT–Cr 22245. **A** habitus, dorsal **B** habitus, ventral **C** habitus, lateral **D** free thoracic somites and abdomen, ventral **E** caudal ramus, ventral **F** oral area **G** leg 1 **H** leg 2. Scale bars = 100 μ m in **A**, **B**, **C**; 50 μ m in **D**; 20 μ m in **E**.

No marked sexual dimorphism in antennule, antenna, and mouth parts, except location of antenna. The base of antenna located anterior to labrum (Figure 7F).

Leg 1 (Figure 7G) biramous; protopod narrower than that of female, with minute basal outer seta; exopodal lobe elongate, tapering into pointed process, carrying 4 outer and 1 inner elements; endopodal lobe small, tipped with minute apical element. Leg 2 (Figure 7H) longer than leg 1; protopod bearing minute basal outer seta; exopodal lobe elongate, tapering into pointed process, bearing 3 outer and 1 inner elements; endopodal lobe tipped with minute element, bearing 1 small outer element. Leg 3 (Figure 7D) represented by single seta. Legs 4 and 5 absent.

Variation of male morphology. The morphology of male paratypes is as in the allotype. The specimens from type series (n = 11) range from 0.32–0.63 (0.53 ± 0.12) in BL.

Site. Both female and male specimens were found in the body cavity of host nudibranchs. The females grasped the host's visceral sac by the lateral processes on the prosome (Figure 1D). Only the posterior tip of the urosome and the egg sacs were exposed from the host's gill circle (Figure 1C).

Etymology. The specific name "*helianthus*" is from the Latin meaning sunflower. The live body color of this new species is yellowish, and the egg sacs attached on the host nudibranch look like flowers.

Remarks. Four species of *Splanchnotrophus* are currently recognized as valid (Huys 2001). *Splanchnotrophus helianthus* sp. n. differs from *S. angulatus* Hecht, 1893, *S. dellachiajei* Delamare Deboutteville, 1950, *S. gracilis* Hancock & Norman, 1863 in the absence of paired posterolateral processes on the prosome and the genito-abdomen bearing lateral lobes in females (vs. the presence of posterolateral processes on the processes on the processes on the prosome and the genito-abdomen without paired lateral lobes, Hancock and Norman 1863; Delamare Deboutteville 1950; Huys 2001). Huys (2001) claimed that, in *S. angulatus*, the shape of the female's genito-abdomen is constant, irrespective of prosome valiability, and the size and shape of the posterolateral lobe of the prosome certainly shows variability. Nevertheless, this species always possesses the posterolateral lobe, which is regarded as a useful identification character. The original description of *S. willemi* by Canu (1891) has no illustration and includes only a minimum amount of information. However, the presence of pleural wings on the third pedigerous somite in *S. willemi* is not shared with the new species (Canu 1891).

Splanchnotrophus imagawai sp. n.

New Japanese name: uzu-himawari-umiushi-yadori urn:lsid:zoobank.org:act:5DD5D5DA-2D24-4A69-A64D-6C3A5004920D http://species-id.net/wiki/Splanchnotrophus_imagawai Figures 1E, F, 8–9

Type material. Holotype: female, ex body cavity of *Trapania miltabrancha* Gosliner & Fahey (Nudibranchia: Goniodorididae), off Red Beach, Kin, Okinawa-jima Island, North Pacific Ocean, Japan (26°26'41"N, 127°54'39"E), 15 m depth, 23 April

2009 (NSMT–Cr 22249). Paratype: 1 female, ex body cavity of *T. miltabrancha*, off Red Beach, Kin, Okinawa-jima Island, North Pacific Ocean, Japan (26°26'41"N, 127°54'39"E), 15 m depth, 29 May 2008 (RUMF–ZC–02105).

Type locality. off Red Beach, Kin, Okinawa-jima Island, North Pacific Ocean, Japan (26°26'41"N, 127°54'39"E).

Description of holotype female. Body (Figure 8A) 1.86 long, composed of swollen prosome and small 2-segmented urosome. Prosome composed of anterior region as cephalosome, middle region comprising first and second pedigerous somites, and posterior region as third pedigerous somite. Cephalosome (Figure 8B) not elongated, broad and unarmed with protruded rostral region (Figure 9A). Middle region (Figure 8B) compact, about as wide as long, bearing 3 pairs of lateral processes, without posterolateral processes. Posterior region (Figure 8B, C) broad, bearing paired bulbs carrying leg 3 on tip. Lateral processes (Figure 8A) long and slender, about twice as long as body length. Urosome (Figure 8C, D) small; genito-abdomen ampulla-like posterior portion bearing paired apertures without posterolateral lobes; opercula bearing small shield-like structure with 2 spiniform processes. Caudal ramus (Figure 8E) small, about 1.5 times as long as wide, bearing 6 setae and 2 dorsal spiniform processes; apical seta long, styliform.

Antennule (Figure 9A, B) 2-segmented; terminal segment divided by 2 constrictions making it appearing as original segmentation; proximal segment bearing 2 blunt spines; terminal segment bearing 2 blunt spines and 1 seta in proximal part, 3 setae and 1 blunt element in middle part, and 9 setae and 2 blunt elements in distal part. Antenna (Figure 9A, C) 3-segmented; coxo-basis broad, bearing 1 medial spine; proximal segment of endopod bearing 1 medial spine; terminal segment of endopod drawn out into strong apical claw, with 2 spines and 2 setal elements. Labrum (Figure 9A, C) bilobate, bearing flat surface. Mandible (Figure 9A, C, D) spatulate, tapering into single curved blade without dentiform processes. Labium (Figure 9A, C) with two patches of spinules. Maxillule not observed. Paragnath (Figure 9C) represented by pinnate lobe. Maxilla (Figure 9C, E) 2-segmented; syncoxa unarmed; allobasis tapering into spiniform process and bearing seta. Maxilliped absent.

Legs 1 and 2 (Figures 8B, 9F, G) unsegmented, weakly sclerotized; protopod bearing outer basal seta, largely incorporated into ventral wall of prosome; elongate exopodal lobe separated from small endopodal lobe; exopodal lobe drawn out into long process bearing multiple constrictions, wrinkly surface, and 4 setal elements; endopodal lobe bulbous, bearing spiniform apical element. Leg 3 (Figures 8C, 9H) represented by conical process with apical seta.

Egg sacs (Figure 8F) bilobate, bearing curved side and spiral side; dull white in live color (Figure 1E, F).

Variation of female morphology. The morphology of the female paratype is as in the holotype. The specimens from type series (n = 2) range from 0.71-1.86 (1.28 ± 0.81) BL.

Male. Unknown.

Site. Female specimens were found in the body cavity of host nudibranchs. They grasped the host's visceral sac by the lateral processes. Only the posterior tip of the urosome and the egg sacs were exposed from the host's gill circle (Figure 1E, F).



Figure 8. *Splanchnotrophus imagawai* sp. n., female, holotype NSMT–Cr 22249. **A** habitus, dorsal **B** habitus, ventral, p1 = leg 1, p2 = leg 2 **C** posterior portion of body, ventral, p3 = leg 3 **D** fourth pedigerous somite and genito-abdomen, ventral **E** caudal ramus, ventral **F** egg sac. Scale bars = 1 mm in **A**; 500 μ m in **B**, **F**; 200 μ m in **C**; 100 μ m in **D**; 20 μ m in **E**.



Figure 9. *Splanchnotrophus imagawai* sp. n., female, holotype NSMT–Cr 22249. **A** anterior portion of cephalosome **B** antennule, ventral **C** oral area **D** mandible, posterior **E** maxilla **F** leg 1 **G** leg 2 **H** leg 3. Scale bars = 100 μm in **A**; 50 μm in **B**, **C**; 10 μm in **D**, **H**; 20 μm in **E**, **F**, **G**.

Etymology. The specific name "*imagawai*" honours the collector of this new species, Mr. Kaoru Imagawa who is a professional diver. The discovery of the new species was brought by his extraordinary ability to find small nudibranch gastropods.

Remarks. The female of the new species differs from *S. angulatus, S. dellachiajei, S. gacilis* and *S. willemi* in the absence of posterolateral processes on the prosome (vs. present, see Hancock and Norman 1863; Canu 1891; Delamare Deboutteville 1950; Huys 2001). *Splanchnotrophus helianthus* sp. n. lacks such processes but differs clearly from the new species in the following characters in females: the anterior region of the prosome is elongate and bent to ventral (vs. not elongate); the middle region of the prosome has a constriction posterior to the base of the first lateral processes (vs. without constriction); the genito-abdomen possesses posterolateral lobes (vs. without lobes); the mandible bears dentiform processes (vs. without processes); the endopodal lobe of leg 1 is adpressed to the exopodal lobe via the small protopod (vs. the endopodal lobe separated from the exopodal lobe); and leg 3 is located on the third pedigerous somite directly (vs. leg 3 located on the apex of paired bulbs).

Majimun gen. n.

urn:lsid:zoobank.org:act:844ACF3B-5676-4961-A013-4731BE2023FB http://species-id.net/wiki/Majimun

Diagnosis of adult female. Body elongate, comprising long prosome with 3 pairs of lateral processes and 3-segmented urosome. Prosome composed of anterior region (cephalosome), middle region (including first and second pedigerous somites), and posterior region (including third and fourth pedigerous somites). Cephalosome elongate. Middle region elongate, about twice as long as wide, without posterolateral processes. Posterior region elongate. Lateral processes long and slender. Urosome small; genital double somite cylindrical, narrower at mid region, bearing paired apertures with slightly prominent posterolateral corners; opercula bearing small shield-like structure with 2 spiniform processes. Caudal rami small, bearing 6 setae; apical seta styliform.

Antennule 3-segmented bearing spiniform elements; proximal segment subdivided into basal part with 4 spines and distal part with 3 elements; middle segment bearing 3 elements; terminal segment bearing 11 elements. Antenna 3-segmented; coxo-basis broad, bearing 1 medial spiniform element; proximal segment of endopod bearing 1 inner spiniform element; terminal segment of endopod drawn out into strong apical claw, with 4 and 1 elements along outer and inner margins, respectively. Labrum bilobate, bearing paired extra lobes and small central, conical protrusion. Mandible spatulate, drawn out into blade with 3 dentiform processes. Maxillule not observed. Paragnath bulbous lobe. Maxilla 2-segmented; syncoxa unarmed; allobasis tapering into curved process, with seta. Maxilliped absent. Labium bearing single pointed process, small paired protrusions ornamented with spinules, and posterolateral patches of spinules.

Legs 1 and 2 composed of protopod largely incorporated into ventral wall of prosome, with exopodal and endopodal lobes; protopod bearing outer basal seta, small protrusion at base of endopodal lobe of leg 1; exopodal lobe indistinctly 2-segmented, tapering into spiniform apical process; endopodal lobe cylindrical bearing apical process. Leg 3 represented by conical process with apical seta.

Egg sacs cylindrical and spiral.

Diagnosis of adult male. Body cyclopiform, composed of cephalothorax and 5 cylindrical somites. Cephalothorax large, bulbous, incorporating first and second pedigerous somites, bearing transverse constriction posterior to mouth parts and paired posterolateral outgrowth. Genital somite bearing paired apertures; opercula unarmed. Caudal rami conical, about as long as wide, bearing 6 setae; apical seta styliform. No marked sexual dimorphism in antennule and mouth parts. Shape of antenna as in female except terminal endopodal segment bearing 5 elements; inner margin bearing 2 of 5 elements. Mandible elongate, drawn out into spatulate apical blade with 3 dentiform processes.

