RESEARCH ARTICLE



# The Collothecidae (Rotifera, Collothecacea) of Thailand, with the description of a new species and an illustrated key to the Southeast Asian fauna

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## Abstract

Following previous reports indicating a remarkable high diversity of sessile rotifers in Southeast Asian freshwaters, we report on an extensive study of the diversity of Collothecidae rotifers from fifteen freshwater habitats in Thailand. A total of 13 species, including two additional infraspecific variants, of Collothecidae are recorded, one of which is described as a new species of *Collotheca*. We further add taxonomic remarks on some of the taxa on record and illustrate the uncinate trophi of several representatives by scanning electron microscopic images. Finally, we provide illustrated identification keys to the Collothecidae recorded to date from Southeast Asia.

## Keywords

Diversity, identification key, sessile rotifers, Southeast Asia, uncinate trophi

## Introduction

Family Collothecidae is one of two families of the rotifer Order Collothecacea. The order is diagnosed by the presence of uncinate trophi (Segers 2002, Wallace et al. 2006) and a peri-buccal region expanded into a wide infundibulum, while family Collothecidae is further characterized by having a modified *corona ciliata* (short: corona) consisting of differentiated cilia implanted along the margin of, or grouped on knoblike, lobate or tentacle-like extensions of the infundibulum. The family contains two genera, Collotheca Harring and Stephanoceros Ehrenberg, and these respectively contain 45 and one valid species (Koste 1978, Segers 2007). Collothecid rotifers are essentially ambush predators. Their expanded and elongated corona lobes and cilia lobes form a fyke-like structure by which mobile prey, either zoo- or phytoplankton, are directed towards an enlarged funnel-shaped infundibulum. Once there, prey is trapped by contraction of infundibular sphincter muscles and swallowed through the pumping action of a membrane supported by the rod-shaped trophi. This specialized feeding strategy and its phylogenetic consequence have received considerable attention by rotifer research (e.g., Kutikova and Markevich 1993, Sørensen and Giribet 2006), although large gaps remain in our knowledge of the diversity and evolution of the group.

To date, comparatively little is known on the distribution and diversity of sessile rotifers in general and of Collothecidae in particular, which is due to the fact that these animals require life observation for identification and study. This knowledge gap is especially evident regarding sessile rotifers from tropical regions. These animals are mostly dealt with on an *ad hoc* basis, and much of what little information that exists is contained in more general inventories of rotifers, in which the sessile taxa are represented as chance occurrences (e.g., Chittapun et al. 2007, Sanoamuang and Savatenalinton 2001, Segers and Chittapun 2001, Segers and Sanoamuang 2007). Some recent relevant studies on Southeast Asian sessile rotifers (Koste 1975, Meksuwan et al. 2011, Segers et al. 2010) report a remarkable diversity of the group, including several species of outstanding taxonomical and/or biogeographically interest, which sparked a more comprehensive study on this particular taxon of rotifers. Here we report on the diversity and taxonomy of Collothecidae found during our extensive study of the sessile rotifers of Thailand. Finally, realizing that the only available, relatively recent identification work dealing with Collothecidae is in German (Koste 1978), we present a key to the identification of the Collothecidae recorded from Southeast Asia, to facilitate and promote future studies on