Legs 1 and 2 composed of round protopod with outer basal seta, indistinctly 2-segmented exopodal lobe drawn out into pointed process, and non-segmented endopodal segment with apical small process. Leg 3 represented by conical process with apical seta.

Type and only species. Majimun shirakawai sp. n. by the present designation.

Etymology. The generic name, "*majimun*", refers to a dialect in Okinawa, which means demons. The gender is neuter.

Remarks. Females of Lomanoticola and Splanchnotrophus differ from Majimun gen. n. in having a 2-segmented urosome comprising the genital double somite and the anal somite (vs. a 3-segmented urosome and the genital double somite separated from the abdomen) (Huys 2001; present study). Females of *Ismaila* spp. share a 3-segmented urosome, which includes 1 postgenital somite (vs. 2 somites). There are also differences in the following female characters between Ismaila and the new genus: the antennule is 2-segmented (vs. 3-segmented); the mandible consists of a small rod tipped with a short tooth and a slender spine (vs. drawn out into a blade with dentiform processes); the paragnath is absent (vs. present); the maxillule is made of a lobe with 2 setae (vs. absent); and the maxilla has the allobasis drawn out into a multipinnate endite with 2 accessory elements (vs. with the allobasis drawn out into a curved process with 1 seta) (see Ho 1981; Haumayr and Schrödl 2003). Majimun gen. n. does not show distinct sexual dimorphism in the antennule, the antenna, and the mouth parts. The new genus also possesses a 3-segmented antennule, the mandible, and the paragnath in both sexes and 2 postgenital somites in females. These characters are not shared with Arthurius (see Huys 2001). All of these characters are shared with *Ceratosomicola*, except the 3-segmented antennule. However, Ceratosomicola differs from the new genus by having the following characters in both sexes: the antennule is composed of 4 distinct segments (vs. 3 segments); the antenna is conical (vs. elongate); the mandible is covered with numerous spinules (vs. spatulate bearing a blade with dentiform processes around apex); and the maxilla possesses a lanceolate process without armature (vs. with process and 1 seta) (see Huys 2001; present study).

Majimun shirakawai gen. et sp. n.

New Japanese name: banana-umiushi-yadori for both the genus and the species urn:lsid:zoobank.org:act:4C7B7C83-FFB4-4997-9329-5999D8553200 http://species-id.net/wiki/Majimun_shirakawai Figures 1G, H, 10–12

Type material. Holotype: female, ex body cavity of *Roboastra luteolineata* (Baba) (Nudibranchia: Polyceridae), off Miyagi Beach, Chatan, Okinawa-jima Island, East China Sea, Japan (26°19'44"N, 127°44'35"E), 6 m depth, 14 June 2010 (NSMT–Cr 22250). Allotype: male (NSMT–Cr 22251) collection data same as that of holotype. Paratypes: 1 female and 1 male, ex body cavity of *R. gracilis* (Bergh), off Cape Maeda, Onna, Okinawa-jima Island, East China Sea, Japan (26°26'41"N, 127°46'20"E), 5 m depth, June 2010 (NSMT–Cr 22252); 2 females and 1 male, ex body cavity of *R. luteolineata* (Baba), off Miyagi Beach, Chatan, Okinawa-jima Island, East China Sea, Japan (26°19'44"N, 127°44'35"E), unknown water depth, 14 October 2009 (RUMF–ZC–02106).

Type locality. off Miyagi Beach, Chatan, Okinawa-jima Island, East China Sea, Japan (26°19'44"N, 127°44'35"E).

Description of adult female. Body length (Figure 10A) 4.99, elongate, composed of elongate prosome with 3 pairs of lateral processes and 3-segmented urosome. Prosome composed of anterior region (cephalosome), middle region (comprising first and second pedigerous somites), and posterior region (comprising third and fourth pedigerous somites). Cephalosome rectangular (Figure 10A, B), bearing protruded rostral area (Figure 10G). Middle region (Figure 10A, B) elongate, about twice as long as wide, bearing constriction at base of first lateral processes and dorsal posterior lobe, without posterolateral processes. Posterior region (Figure 10A, B) elongate, third and fourth pedigerous somites covered with anchor-shaped spinules (Figure 10E) along posterior margin (Figure 10C, D). Lateral processes (Figure 10A) long and slender, about 1.3 times as long as body length. Urosome (Figure 10C, D) small; genital double somite cylindrical, narrower at middle length, bearing paired apertures with slightly prominent posterolateral corners; opercula bearing small shield-like structure with 2 spiniform processes. Caudal ramus (Figure 10F) small, fusiform, about twice as long as wide, bearing 6 setae; apical seta styliform.

Antennule (Figure 11A) 3-segmented bearing spiniform elements; proximal segment subdivided basal part with 4 elements and distal part with 3 elements; middle segment bearing 3 elements; terminal segment bearing 11 elements. Antenna (Figure 11B) 3-segmented; coxo-basis broad, bearing 1 inner spiniform element; proximal segment of endopod bearing 1 inner spiniform element; terminal segment of endopod drawn out into strong apical claw, with 4 and 1 elements along outer and inner margins, respectively. Labrum (Figure 11B) bilobate, bearing paired extra lobes along posterior margin and small central, conical protrusion. Mandible



Figure 10. *Majimun shirakawai* gen. et sp. n., female, holotype NSMT–Cr 22250. **A** habitus, dorsal **A'** enlarged view of egg sac **B** habitus, ventral, p1 = leg 1, p2 = leg 2 **C** posterior portion of body, dorsal, p3 = leg 3 **D** posterior portion of fourth pedigerous somite and genito-abdomen, ventral **E** enlarged view of the patch of anchor-like spinules on posterior margin of fourth pedigerous somite **F** caudal ramus, ventral **F** anterior portion of cephalosome, ventral. Scale bars = 1 mm in **A**, **B**; 100 µm in **A'**; 200 µm in **C**, **D**, **G**; 20 µm in **E**, **F**.

(Figure 11B, C) spatulate, drawn out into blade with pointed tip and 3 dentiform processes. Maxillule not observed. Paragnath (Figure 11B, D) bulbous lobe covered with setules. Maxilla (Figure 11B, E) 2-segmented; syncoxa unarmed; allobasis tapering into curved process, with seta. Maxilliped absent. Labium (Figure 11B) bearing single pointed process, small paired protrusions ornamented with spinules, and posterolateral patch of spinules.

Legs 1 and 2 (Figures 10B, 11F, G) composed of protopod largely incorporated into ventral wall of prosome with exopodal and endopodal lobes; protopod bearing outer basal seta, small protrusion at base of endopodal lobe of leg 1; exopodal lobe indistinctly 2-segmented, tapering into spiniform apical process, bearing 4 and 2 elements in legs 1 and 2, respectively; endopodal lobe cylindrical bearing apical process. Leg 3 (Figures 10C, 11H) represented by conical process with apical seta.

Egg sacs (Figure 10A) cylindrical and spiral; orange in live color (Figure 1G, H).

Variation of female morphology. The morphology of body parts of female paratypes is as in the holotype. The specimens from type series (n = 4) range from 3.31-4.99 (3.99 ± 0.77) BL.

Description of adult male. Body (Figure 12A–C) 1.02 long, cyclopiform, composed of cephalothorax and 5 cylindrical somites. Cephalothorax (Figure 12A–C) large, bulbous, incorporating first and second pedigerous somites, bearing transverse constriction posterior to mouthparts and paired posterolateral outgrowths. Posterior margin of third and fourth pedigerous somites (Figure 12C, D) covered with anchorshaped spinules (Figure 12E) on both dorsal and ventral surface. Genital somite (Figure 12D) bearing paired apertures; opercula unarmed. Caudal ramus (Figure 12F) conical, about as long as wide, bearing 6 setae; apical seta styliform. No marked sexual dimorphism in antennule and mouthparts. Shape of antenna (Figure 12G) as in female except terminal endopodal segment bearing 5 elements; inner margin bearing 2 of 5 elements. Mandible (Figure 12H) elongate, drawn out into spatulate apical blade with 3 dentiform processes.

Legs 1 and 2 (Figure 12B, I, J) composed of round protopod with outer basal seta, indistinctly 2-segmented exopodal lobe drawn out into pointed process with 4 and 3 elements on legs 1 and 2, respectively, and non-segmented endopodal segment with apical small process. Leg 3 (Figure 12D, K) represented by conical process with apical seta.

Variation of male morphology. The morphology of male paratypes is as in the allotype. The specimens from type series (n = 3) range from 0.50-1.02 (0.75 ± 0.26) in BL.

Site. All specimens of both sexes were found in the body cavity of the host nudibranchs. The lateral processes on the prosome of females grasped the host's visceral sac, and their mouthparts were in touch with the host's gonads. The posterior tip of the urosome and the egg sacs were exposed from the posterior region of the host's gill circle (Figure 1G). Males were attached to the posterior part of the female prosome (Figure 1H). Both females and males bear patches of hook-like spinules (Figures 10E, 12E) on the posterior margin of the third and fourth pedigerous somites.

Etymology. The new species is named after Mr. Naoki Shirakawa, an expert diver who finds remarkable animals. He collected the nudibranchs infected by the new species.



Figure 11. *Majimun shirakawai* gen. et sp. n., female, holotype NSMT–Cr 22250. **A** antennule, anterior **B** oral area **C** mandible **D** paragnath **E** maxilla **F** leg 1 **G** leg 2 **H** leg 3. Scale bars = 50 μ m in **A**, **E**; 100 μ m in **B**; 20 μ m in **C**, **D**, **F**, **G**; 10 μ m in **H**.



Figure 12. *Majimun shirakawai* gen. et sp. n., male, allotype NSMT–Cr 22251. **A** habitus, dorsal **B** habitus, ventral, p1 = leg 1, p2 = leg 2 **C** habitus lateral **D** posterior portion of body, ventral, p3 = leg 3 **E** anchor-like spinule on posterior margin of fourth pedigerous somite **F** caudal ramus, ventral **G** antenna, anterior **H** mandible **I** leg 1 **J** leg 2 **K** leg 3. Scale bars = 200 μ m in **A**, **B**, **C**; 100 μ m in **D**; 20 μ m in **F**, **H**, **I**, **J**, **K**; 10 μ m in **E**; 50 μ m in **G**.

Discussion

Despite the fact that Splanchnotrophidae comprises 23 species in 5 genera, only 4 species in 3 genera have been recorded from the North Pacific Ocean: 2 species of *Ismaila* from the East coast and another 2 species in 2 other genera from the western North Pacific (Huys 2001; Salmen et al. 2008a). One species of *Ceratosomicola* has been reported from Japanese waters (Fujita 1895; see Huys 2001) and this species is described herein as *C. japonica* sp. n. The other species, *Arthurius bunakenensis* Salmen, Kaligis, Mamangkey & Schrödl, 2008 was described from Gangga Island off northern Sulawesi, Indonesia (Salmen et al. 2008a). With the descriptions of 4 new species and 1 new genus in this paper, there are now at least 4 species in 3 genera of splanchnotrophids in Japanese waters and 5 species in 4 genera in the western North Pacific Ocean.

Currently, 4 species of *Splanchnotrophus* are recognized, and all of them have been described or reported from European waters (Huys 2001). Thus, *S. helianthus* sp. n. and *S. imagawai* sp. n. are the first and second species found from North Pacific Ocean. On the other hand, the host nudibranchs of these 2 new species, *Thecacera pennigera* and *Trapania miltabrancha*, are widely distributed: *T. pennigera* is known from the subtropical Atlantic and Pacific region (Debelius and Kuiter 1998), and *T. miltabrancha* is probably widely distributed in the Indo-West Pacific because this species was originally described from Indonesia (Gosliner and Fahey 2008). We infer that the 2 *Splanchnotrophus* spp. described in this paper are not distributed only in a limited region. In fact, with the recent spread of SCUBA diving, many opisthobranch gastropods infected by splanchnotrophids have been found and their pictures have been taken from temperate to tropical waters around the world. Because of the shape of the egg sac, some of such splanchnotrophids are surmised to be undescribed species of *Splanchnotrophus*.

The original descriptions of the 4 known species of Splanchnotrophus lack adequate illustrations of mouthparts and the swimming legs (Hancock and Norman 1863; Canu 1891; Hecht 1893; Delamare Deboutteville 1950). Therefore, the shape of the prosome and the urosome has been used for species identification, and the shape of the genito-abdomen is especially important. Nevertheless, some characters in the mouthparts and the swimming legs are useful to separate species of Splanchnotrophus from each other: the mandibles of S. angulatus, S. gracilis, and S. helianthus sp. n. carry several dentiform processes on the apical parts (Huys 2001; present study) but that of S. imagawai sp. n. lack such armature. The shape of legs 1 and 2 in females also differs between S. angulatus, S. helianthus sp. n., and S. imagawai sp. n. Splanchnotrophus spp. have been described based on female specimens, and male specimens were described only for 2 species, S. angulatus and S. gracilis (Hancock and Norman 1863; Huys 2001). Splanchnotrophus helianthus sp. n. is a third species described based on both sexes, and the male anal somite of this new species nearly completely withdrawn into genital somite is not shared with the other 2 species. It is, therefore, considered that the male morphology can also serve as useful character to distinguish between morphologically similar species of Splanchnotrophus.

Majimun gen. n. and Ceratosomicola share 2-segmented postgenital somites, cylindrical egg sacs, and posterolateral lobes on the male's prosome (see Huys 2001; present study). On the other hand, Majimun gen. n. shares the following characters in both sexes with Splanchnotrophus: the antenna has elongate middle and terminal segments; the mandible bears a blade that is not recurved and not covered with spinules; the paragnath is present; the maxilla is 2-segmented with a seta; legs 1 and 2 possess exopodal and endopodal lobes; and leg 3 is represented by a conical projection with 1 apical seta (see Huys 2001; present study). Ceratosomicola has a globular caudal ramus with 5 short elements, but that of Splanchnotrophus is elongate with 5 setae and 1 terminal spiniform seta. The somewhat elongate, fusiform caudal ramus with 5 minute and 1 short spiniform terminal setae of *Majimun* gen. n. shows just an intermediate type between that of Ceratosomicola and Splanchnotrophus. However, the antennule of Majimun gen. n. is 3-segmented, which differs from that of both genera. Huys (2001) mentioned that the ancestral splanchnotrophid antennule is 5-segmented, and the proximal segment in Ceratosomicola is homologous to the first 2 segments in ancestral one. In Majimun gen. n., the proximal segment is large and bears 4 spines and 3 elements on the proximal and distal parts, respectively, and this segment also corresponds to the first 3 segments in the ancestral antennule.

Key to genera of Splanchnotrophidae, based on females:

| 1 | Postgenital somites at most 1-segmented |
|---|--|
| _ | Postgenital somites 2-segmented |
| 2 | Prosome with 1 pair of relatively small anteroventral processes; antennule short, 1-segmented; mandible absent |
| _ | Prosome without paired anteroventral processes; antennule at least 2-seg- mented; mandible present |
| 3 | Prosome with 1 elongate dorsal process; antennule 2-segmented; mandible represented by single rod with 2 elements on tip <i>Ismaila</i> |
| _ | Prosome without such dorsal processes; antennule indistinctly 4-segmented; mandible drawn out into curved blade with or without dentiform processes4 |
| 4 | Egg sacs attached at midlength with well-developed anterior and posterior lobes; prosome with lateral processes longer than body <i>Splanchnotrophus</i> |
| _ | Egg sacs attached at subterminally, cylindrical, and slightly curved; prosome with conical lateral processes shorter than or as long as body <i>Lomanoticola</i> |
| 5 | Antennule 3-segmented; antenna with elongate middle segments; mandible drawn out into spatulate apical blade with dentiform processes |
| | |
| _ | Antennule 4-segmented; antenna conical with short middle segments; man- |
| | dible represented by incurved blade covered with numerous spinules |
| | Ceratosomicola |

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RESEARCH ARTICLE



High mitochondrial DNA sequence divergence in New Guinea Carabdytes stream beetles and the taxonomist's dilemma when other evidence is kind of subtle... (and collecting localities are far far away)

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Abstract

Carabdytes upin tindige **ssp. n.** is described from the Arfak Mountains, Bird's Head, Indonesian Papua. It is morphologically very similar to *Carabdytes upin upin* Balke et al., 1992, known from eastern Indonesian Papua eastward to the western limits of the Papuan Peninsula of Papua New Guinea. For 726 bp at the 3' end of the mitochondrial *cox1* gene, the subspecies differ by 8.1–9.2% uncorrected *p*-distance. However, we also document considerable *cox1* divergence within *Carabdytes upin upin*. We find few diagnostic positions in the nuclear genes argenine kinase as well as elongation factor 1 alpha that suggest there are indeed two isolated groups of *Carabdytes*, but evidence in elongation factor 1 alpha is not unambiguous. We decided to highlight this phenomenon of ambiguous evidence for ongoing/just attained speciation by describing a subspecies. We argue that such cases are actually common once mitochondrial sequence data are routinely added to the taxonomist's toolkit, and sometimes simply adding data from few nuclear genes will not suffice the solve taxonomic riddles. Here, detailed population genetic investigations would be required – for which sufficient numbers of specimens from a sufficiently wide geographical sampling might be nearly impossible to acquire.

Keywords

Coleoptera, integrative taxonomy, cryptic species, DNA sequencing, DNA barcoding

Introduction

Carabdytes Balke et al., 1992, is a genus of New Guinea Colymbetinae diving beetles which to date only contains *C. upin* (Balke et al. 1992; Balke 2001). The species inhabits fast flowing, cold mountain rivers, where the beetles hide under large stones at the edge, but still in the water. The beetles also inhabit smaller, shaded, low order streams where they also tend to hide under stones or creep about in small stream pools and between stones in the stream bed. We also collected the species from deep, high altitude blackwater *Sphagnum* pools on peat, *c.* 2800–3400 m high (PNG, Kumul Lodge, see below). The beetles are dorso-ventrally flattened, and with their long legs with only few swimming hairs, and the basally constricted pronotum rather resemble ground beetles than other diving beetles (Fig. 3). Given its highly specialized, higher altitude-related ecology, it is surprising that the species is comparably widespread (Fig. 1), the easternmost localities in the Wau area of Papua New Guinea and the westernmost localities in Indonesian Papuan highlands being roughly 800 kilometers apart.

Recent molecular phylogenetic analyses revealed that *Carabdytes upin* belongs to an isolated clade of the Colymbetinae (Balke et al. 2007, 2009) which also contains several Oceanian *Rhantus* species such as *R. novaecaledoniae* Balfour-Browne, 1944 and *R. alutaceus* Fauvel, 1883, from New Caledonia. These species should be probably all assigned to *Carabdytes*, but the redefinition of *Carabdytes* will be possible only when a robust, global analyses of the Colymbetinae is finished (Balke et al., in prep.).

During an extensive survey across the island of New Guinea, we obtained *Carab-dytes* samples from several new localities, pushing its range boundary approximately 700 kilometers westward to the Bird's Head peninsula (Fig. 1). The fresh tissue was used for DNA purification and sequencing to study intraspecific variation. Here, we report surprisingly high mitochondrial DNA divergence in *Carabdytes upin*. We present evidence from nuclear protein coding genes and morphology that the Bird's Head beetles might belong to a different species. We also describe the taxonomist's dilemma when there is some evidence for the presence of cryptic species but perhaps not enough and there is no straightforward solution as the required additional localities are very remote and extremely difficult to visit. We argue this scenario might be not so rare, and new technology eagerly awaited by the traditional taxonomist does not always provide a fast and complete solution of the "old problems".

Material and methods

Beetles were preserved in 95% ethanol and flight muscle tissue was used for DNA purification. The laboratory methods employed are detailed on our DNA laboratory wiki: http://zsm-entomology.de/wiki/The_Beetle_D_N_A_Lab. PCR conditions with Mango Taq (Bioline) were for *cox1*: (primers: Jerry/Pat, Simon et al. 1994) 1' 94°C – $40 \times (30s \ 94^{\circ}C - 30s \ 47^{\circ}C - 1' \ 72^{\circ}C) - 10' \ 72^{\circ}C$; for Elongation Factor 1 α (EF1 α): (primers: efs372/efa754, McKenna and Farrell 2005; Normark et al. 1999) 5' 95°C



Figure 1. Distribution of *Carabdytes upin*, blue dots = sequenced specimens, white dots = other records. Lowland gap indicated by bars in the Bird's neck. Below, detailed map of central Papua New Guinea with localities for sequenced specimens.

 $- 8 \times (308\ 95^{\circ}C - 1'\ 58^{\circ}C - 1'\ 72^{\circ}C - 308\ 95^{\circ}C - 1'\ 58^{\circ}C - 1'\ 72^{\circ}C - 308\ 95^{\circ}C - 1'$ $58^{\circ}C - 1'\ 72^{\circ}C) - 18 \times (308\ 95^{\circ}C - 1'\ 42^{\circ}C - 1'\ 72^{\circ}C)$; for Arginine Kinase: (primers: AK183F/AK939R, Wild and Maddison 2008) 3' 94°C - 35× (308 94°C - 308 53°C - 1'\ 72^{\circ}C) - 10'\ 72^{\circ}C.

We use GARLI V.0.951 (Zwickl 2006) with default settings (using the GTR model of evolution with parameter estimation) to obtain a maximum likelihood tree of the *cox1* data. The SpeciesIdentifier module of TaxonDNA software v.1.6.2 was used to study the genetic divergences in our dataset and to cluster sequences at different preset thresholds using uncorrected *p*-distances (Meier et al. 2006; http://code.google.com/p/taxondna/). SpeciesIdentifier accounts for threshold violations according to triangle inequity (i.e., when the divergence between A - B and B - C is 3% or less, but A - C exceeds 3%, then A, B and C would still be grouped into one 3% cluster by Taxon DNA. We routinely use 3% as a preset threshold, as this value captures species boundaries comparably well for Dytiscidae (Hendrich et al. 2010).

Digital images were taken with a Nikon D3X with a Voigtländer Apo Lanthar 90 mm attached to a bellows; fitted to a custom built macro rail (image steps used: 0.4 mm). Image stacks were aligned and assembled with the computer software Helicon Focus 4.77TM.

Institutional abbreviations:

| CSH | Coll. Andre Skale, Hof/Saale, Germany |
|-----|---|
| MZB | Museum Zoologicum Bogoriense, LIPI, Cibinong, Indonesia |
| NMW | Naturhistorisches Museum Wien, Austria |
| ZSM | Zoologische Staatssammlung München, Germany |

Other abbreviation:

PNG Papua New Guinea

Locality data for specimens of *C. upin* studied for this paper (* sequence data available, see Table 1):

Papua New Guinea

- * Papua New Guinea: Simbu, Mt Wilhelm, lower lake from Keglsugl, 3500–3700 m, 23.ix.2002, ca. 05.53.733S, 145.02.742 E, Balke & Sagata leg. (PNG 020) (ZSM)
- * Papua New Guinea: Eastern Highlands, Goroka, Daulo Pass, 2500m, 19.v.2006, 06.02.432S, 145.13.333E, John & Balke leg. (PNG 67) (ZSM)
- Papua New Guinea: Southern Highlands, Sopulkul, 30–35Km NE Mendi, from swamp that drains into stream, 2679m, 16.vi.2006, 06.02.944S, 143.46.485E, John leg. (PNG 79) (no DNA sequence data) (ZSM)
- * Papua New Guinea: Enga, Kumul Lodge @ foot of Mt Hagen, 2700m, 5.xii.2006, 05.47.548S, 143.58.761E, Balke & Kinibel leg. (PNG 124) (ZSM)
- * Papua New Guinea: Western Highlands, Simbai, 1800–2000m, 1.iii.2007, 05.14.276S, 144.28.741E, Kinibel leg. (PNG 138) (ZSM)
- * Papua New Guinea: Western Highlands, Simbai area, 2500m, 8.iii.2007, 05.14.202S, 144.33.651E, Kinibel leg. (PNG 150) (ZSM)

Indonesia: Papua

Jayawijaya Mts., Aipomek-Diruemna, 2600m, 3.ix.1992, 04.26S, 139.57E, Balke leg. (no DNA sequence data) (NMW)

Indonesia: West Papua

* Bird's Head, Manokwari, Mokwam (Siyoubrig), 1400–1800m, 24.–28.II.2007, 01.06.26S, 133.54.41E, Skale leg. (CSH, MZB, ZSM)

| | | cox1 | EF1a | ARK |
|-------------------------|----------------------|------------|----------|----------|
| C. upin tindige MB 3084 | Papua: Arfak | HF558675 | HF558686 | HF558698 |
| C. upin MB 3328 | PNG 138: Simbai | HF558676 | HF558687 | HF558699 |
| C. upin MB 3354 | PNG 067: Daulo Pass | HF558677 | HF558688 | HF558700 |
| C. upin MB3452 | PNG 150: Simbai | HF558678 | HF558689 | |
| C. upin MB3453 | PNG 150: Simbai | HF558679 | HF558690 | |
| C. upin MB3454 | PNG 150: Simbai | HF558680 | HF558691 | |
| C. upin MB3455 | PNG 150: Simbai | HF558681 | HF558692 | |
| C. upin MB3045 | PNG 124: Mt. Hagen | HF558682 | HF558693 | HF558701 |
| C. upin MB4316 | PNG 067: Daulo Pass | HF558683 | HF558694 | |
| C. upin MB4317 | PNG 138: Simbai | HF558684 | HF558695 | HF558702 |
| C. upin MB4318 | PNG 138: Simbai | HF558685 | HF558696 | HF558703 |
| C. upin MB0306 | PNG 020: Mt. Wilhelm | FN263070.1 | HF558697 | |

Table 1. Sequenced Carabdytes specimens and EMBL accession numbers.

Results

Analysis of genetic and morphological variation in C. upin

Elongation factor 1 alpha. We obtained a 555 bp fragment in the 5' region of EF1 α for all specimens shown in Figure 2. There are 3 diagnostic nucleotide substitutions for a specimen from the Bird's Head (MB3084, see Fig. 1) (positions 36, 75, and 357, all 3^{rd} codon positions) delineating this specimen from the other *Carabdytes*.

Specimens MB3084 (Bird's Head) and MB4317, 4318 (PNG: Simbai) share a non synonymous substitution in position 406 (1st codon position).

Arginine Kinase. We obtained 656 bp of sequence data for 6 individuals of *Carabdytes* (Table 1). There was one diagnostic character, a 3rd codon substitution in position 414 of our alignment. This character delineates the Bird's Head specimen from the other *Carabdytes*. The sequences are otherwise identical.

Cytochrome c oxidase 1. We obtained a 726 bp fragment at the 3' of *cox1* for 12 individuals of *Carabdytes* (Table 1). Sequence data were surprisingly divergent, although most of the samples all originate from one major region in eastern New Guinea (Figs 1, 2). Uncorrected *p*-distances were 0–9.23%. There are 29 unambiguous diagnostic characters delineating the Bird's Head specimen from the other *Carabdytes*, all of them in 3^{rd} codon positions.

The clade MB3328 / MB3354 / MB4316 has 20 diagnostic characters (and two 1st codon substitutions resulting in amino acid change, pos. 316 and 415 in our alignment) and MB0306 / MB 3045 / MB3452–55 has 5 diagnostic characters (Fig. 2).

Cluster analysis. At 3% preset threshold, SpeciesIdentifier finds four *cox1* clusters, which agree with the three main lineages of the tree in Fig. 2 (MB4317 and MB4318 form one cluster). For the nuclear markers, all data only form a single cluster at 3%.



Figure 2. Maximum likelihood tree based on *cox1* data, the tree was rooted with *Rhantus guadalcanalensis* Balke (pruned here), colored buttons represent diagnostic nuclear DNA characters and their positions in our sequence alignment (there are found characters supporting the node between *Carabdytes* 3084 and all others, and one that is shared between 3084 as well as the two specimens 4317 & 4318 in Simbai 138).

Morphology. Four specimens were available from the Bird's Head for morphological study. The distinguishing feature between these specimens and *C. upin* from eastern localities in New Guinea is: Pronotum and elytra conspicuously shining with very indistinct punctuation (the elytra have a conspicuous coarse punctuation, especially on the apical half, in eastern *C. upin*) (Fig. 3). Specimens of *Carabdytes upin* studied for this comparison come from the localities mentioned above, covering most of its range (no specimens studied from Huon Peninsula and Wau). The specimens from Simbai (locality PNG138) have an intermediate elytral punctation, with only few coarse punctures on the apical part, while specimens from Simbai (loc. PNG150) which is less than 20 kilometers apart (Fig. 1) are coarsely punctate. The Simbai localities both have specimens with attached sequence data.

Taxonomic treatment

For *Carabdytes upin*, we do suggest to flag the Bird's Head beetles with a subspecies name, assuming the combined, congruent observations described above are evidence for longer periods of interrupted gene flow. We suggest the use of a subspecies name to stimulate further investigation to verify or falsify this hypothesis.


Figure 3. Morphological characters, above habitus, left *Carabdytes upin tindige* from the Bird's Head (12.0 mm long), right *Carabdytes upin upin* from Papua: Aipomek area (12.9 mm long); below left median lobe of aedeagus in lateral view of *Carabdytes upin tindige* (*Carabdytes upin upin* is identical), its paramere, right, paramere of *Carabdytes upin upin* from Papua: Aipomek area. Scale for genitalia is 0.1 mm.

Carabdytes upin tindige ssp.n.

http://species-id.net/wiki/Carabdytes_upin_tindige

Type locality. West Papua, Arfak Mountains, Siyoubrig, 01°06.26'S, 133°54.41'E.

Description. Holotype: 3° (MZB): INDONESIA, West Papua, Arfak Mountains, stream near Siyoubrig 01°06.26'S, 133°54.41'E, 1400–1800 m, 24. –28.II.2007, leg. A. Weigel. **Paratypes:** $23^{\circ}3^{\circ}1^{\circ}$ (CSH, ZSM): same locality data as holotype (the female was sequenced).

Habitus as in Fig. 3; total length: 11.6–12.0 mm; total width: 5.3–5.5 mm. Dark brown to almost black; labrum, lateral margin of pronotum and all body appendages paler reddish brown; elongate.

Colour. Head black, labrum reddish brown, clypeus with indistinctly reddish colour almost reaching eyes; head with indistinctly reddish patch on frons. Pronotum black, with indistinctly median reddish patch, posterior angles reddish. Ventral surface blackish. Venter dark brown.

Structures. Head with fine, sparse punctation interspersed with coarser punctures between eyes and behind anterior clypeal margin. Pronotum shining, posterior angles with irregular, coarse punctures, lateral margin very strongly. Elytra shining with very indistinctly punctures; each elytra with four rows of coarser, moderately arranged punctures. Lateral wing of metaventrite broad and tongue-shaped; outer margin slightly sinuate; last abdominal ventrite medially emarginate. Legs long and slender.

Male. Pro- and mesotarsal claws of similar structure; anterior and posterior claws moderately long and evenly curved; Median lobe of aedeagus relatively slender (Fig. 3, shape in *Carabdytes upin upin* is identical); paramere slender, with distinctly longitudinal striation; setation more or less long (Fig. 3), the setation might be basally shorter in some specimens of *Carabdytes upin upin* (Fig. 3), but this difference does not appear constant.

Diagnosis. Distinguished from *C. upin upin* through the molecular and morphological characters mentioned in the results section under "morphology". With *C. upin upin* there is no overall size difference (10.1–12.2 mm).

Habitat. Two individuals collected with an aquatic net from the rough gravel at the edge of a stream bed, the stream was rather dry at the time of collection (Fig. 4). The species co-occured with *Platynectes* spp., *Exocelina* (*=Papuadytes*) spp. and *Hy-draena cristatigena* Jäch & Diaz. Two exemplars were collected with the help of a light trap, approx. 50 m away from the stream.

Distribution. So far known only from the type locality (Figs 1, 4).

Etymology. In loving memory of Samkris "Kris" Tindige, relentless conservationist in Papua, who left us too early. The beetles were collected in the stream bed very close to a birdwatching guesthouse set up by Kris and Shita Prativi above Siyoubrig village.



Figure 4. Type locality of *Carabdytes upin tindige* ssp.n.

Discussion

Here, we document mitochondrial DNA divergence of up to 9% within *Carabdytes upin*. Our samples mainly originate from the core range of this species, from eastern New Guinea. One specimen from the Bird's Head Peninsula in the west of New Guinea, about 700 km west of the next known locality for *Carabdytes upin*, is well separated geographically from other populations. It is also most divergent genetically. The mountain regions between the known localities are understudied, but some (wider Wamena area eastwards to Diruemna; Nabire area up to Enarotalia; Cyclops Mountains near Jayapura) have specifically been screened for diving beetles. *Carabdytes upin* was not yet collected there. The vast expanse of karst as well as tropical lowland in the Bird's neck region, roughly from Lake Yamur westwards to Arfak Mountains, offers few obvious habitats for *C. upin*, with Wandammen Peninsula as a potential stepping stone (though the species was not yet detected there) (Fig. 1, "lowland").

Intraspecific mitochondrial *cox1* divergences >3 % are considered high in Dytiscidae. For the Australian fauna, largest intraspecific distances reported by Hendrich et al. (2010) were well below that (median 1.25%, mean 1.94%, SD 2.37%), and <u>average</u> distances even lower (median 0.50%, mean 0.71%, SD 0.80%). However, there are exceptions. Morphologically identical populations of *Copelatus* diving beetles from northern South America diverge up to 8% in *cox1*, with strong geographical signal (Balke et al. 2008). However the authors acknowledged that additional investigation was certainly warranted to understand how many species there really are. Such additional investigations were conducted here for *C. upin*, in this case sequencing of nDNA loci.

Cryptic species are apparently more common and phylogenetically more widespread than assumed previously (Pfenninger and Schwenk 2007). The use of molecular methods, namely extensive mitochondrial DNA sequencing or barcoding, routinely uncovers strong genetic subdivision among morphologically highly similar or indiscernible populations. In many cases, this even concerns species in well-studied faunas which were supposedly widespread and abundant, and not necessarily understudied faunas or taxa only (e.g. Hebert et al. 2004). However, detection of unusually high mitochondrial divergence *per se* does not satisfyingly support cryptic species hypotheses and additional lines of evidence should be followed, no matter which species concept is used (Hawlitschek et al. 2011; Tänzler et al. 2012).

In the morphologically highly similar *Carabdytes upin*, we find geographical separation and high *cox1* divergence. In the nDNA marker Arginine Kinase, we find one diagnostic character for the Bird's Head beetle, in elongation factor 1 alpha (EF1 α) there are three, but all of these are synonymous substitutions not altering the amino acid sequence and thus protein derived from the nucleotide sequence. For EF1 α , there is another substitution, but this one is shared between the Bird's Head specimen and two specimens from eastern New Guinea (Simbai, PNG138, MB4317 & 4318) (Figs 1, 2). A third specimen from the Simbai PNG138 locality has the same EF1 α genotype as all other *Carabdytes upin*. Within *Carabdytes upin* from eastern New Guinea, we also observe considerable mtDNA variation, up to 7.7% (Table 2). Importantly, this also concerns close localities such as Simbai PNG138 and PNG150, less than 10 km apart. Moreover, haplotypes from locality Simbai PNG138 also differ around 7% from each other.

Thus, there is considerable *cox1* variation, as expected in running water organisms, or species in highly fragmented habitats in general (Abellan et al. 2007; Engelhardt et al. 2008), but here this variation is apparently only partially structured geographically. Most Melanesian running water beetles exhibit pronounced endemism and microendemism (Balke 1999), and species are usually similar morphologically yet with clear differentiation in genital structure and often in terms of body size, color and fine sculpture. This is not the case in *Carabdytes upin*. There are diagnostic nDNA characters delineating the Bird's Head sample and other *Carabdytes upin*, as well as geographic separation. This case would now warrant in-depth study of population level processes, but it is not possible to collect the amount of specimens from the higher number of localities required for such approaches (see Abellan et al. 2007 for an adequate sampling design).

What are the practical implications from the beetle taxonomist's point? Mitochondrial DNA variation alone does not provide sufficient evidence. While divergence between eastern and western localities is high, such is divergence even within *one* of the eastern localities, as well. Thus, we tried to find other *congruent* evidence that

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---------------------------------------|----|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|----|
| 0306 Carabdytes PNG Mt Wilhelm | 1 | | | | | | | | | | | | |
| 3045 Carabdytes PNG Mt Hagen | 2 | 0.010 | _ | | | | | | | | | | |
| 3452 Carabdytes PNG150 Simbai | 3 | 0.010 | 0.011 | | | | | | | | | | |
| 3453 Carabdytes PNG150 Simbai | 4 | 0.010 | 0.011 | 0.000 | | | | | | | | | |
| 3454 Carabdytes PNG150 Simbai | 5 | 0.010 | 0.011 | 0.000 | 0.000 | | | | | | | | |
| 3455 Carabdytes PNG150 Simbai | 6 | 0.010 | 0.011 | 0.000 | 0.000 | 0.000 | | | | | | | |
| 3328 Carabdytes PNG138 Simbai | 7 | 0.077 | 0.077 | 0.073 | 0.073 | 0.073 | 0.073 | | | | | | |
| 4316 Carabdytes PNG067 Daulo | 8 | 0.076 | 0.076 | 0.072 | 0.072 | 0.072 | 0.072 | 0.001 | _ | | | | |
| 3354 Carabdytes PNG067 Daulo | 9 | 0.076 | 0.076 | 0.072 | 0.072 | 0.072 | 0.072 | 0.001 | 0.000 | | | | |
| 4318 Carabdytes PNG138 Simbai | 10 | 0.040 | 0.047 | 0.039 | 0.039 | 0.039 | 0.039 | 0.068 | 0.066 | 0.066 | | | |
| * 3084 Carabdytes West Papua Arfak | 11 | 0.087 | 0.080 | 0.086 | 0.086 | 0.086 | 0.086 | 0.093 | 0.091 | 0.091 | 0.090 | | |
| 4317 Carabdytes PNG138 Simbai | 12 | 0.032 | 0.039 | 0.030 | 0.030 | 0.030 | 0.030 | 0.070 | 0.069 | 0.069 | 0.014 | 0.084 | |

Table 2. Uncorrected *cox1 p*-distances for *Carabdytes* specimens (* the new subspecies from the Bird's Head).

might indicate presence of a cryptic species. In the nuclear genes Arginine Kinase and elongation factor 1 alpha, we count a total of 4 diagnostic characters (Fig. 2). This is additional evidence, combined with the high mtDNA divergence, of interrupted gene flow over longer periods. It is interesting to note that two specimens from Simbai (locality PNG138) which also diverge highly from other *C. upin* share 1 diagnostic EF1 α position with the Bird's Head specimen. As described above, the Simbai (PNG138) specimens are morphologically intermediate between the Bird's Head specimens and other studied *C. upin*. Overall molecular evidence suggests they belong to the eastern clade, presence of a shared substitution in EF1 α between PNG138 and EF1 α can not be explained based on the available data. The generally high mitochondrial divergence indicates complex mechanisms are at work, and the mtDNA data are not necessarily the answer but rather a starting point for a population genetic study in its own right.

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RESEARCH ARTICLE



A new species of Rhytidognathus (Carabidae, Migadopini) from Argentina

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Abstract

The Migadopini are a small tribe of Carabidae with 47 species that occur in South America, Australia, and New Zealand, in the sub-Antarctic areas. In South America, most of the genera inhabit areas related to sub-Antartic *Nothofagus* forest except two monogeneric genera, the Ecuadorian genus *Aquilex* Moret and the Pampean genus *Rhytidognathus* Chaudoir. These two genera are geographically isolated from the remaining five South American genera. New material of *Rhytidognathus* from the northeast of Buenos Aires province and from Entre Ríos province permits establishing that the previous records of *Rhytidognathus ovalis* (Dejean) for Argentina were erroneous and that it belongs to a new species. Based on external morphological characters and from male and female genitalia we describe *Rhytidognathus platensis* as a new species. In this contribution we provide illustrations, keys, habitat characteristics and some biogeographic considerations on the distribution of *Rhytidognathus*.

Keywords

Migadopini, Rhytidognathus, New species, Male and female genitalia, Distribution

Introduction

The Migadopini are a small tribe of Carabidae, with 16 genera and 47 species. This tribe was considered related to the Holarctic tribes Elaphrini and Loricerini (Jeannel 1938), and Loricerini (Maddison et al. 1999). Ball and Erwin (1969) considered that the characters shared with Loricerini are convergent and do not show an ancestral relationship. The most modern classification considers the Migadopini as constituting the subfamily Migadopinae, together with the tribe Amarotypini (Johns 2010).

The species of Migadopini are distributed over fragments of the austral Gondwana, called Paleantarctic by Jeannel (1938). These species occur in southern South America (eight genera with con 17 species) (Roig-Juñent 2004), one monotypic genus in the Andean region of northern South America (Moret 1989), four genera with seven species in Australia (Baher 2009) and four genera with 19 species in New Zealand and circum-Antarctic islands (including a new genus and several new species not yet described) (Johns 2010). The only complete revision of the tribe is that by Jeannel (1938). Later, for South America, Straneo (1969), Négre (1972), and Baher (1997; 1999) described new species or subspecies, Moret (1989) described a new genus and species and finally Roig-Juñent (2004) redescribed all the austral South American genera including male and female genitalia characters and developed a cladistic and biogeographic analysis of the genera. For Australia, Baher (2009) described a new genus with two species, and for New Zealand, Johns (2010) described 11 new species.

The number of species per genus is low. Of the 16 genera, eight are monospecific, four have two species and the most diverse in number of species is *Taenarthrus* Broun with 12 species (Johns 2010).

Migadopines constitute a characteristic element of the sub-Antarctic biota, and except some frequent species such as the South American *Migadops latus* (Guérin-Ménéville) the others are scarce in natural history collections, with just a few specimens of several species known. This is the case for the genus *Rhythidognathus* Chaudoir of which only 12 specimens are known: the holotype of *R. ovalis* (Dejean), nine more specimens from Uruguay, and two from Argentina. Of these last two specimens, one is lost, and we only have the account by Tremoleras (1931). Strange as well is the particular distribution of the genus *Rhytidognathus*, because it does not inhabit sub-Antarctic habitats, and its phylogenetically related genera are about 3000 km to the south.

Ecological studies conducted in the area of La Plata (Buenos Aires, Argentina) yielded the discovery of new specimens of *Rhytidognathus*, and particularly the capture of males allowed establishing that the previously cited species of *Rhytidognathus* from Argentina (Tremoleras 1931, Roig Juñent 2004) is not *R. ovalis* but instead a new species.

The objective of the present contribution is to describe this new species, including new data on its habitat, and discuss some biogeographic considerations.

Material and methods

Material examined. The material is held in the following institutions: IADIZA: Instituto Argentino de Investigaciones de las Zonas Áridas (Mendoza, Argentina, Sergio Roig-Juñent); MACN: Museo Argentino de Ciencias Naturales "Bernandino Rivadavia" (Buenos Aires, Argentina, Arturo Roig-Alsina); MLP: Museo de La Plata (La Plata, Argentina, Analía Lanteri).

Dissection methods, measurements, and the terminology used follow previous revisions of Migadopini (Jeannel 1938, Moret 1989, Roig-Juñent 2004, Johns 2010).

Predictive species distribution models were built using the MAXENT program version 3.4.1 (Phillips et al. 2006), because MAXENT performed well with small sample sizes (Tognelli et al. 2009), which is the case of *Rhytidognathus*. Also because of the low number of known species localities, we performed the analysis at generic level.

Rhytidognathus Chaudoir, 1861

http://species-id.net/wiki/Rhytidognathus

Type species. *Nebria ovalis* Dejean, 1831, by monotypy.

Redescription. Habitus. Body shape rounded, depressed (Fig. 1)

Head. Labrum short, transverse, bilobate at anterior margin; clypeus with two subparallel lateral sulci slightly developed, projected at the base of the frons (Figs 2, 5); mentum and submentum not fused, mentum with four setae, two lateral to the tooth, and two at the base; mentum-tooth bifid; glossa with a central carina, with two apical setae; glossa with two setae, paraglossae rounded, not projected; galea biarticulate, distal article as long as anterior one; mandibles with several dorsal transverse sulci; last maxillary and labial palpomeres long and truncate at apex; antennomeres three times as long as wide; antennae long, reaching the base of the elytra (Fig. 5); antennomeres fusiform, pubescent from the fifth antennomere (Fig. 8).

Prothorax. Pronotum wide, wider than head, with anterior angles projected forward (Figs 2, 5); median line slightly delimited; base of pronotum with strong punctures (Figs 2, 5); pronotum without setae on lateral margin; lateral margin rounded, without sinuosity, base bisinuate; prosternal apophysis with a longitudinal sulcus at apex, and a small protuberance or carina; prosternal apophysis projected posteriorly, but short, not touching the mesosternum, border of apophysis straight (Fig. 3) or concave (Fig. 6).

Pterothorax: mesoepisternum with deep punctures (Fig. 9); metaepisternum with a row of punctures and two apical sulci (Fig. 9); elytra twice as wide as than pronotum, without shoulders (Figs 4, 7), with borders rounded, elytra increasing in width to the apex, the widest part on apical third (Fig. 1); elytral epipleura more than twice wider at base than at apex, decreasing in width from base to apex; scutellar stria complete; striae with punctures, deep on the basal third, shallower on the second third and on apical



Figure 1. Dorsal aspect of male *Rhytidognathus platensis* (Scale = 5 mm).

third imperceptible, striae well delimited and deep all along their length (Fig. 4); setae only on ninth interval, with six or seven setae. Apterous.

Legs. Protarsomeres 1-4 and mesotarsomeres 1-3 of male with adhesive setae, wider than in females. Protrochanters with one seta present. Protarsomeres 2 and 3 of male wider than long; metatarsomeres long.

Abdominal sterna. Sterna III-V constituting more than two thirds of the length of abdomen; sulcus of separation of sterna III-IV and IV-V not reaching the center; female sterna VIII without apical sulcus, with two apical setae. Sternite III and IV with deep basal punctures.



Figures 2–7. *Rhytidognathus ovalis:* **2** Head and pronotum, dorsal view (Scale = 1 mm) **3** Lateral view of prosternal apophysis (Scale = 1 mm) **4** Dorsal view of elytra **5** Head and pronotum, dorsal view (Scale = 1 mm) **6** Lateral view of prosternal apophysis (Scale = 1 mm) **7** Dorsal view of elytra.



Figures 8–9. *Rhytidognathus platensis*: **8** Lateral view of head showing the eyes (Scale = 1 mm) **9** Lateral view of meso-metathorax and abdomen (Scale = 5 mm).

Comparative notes. The genus *Rhytidognathus* shares with *Pseudomigadops* Jeannel the characteristic of having the elytral striae punctured and differs from it by having the articles of maxillary and labial palpi elongated and thin, as well as by having the mandibles carined dorsally. This last character is exclusive to the genus within the tribe.

Key for differentiating the species of Rhytidognathus

Elytra oval, completely black; labrum black; elytral striae deep, interstriae convex (Fig. 4); superior border of eyes straight; prosternum with a median apical prolongation that projects dorsally (Fig. 3) *Rhytidognathus ovalis* Elytra more rounded, with interstria 8 reddish; eytral striae marked but not deep, interstriae flat (Fig. 7); labrum with lateral borders yellowish; upper border of eye rounded (Fig. 8); prosternum with a slight swelling in the apical region (Fig. 6)......*Rhytidognathus platensis*

Rhytidognathus ovalis (Dejean, 1831)

http://species-id.net/wiki/Rhytidognathus_ovalis

Nebria ovalis Dejean, 1831: 581. Rhytidognathus ovalis: Chaudoir 1861.

Material. Male and female, Cerro Colorado Uruguay, Florida (MLP); male Banda Oriental (IADIZA).

Diagnosis. Head with deep punctures in front, as well as at the base and apex of pronotum; elytra black, concolor; labrum concolor; legs black or dark red, tarsi reddish; apex of median lobe rounded.

Description. Body shape oval. Length: 12–13 mm; coloration: black; with antennae light colored, reddish, and legs testaceous or dark reddish. Elytra black, concolor.

Head. Head with deep punctures in front, eyes slightly protruding, sub-quadrangular. Maxillary palpi black or dark red.

Prothorax. Wider than long, maximum width at middle (Fig. 2); dorsal surface with deep punctures at base and apex (Fig. 2); lateral margins narrow, curved; central longitudinal sulcus slightly developed; posterior transverse foveae impressed, with deep punctures (Fig. 2); prosternum with punctures; prosternal apophysis prolonged into a carina, which extends straight toward the dorsal region (Fig. 3).

Pterothorax: Elytra. Humeral angles rounded (Fig. 4); striae well impressed, and deeply foveate on basal third (Fig. 4), being less marked toward the apex; six to seven setae only in the ninth interval.

Male genitalia (Figs 10–12). Median lobe wide, with apex rounded (Figs 10-12), apical orifice small, opening laterally to the right with a sclerified plate. Basal orifice wide, closed dorsally (Fig. 10), without basal keel. Left paramere wide with apex round-



Figures 10–18. *Rhytidognathus ovalis*. 10 Median lobe and left paramere 11 Right paramere 12 Median lobe, right view 13 Female genital track, ventral view. *Rhytidognathus platensis*. 14 Median lobe, left view 15 Median lobe, right view 16 Apex of median lobe 17 parameres 18 female genital track, ventral view. Scale 1 mm.

ed (Fig. 11), with setae on apical third (Fig. 11). Right paramere straight and thin, the same width all along its length, with several setae from middle to apex (Fig. 11).

Female genital track (Fig. 13). With gonopod VIII small. Gonopod IX dimerous, the base with two sclerified plates, the apex small and without setae, with subapical setose organ (Fig. 13). Bursa copulatrix big, without accessory glands. Spermatheca on the base of oviduct, digitiform. Bursa copulatrix with a well developed sclerite.

Intraspecific variation. Jeannel (1938) found some intraspecific variation in the intensity of basal punctures of the pronotum and also in the coloration of the legs.

Distribution. Uruguay: Montevideo: Montevideo (Chaudoir 1861). Florida: Cerro Colorado (MLP).

Rhytidognathus platensis sp. n.

urn:lsid:zoobank.org:act:89A5BF3B-FB4B-4B75-95DA-D86FD0F667C8 http://species-id.net/wiki/Rhytidognathus_platensis

Type material. Holotype: male, Argentina: Buenos Aires, Los Olmos (MLP); Paratypes, same date, one male two females (MELP, IADIZA); Entre Ríos (MACN), one female.

Diagnosis. Head with small punctures, on the borders; elytra black with interstria 8 reddish; labrum with the borders yellowish; interstriae flat; apex of median lobe sub-quadrangular.

Description. Habitus as in Fig. 1. Length: 10.3 mm. Coloration: black; with antennae light colored, reddish, and legs testaceous, dark reddish. Labrum with borders yellowish; elytra black with interstria 8 reddish.

Head. Head with small punctures in front; eyes slightly protruding, rounded (Fig. 8). Maxillary palpi black or dark red.

Prothorax. Wider than long, maximum width at middle (Fig. 5); dorsal surface with punctures on the base (Figs 1, 5), apex with small or no punctures. Lateral margins narrow, curved; central longitudinal sulcus slightly developed; posterior transverse foveae slightly impressed. Posterior angles rounded. Prosternum without punctures or one or two on the apex. Prosternal projections not marginate, with a small apical tubercle, sinuate dorsally (Figs 6, 9).

Metathorax. Elytra with humeral angles rounded (Fig. 7); striae on basal third well impressed, and foveate, less impressed at apex. Ninth interval with six setae; elytral interval flat.

Male genitalia (Figs 14–17). Median lobe wide, with apex sub-quadrangular (Figs 14-16), apical orifice big, open dorsally and straight; basal orifice wide, closed dorsally (Fig. 14), without basal keel. Left paramere wide with apex rounded (Fig. 16), setae on apical third (Fig. 16). Right paramere thin, constricted in the middle, with setae from middle to apex (Fig. 16).

Female genital track (Fig. 18). With gonopod VIII small. Gonopod IX dimerous, the base with two sclerites, the apex small without setae, with apical setose organ (Fig. 18). Bursa copulatrix large, without accessory glands. Spermatheca on the base of oviduct, digitiform. Bursa copulatrix with a large sclerite.

Etymology. The name of the new species is related to the area where it was collected, La Plata district, near the La Plata river in Buenos Aires Province, Argentina.

Taxonomic considerations. Tremoleras (1931) cited *Rhytidognathus ovalis* for Argentina. Tremoleras' specimen was held in his collection and now we can not find it. The description by Tremoleras (1931) does not allow a clear identification of this material. Roig-Juñent (2004) cited also *Rhytidognathus ovalis* for Entre Ríos province (Argentina), based on a female. In the present contribution, this female specimen is now considered as being R. *platensis.* Taking into account that *R. platensis* is distributed along the western shore of the La Plata river, we considered it more likely that Tremoleras' specimen belongs to the new species, *R. platensis*, and not to *R. ovalis*.

Distribution. Argentina: *Buenos Aires:* San Isidro (Tremoleras 1931); Los Olmos (La Plata); *Entre Ríos.*

Habitat. The new material was collected in the locality of Lisandro Olmos (La Plata, Buenos Aires) at "La Nueva Era" farm (35°01'18"S, 58°02'07"W) (Fig. 20), devoted to horticultural production under organic management (Fig. 21). The area has elevations of about 30 m, with soils derived from the Buenos Aires belt corresponding to grassland soils. It is surrounded by horticultural crops grown under cover and in the open, primarily tomato, pepper, leafy vegetables, celery, eggplant and small plots of corn, among others. Cut flower production in greenhouse conditions is also important in this area.

Samples were collected by pitfall traps set up in a 2000 m²-area cultivated with lettuce (*Lactuca sativa*), onion (*Allium cepa*), radish (*Raphanus sativus*), rocket (*Diplotaxis sp.*), cabbage (*Brassica oleracea*) and different types of weeds. This habitat has no native vegetation. Probably *Rhytidognathus platensis* inhabits the patches of semi-natural vegetation surrounding the crops. It has been proven that carabids move between cultivated and uncultivated patches (Marshall and Moonen 2002, Magura 2002).

On the shores of La Plata river in Buenos Aires province we found two natural habitats. One habitat is close to the river and includes: a) cliffs, with small forest of *Celtis tala* and other arboreal species, b) riparian shallows extending between the cliffs and the river and constituting a low plain that gets flooded, similar to the marshes of the Paraná river delta. The soil is clay and salty, and the vegetation is characterized by halophytic steppe with dominance of low grasses such as *Distichlis spicata*. The second habitat, the Pampean plain, lies above the cliffs. This lowland has a temperate climate, with an even year-round precipitation regime, soil type is loam, and the plants that dominate the landscape are herbs that compose the extensive Pampean grassland, a steppe. The typical original plant community comprises species of the genera *Stipa* and *Piptochaetium*. This landscape is accompanied on different sites by low shrubs of several species of *Bacharis*.

Predictive models of distribution show that the genus *Rhytidontahus* is restricted to the coast and areas close to the La Plata river and the delta of the Paraná and Uruguay Rivers (Fig. 20), occupying shore habitats and the Pampean grassland near the shore. This Pampean plain has been strongly modified, allowing for great agricultural development with establishment of annual crops and pastures, leaving hardly any native vegetation in the region. The Pampean grassland and forest close to the



20

Figures 19–20. Habitat of *Rhytidognathus platenesis.* **19** Aerial view of the collecting area **20** Area where the study was developed, showing the crops.

La Plata river and to the high Paraná River differ in species and habitat conditions from the areas inhabited by nearly all sister groups of *Rhytidognathus*, the genera *Lissopterus* Waterhouse, *Migadopidius* Jeannel and *Pseudomigadops*. *Migadopidius* occupy temperate *Nothofagus* forests (Fig. 24, Table 1). *Lissopterus* and *Pseudomigadops* (Figs 22-23) occur in habitats closer to the shore, principally sub-Antarctic forest or moorlands (Figs 22–23, Table 1). The unique genus of the sister group inhabiting grassland is *Pseudomigadops*, in some part of Malvinas Islands. As we can see, *Pseudomigadops* inhabits coastal forest and grassland, like *Rhytidognathus*, but species composition in their habitats is far from being the same, as the former is of sub-Antarctic origin and the other of Neotropical origin (Morrone 2004). Climatic conditions are not the same either, and if we look at the variables that explain the predictive models of distribution of these four Migadopini genera, the most important variable is temperature (Table 1).



Figures 21–24. Potential distribution of: 21, *Rhytidognathus* 22 *Pseudomigadops* 23 *Lissopterus* and 24 *Mi-gadopidius*. Known localities are in white, probabilities of occurrence are indicated in different shades of grey.

| | Habitat | variables |
|----------------|---|--|
| Rhytidognathus | Lowlands, 30-m altitude, in Pampean grasslands, and probably in riparian forests along the La Plata river and the Paraná river delta. | 67.3%: Isothermality: 17.0% :Precipitation Seasonality (Coefficient of Variation) 10.1 Mean Temperature of Wettest Quarter |
| Pseudomigadops | Lowlands, sea level to 10-meter altitude; in Malvinas grasslands (mainly of <i>Poa</i> <i>flabellata</i>) and Magellanic moorland (of <i>Empetrum rubrum</i>). In Navarino, southern Tierra del Fuego (near Beagle Channel), Isla de los Estados and Cape Horn <i>Nothofagus betuloides</i> forest on the coast and Magellanic moorland (<i>Empetrum rubrum</i>) (Niemela 1990) | 46.9% Max Temperature of Warmest Month 14.7 % Mean Temperature of Driest Quarter 11.8 % Mean Annual Temperature |
| Lissopterus | Lowlands, sea level to 5-meter altitude; in Malvinas grasslands (mainly of <i>Poa</i> <i>flabellata</i>) and Magellanic moorland (of <i>Empetrum rubrum</i>). In Navarino, southern Tierra del Fuego (near Beagle Channel), Isla de los Estados and Cape Horn <i>Nothofagus betuloides</i> forest on the coast and Magellanic moorland (<i>Empetrum</i>) (Niemela 1990). Sub-Antarctic maritime areas including off-shore and more remote islands (Erwin 2011) | 66.0% Max Temperature of Warmest Month 11.9% Altitude 6.1% Annual Temperature Range |
| Migadopidius | Nothofagus forest and Araucaria habitat; mixed forest (Araucaria araucana, Nothofagus dombeyi, N. antarctica and N. pumilio) (Dapoto et al. 2005) | 63.0% Mean Temperature of Wettest Quarter 29.0% Precipitation of Coldest Ouarter |

Table 1. Habitat characterization and the major variables explaining the predictive model of distribution obtained by Maxent

Biogeographic considerations

Because of its particular distribution pattern and its phylogenetic relationships with other tribes, the Migadopini have been used to explain some very different biogeographic views, such as an austral origin and separation by vicariance (Jeannel 1938, Brundin 1966) or a Holarctic origin, separate dispersal to the southern continents, extinction in tropical and subtropical regions (Darlington 1965). Beyond the different proposals regarding the origin of the tribe, everybody considers that its current restricted distribution is relictual (Jeannel 1938, Darlington 1965). Upon the advent of the theory of plates as applied to the continental drift, it was put forward that many groups with distribution patterns similar to those of migadopines be considered of austral origin, whose fragmentation led to their present distribution. By applying a Dispersal and Vicariance analysis, Roig-Juñent (2004) put both hypotheses to test and his conclusions concur with Jeannel's saying that the tribe has had an origin in the southern hemisphere and that its current distribution across the southern continents has been due to vicariant events. Notwithstanding, the analysis yielded no support for the existence of three separate phyletic lines (monophyletic groups): Australian, New Zealander and American, as Jeannel proposed (1938). This shows that some clades would have originated before the fragmentation of some parts of Gondwana.

Regarding the present distribution of the Migadopini in South America, it is restricted to three disjunct areas. The first is in the Ecuadorian Andes, where the genus Aquilex occurs at about 4300 m elevation at Páramo (Moret 1989); the second is on the shores of the La Plata river where *Rhytidognathus* lives in Pampean grassland and riparian forest environments; and the third, which is the largest in surface area and coincides with the sub-Antarctic region in Chile and Argentina, includes all Nothofagus forests and sub-Antarctic regions up to Cape Horn. The latter is the area with highest number of Migadopini genera, and where most taxa show more phylogenetic affinity to other taxa from southern regions (New Zealand, Australia) than to those from the rest of the Neotropics. Although the present distribution of the Migadopini is largely restricted to the sub-Antarctic region in South America, it is likely that, at some point of the Cenozoic, the tribe may have had a broader distribution. The sub-Antarctic biota expanded to more northern areas and its later retraction left areas with relictual distributions. Such is the case of the Fray Jorge forests in Chile (30° 40' 44" S, 71° 40' 54" W) or the Araucaria forests in the south of Brazil and north of Argentina (26° 27"S, 53° 37'W). This expansion might explain the presence of Rhytidognathus in the La Plata river because, being apterous and large-sized, this taxon has almost no capacity for dispersal. Moret (1989) considers the same situation for the genus Aquilex, which would have originated from its southern ancestors in the pulses of northward expansion of the sub-Antarctic biota during the Cenozoic.

Considering the particular distribution of *Rhytidognathus*, the biogeographic analysis carried out by Roig-Juñent (2004) shows that this genus would have been split by a vicariant event from its sister group (*Lissopterus* + *Pseudomigadops* + *Migadopidius*) which now inhabits the Magellanic region or the northern *Nothofagus* forests. Although the distance to the Magellanic region exceeds 3000 km and is 1000 km to the *Nothofagus* forest region, the possibility of a vicariant event is feasible because, as mentioned for the austral region of South America, its cold austral biota experienced expansions during the Cenozoic whereby the genus came to occupy areas more northern than the current ones (Romero 1986, Barrera and Palazzesi 2007). So the separation of *Rhytidognathus* may have been caused either by vicariance or by isolation upon the southward retraction of the austral biota. Numerous are the relictual taxa than can be found in the Pampean region and south of Brazil, such is the case among carabids of the tribe Broscini.

In analyzing the environmental features of each genus, we find that there could also have been environmental features involved in the split. Figures 21–24 show the potential distribution range of *Rhytidognathus* and that of its sister genera. For these four genera, we find three clearly separate areas, one is austral sub-Antarctic, another one comprises the cold-temperate forests, and the third one encompasses the Pampean steppe and riparian forests along the La Plata river. The Pampean region is the exception with respect to the other habitats where migadopines occur in South America, and to the remaining circum-Antarctic regions, because most are from cold-temperate or cold environments, such as the

species of *Loxomerus* Chaudoir (Johnson 2010). Although the Pampean grassland is a temperate area, it has warm summers and the vegetation is Neotropical in origin, not austral.

In other cases, it has been put forward that there often is niche conservation, commonly observed in species of the same genus whose potential distributions show areas occupied by other species of the genus rather than by them. However, we see that a shift has occurred among these four genera regarding the environment occupied by some of them. We propose that the environment occupied by the ancestor of *Rhytidognathus* and the sister group could have been cold-temperate coastal or riparian habitats, either forest or grassland (present in *Rhytidognathus* and *Pseudomigadops*). An arid barrier formed during the Cenozoic between the Pampean and sub-Antarctic regions (Barreda and Palazzesi 2007), isolating *Rhytidognathus*, and the current species of this genus would have had to become adapted to this more temperate climate.

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FORUM PAPER



Known unknowns, Google Earth, plate tectonics and Mt Bellenden Ker: some thoughts on locality data

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Abstract

Latitude/longitude data in locality records should be published with spatial uncertainties, datum(s) used and indications of how the data were obtained. Google Earth can be used to locate sampling sites, but the underlying georegistration of the satellite image should be checked. The little-known relabelling of a set of landmarks on Mt Bellenden Ker, a scientifically important collecting locality in tropical north Queensland, Australia, is documented as an example of the importance of checking records not accompanied by appropriately accurate latitude/longitude data.

Keywords

Spatial data, latitude/longitude, georeferencing

Missing numbers

With the debut of *Biodiversity Data Journal* (http://www.pensoft.net/journals/bdj) not far off, I have started thinking, as a Pensoft editor, about the tables of locality data that are likely to be submitted. Will the authors have read the "Guide to Best Practices in Georeferencing"? (Chapman and Wieczorek 2006) produced for GBIF? Will the locality records be tabulated using Darwin Core categories for location (http://rs.tdwg. org/dwc/terms/index.htm#locationindex), or at least with fields that can easily be converted to their Darwin Core equivalents?

I suspect not. To judge from what I often see in recent research papers, a location will be specified simply as a latitude/longitude from a single reading of a handheld

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GPS receiver (standalone operation), together with an inexact description that checks the latitude/longitude by giving a general idea of the location, something like ca 5 km S of Woop Woop [an imaginary town], 22°48'20.6"S, 124°33'10.4"E

There are two numbers missing here. One is the datum for the latitude/longitude. While most GPS users set their receivers to the WGS84 datum, another datum may have been used in order to allow checking of the latitude/longitude or grid reference on a paper map based on a local datum. Datum differences are not trivial. For example, there is ca 200 m distance between the WGS84 location of a site in Australia and its corresponding location on a map referenced to the long-used AGD66 datum. Locality data tables should therefore include a field for specifying the datum or for noting that the datum is unknown. Compilers should not assume that if no datum is specified, then the datum for that location must have been WGS84.

The second missing number is an uncertainty estimate. We don't know whether the location is a spot or a large area, and if the latter, what relation the latitude/longitude has to the area sampled. We can be reasonably sure that the latitude/longitude is too accurate, even for a spot sample. The implied uncertainty is ±0.05", which at the given latitude represents about 1.5 m in either latitude or longitude. The GPS receiver's manufacturer probably didn't claim that high an accuracy. For example, the owner's manual for the Garmin GPSMap[®]62S, a popular handheld GPS receiver, says only that 95% of waypoints will be within 10 m of the stated position in typical use.

Good practice is to specify an uncertainty, and not just to imply one. A possible entry for an uncertainty field is the accuracy as reported by the GPS receiver for that particular reading; a column heading in the data table might be "GPS accuracy declaration" or something similar. In my part of the world, GPS accuracy declarations after a few minutes at a spot are generally in the range 5-10 m in the open air and 15–30 m in forest.

For many biological samplers, however, a collecting site is not a spot, but a small area over which a number of specimens are collected. A good way to report that location is to give the latitude/longitude of the centre of the site. The uncertainty can then be approximated as the radius of a circle containing the area searched, following the Darwin Core definition of uncertainty: "The horizontal distance (in meters) from the given decimalLatitude and decimalLongitude describing the smallest circle containing the whole of the Location" (http://rs.tdwg.org/dwc/terms/index.htm#coordinateUnc ertaintyInMeters).

ca 5 km S of Woop Woop, 22°48'20.6"S, 124°33'10.4"E, ±15 m, WGS84. That's better, but something is still missing.

Outrageous numbers

I recently audited some museum database records, one of which said the collecting site was at 22°06'57.54"S 117°53'15.31"E. At the latitude/longitude involved, those last

0.01" figures correspond to about 30 cm in latitude and longitude, or ± 15 cm. How did the collector get those numbers?

Possibly from Google Earth. With coordinates set to degrees, minutes and seconds, the status bar at the bottom of a Google Earth window reports the cursor location to the nearest 0.01 second of latitude and longitude. You can zoom in as much as you like to see a collecting site, then just place the cursor on the site and read off the latitude/ longitude, which will be outrageously accurate.

Since the latitude/longitude is not a GPS reading, it would be sensible to round the figures off to the nearest second. Uncertainty in this case depends in part on how accurately Google Earth has placed the satellite image on its mathematical model of the globe, a procedure known as georegistration. The accuracy of georegistration can vary from image to image and from date to date in an image series. One site I looked at in Queensland had shifted more than 100 m between image dates.

Google Earth can be very useful for locating sampling sites if GPS reception is poor or if GPS accuracy declarations are large. It's a good idea, however, to check the georegistration by getting at least one GPS reading at a spot (somewhere near the site) which will be clearly distinguishable on the satellite image. Even better, compare the known location of an official survey mark in the vicinity with its Google Earth location. In either case, the uncertainty specified for a position located using Google Earth should be at least the difference found between the Google Earth latitude/longitude and the corresponding figures for a GPS reading or a survey mark. (I recently checked a survey mark in my home town. Google Earth put it 2-3 m from its actual location. Not bad, but not as good as the implied ±15 cm.)

Here in Australia, at least, there is an additional complication. The Australian Plate is moving northeast towards Papua New Guinea at about 7 cm/yr and carrying with it the survey reference framework, GDA, standardised in 1994. Google Earth, like the GPS system, is based on the WGS84 framework, which for all practical purposes is independent of earth movements. In 2012, there is a more than a metre horizontal difference between GDA and WGS84, thanks to plate tectonics.

Complications aside, locality data tables should always contain a field for the source of latitude/longitude data, i.e. map, GPS, Google Earth, etc. The source will then stay with the record if the record is separated from the table's metadata.

ca 5 km S of Woop, 22°48'20.6"S, 124°33'10.4"E, ±15 m, WGS84, GPS. Much better!

Numbers helped by words

A description like *ca 5 km S of Woop Woop* is obviously not as accurate as a GPS reading, but is important in a locality record as a check on latitude/longitude. The better the description, the closer the check. Description writing has been very well summarised by Chapman and Wieczorek (2006, p. 7): – "Provide a descriptive locality, even if you have geographic coordinates. The locality should be as specific, succinct, unambiguous, complete, and as accurate as possible, leaving no room for uncertainty in interpretation."

– Localities used as reference points should be stable – i.e., places (towns, trig points, etc.) that will remain for a long time after the collection events. Do NOT use temporary locations or waypoints as the key reference location. You may have made an accurate GPS recording for the temporary location and then referenced future collections from that point (e.g., 200 m SE of the Land Rover), and that may make perfect sense for that series of collections. It is meaningless, however, when those collections are later broken up and placed in a museum under a taxonomic arrangement, and no longer have a link to where the 'Landrover' was.

 If recording locations along a path (road, river, etc.) it is important to also record whether the distances were measured along the path ('by road') or as a direct line from the origin ('by air').

- Hint: The most specific localities are those described by a) a distance and heading along a path from a nearby and well-defined intersection, or b) two cardinal offset distances from a single persistent nearby feature of small extent. "[Example given for the latter: "ice field below Cerro El Plomo, 0.5 km S and 0.2 km W of summit, Region Metropolitana, Chile. "]"

Nevertheless, even the best landmark-based descriptions can stray from the truth if the landmarks themselves change, as the following example shows. I am describing it in some detail because the locality involved has yielded numerous new animal and plant species, and avoidable georeferencing errors are possible at this scientifically important locality.

Unstable numbers

The Bellenden Ker Range in tropical north Queensland has attracted scientific explorers and collectors since the late 19th century. Much of the Range lies less than 10 km from the Coral Sea, and access is relatively easy from the coastal Bruce Highway between the towns of Gordonvale and Innisfail, and from the Atherton Tableland to the west. The densely vegetated Bellenden Ker Range has Queensland's highest mountain (Mt Bartle Frere, 1622 m) and Australia's wettest weather station (Mt Bellenden Ker, 8150 mm/yr).

In the early 1970s a cableway was built to access a telecommunications and broadcast facility on the summit of Mt Bellenden Ker (Fig. 1). Formerly operated by Telecom Australia (now Telstra), the Mt Bellenden Ker Cableway and Transmission Facility is currently owned and managed by Broadcast Australia.

Over the past four decades the cableway has not only offered access to Mt Bellenden Ker for biological sampling, but has also provided a simple system of landmarks, namely the nine cableway support towers. These landmarks have often been used to georeference collecting localities, with no accompanying latitude/longitude or only an approximate one. A botanical example is the type locality of *Morinda constipata* Hal-



Figure 1. View of the Mt Bellenden Ker cableway from the east in June, 1976. Lower towers are labelled with their current numbers. Image by Len Webb, reproduced with the permission of the copyright holder, Griffith University.



Figure 2. Plan of the Mt Bellenden Ker cableway showing current and former tower numbers. SS = summit station, BS = base station. Contours (100 m) and streamlines are only approximate and are from the 1:50000 scale 'Bartle Frere' map produced by the Royal Australian Survey Corps in 1986.

ford & A.J. Ford, 2009 (Rubiaceae): 'National Park Reserve 904, Wooroonooran, just S of tower 9, Mt Bellenden Ker cableway' (Halford and Ford 2009).

It is not generally known, however, that the tower numbering was reversed in about 1997 when the cableway changed owners. Whereas Telecom numbered the towers from the top of the mountain to the bottom, the new system numbers the towers from bottom to top. Figure 2 shows the current and former tower numbering, and Table 1 gives latitude/longitude for each of the cableway landmarks.

Table 2 lists correct locations for the principal cableway sites sampled during the 1981 Earthwatch/Queensland Museum expedition, the source of a very large number of insect and other zoological samples (Queensland Museum 1982, Monteith and Davies 1992). The sites were referenced to the older tower numbering, and were offset various distances from the landmarks indicated on specimen labels.

| Landmark | Latitude | Longitude | Approx. elevation (m) | |
|-----------------------|------------|-------------|-----------------------|--|
| Cableway base station | 17°16'12"S | 145°54'00"E | 100 | |
| Tower 1 (formerly 8) | 17°16'11"S | 145°53'46"E | 210 | |
| Tower 2 (formerly 7) | 17°16'10"S | 145°53'37"E | 300 | |
| Tower 3 (formerly 6) | 17°16'07"S | 145°53'22"E | 360 | |
| Tower 4 (formerly 5) | 17°16'04"S | 145°53'00"E | 500 | |
| Tower 5 (formerly 4) | 17°15'59"S | 145°52'20"E | 970 | |
| Tower 6 (formerly 3) | 17°15'58"S | 145°52'13"E | 1030 | |
| Tower 7 (formerly 2) | 17°15'53"S | 145°51'31"E | 1450 | |
| Tower 8 (formerly 1a) | 17°15'51"S | 145°51'18"E | 1530 | |
| Tower 9 (formerly 1) | 17°15'51"S | 145°51'15"E | 1550 | |
| Summit TV station | 17°15'50"S | 145°51'13"E | 1550 | |

 Table 1. Spatial data (WGS84) for the landmarks mapped in Fig. 2. Positional uncertainty is ±50 m.

Table 2. Spatial data (WGS84) for the principal cableway sampling sites of the 1981 Earthwatch/ Queensland Museum expedition, based on the older tower numbering. Positional uncertainty is ±100 m.

| Landmark | Latitude | Longitude | Approx. elevation (m) |
|----------------------------------|------------|-------------|-----------------------|
| 'Cableway base station' | 17°16'06"S | 145°54'00"E | 110 ('100') |
| '1 km S of towers 6/7' | | | |
| = '0.5 km S of tower 7 ' (now 2) | 17°16'33"S | 145°53'15"E | 500 ('500') |
| = '1 km SW of tower 6' (now 3) | | | |
| 'Tower 3' (now 6) | 17°16'02"S | 145°52'12"E | 1020 ('1054') |
| 'Summit TV station' | 17°15'50"S | 145°51'14"E | 1550 ('1560') |

Conclusion

Locality databases have grown enormously in recent years, and the georeferencing of legacy localities from old specimen labels has become a well-understood practice for database compilers (Chapman and Wieczorek 2006). Many new localities are now being added to databases by collectors who have not been trained in georeferencing. There is more to capturing spatial data than pushing a button on a GPS receiver, and as an editor for the forthcoming *Biodiversity Data Journal* I hope to see locality records submitted with appropriate uncertainties, datum used, clear indications of data source and spatially explicit site descriptions.

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BOOK REVIEW



Book review: The ants of Poland with reference to the myrmecofauna of Europe - Fauna Poloniae (New series) vol. 4

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The four authors are well-known in the field of European myrmecology. Research on the myrmecofauna of Poland has a long history. Due to the continuity in the work of myrmecologists of different generations, it seems likely that their good work will be extended successfully into the future. The fruitful work of the late Prof. Bohdan Pisarski has been continued successfully by Wojciech Czechowski and Wiesława Czechowska. They and their students have worked intensively on the Polish myrmecofauna for several decades and they have amassed a great database on every part of Poland. The well-known Palaearctic myrmecologist Aleaxander Radchenko has made outstanding contributions to the knowledge of European myrmeco-

fauna. Kari Vepsäläinen and his team in Finland have had a longstanding collaboration with their Polish colleagues.

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The present book is the fourth volume of the new series of Fauna Poloniae. The former monograph "The ants of Poland" (Czechowski et al. 2002) has been considerably updated. It incorporates the latest developments in the taxonomy of ants and has been improved by the inclusion of scanning photographs, detailed drawings of representative ant species by castes, notes on the conservation status of the species, spatial analysis of the Polish ant fauna by regions as well as faunistical, zoogeographical and ecological comparisons of the Polish myrmecofauna with European fauna as a whole and with that of other countries.

The monograph includes the 103 species of ants from 25 genera reported for Poland up until 2010.

The book is in English, separated into 4 chapters:

Chapter 1: Checklist of the ant taxa of Europe. A checklist for European myrmecofauna is published with nine subfamilies, 57 genera and 613 valid species. The list is in alphabetical order. This is the most recently updated list of the European ants, and shows the recent changes in ant taxonomy.

Chapter 2: Faunistic catalogue of the ants of Poland. This chapter includes a taxonomic survey of the Polish ant species. For every species there is enclosed: Latin name, taxonomic history, general distribution in the Palaearctic region with map, the distribution in 21 geographical regions in Poland with map and joint table, notes on the biology and conservation status of the species. Drawings of a representative species are given for every genus including worker, queen and male. A list with 17 species excluded from the Polish myrmecofauna is given. The reason for their elimination is noted for each species.

Chapter 3: Characteristics of the Polish myrmecofauna. The chapter is devoted to analyses of species richness and composition, zoogeographical and ecological composition of the Polish myrmecofauna, with reference to the European one. Among 103 Polish ant species, 97 are outdoor and six are entirely synanthropical species.

Contemporary statistical ecological methods are used for spatial analysis of Polish myrmecofauna by regions and among other 12 European countries (with recent check-lists).

The core of the ant fauna is formed by common species occurring in almost all Polish geographical regions. The richest region is Pieniny Mountains with 64 known species. No significant difference is calculated between the mean numbers of ant species in lowlands and uplands. A Central-European myrmecofauna and species with wide range of ecological tolerance prevail in Poland.

The most abundant zoogeographical element in Polish myrmecofauna is the Euro-Caucasian one followed by Euro-West Siberian and Boreo-Montane elements.

Information about the environmental requirements and habitats is reported for each species. With regards to ecological plasticity, oligotopic ant species are observed to prevail in Poland. Three species are classified as everytopic. In relationship to humidity conditions, mesohygro-xerophilic and mesohygrophilic categories are the richest. In respect to temperature conditions species with moderate requirements prevail. No significant differences are shown among the geographical zones in Poland by zoogeographical and ecological composition.

Comparing the Polish myrmecofauna with the European as a whole, the genera *Formica*, *Lasius* and *Myrmica* are significantly overrepresented. The authors investigated changes in species number and presence-absence of outdoor myrmecofauna among 12 European countries along a North-South gradient with detailed discussions.

Chapter 4: Keys for identification. In this chapter are included keys for identification of subfamilies, genera and species of ants, separately for workers, queens and males (when they are distinguishable). The keys for subfamilies and genera include all European taxa of these ranks. The keys for species include all ant species known in Poland as well as those present in adjacent countries that are recognized as possibly to be found in Poland in the future. The keys are illustrated with excellent quantity SEM photographs of key exterior body characters.

Ant identification is a difficult and somewhat uncertain task. Myrmecologists and students do need a good key for European myrmecofauna. The recent key for Polish ant species will be a useful reference book for European, especially for Central European, specialists.

The present book devoted to the ant fauna of Poland will be very valuable for the libraries of universities and scientific institutions, and thus, it will be of use to European myrmecologists, entomologists, ecologists, conservation biologists, students and all the people interested in the world of ants.

Price: 95 € (173 × 243 mm, hardcover, 496 pages) ISBN 978-83-930773-4-2

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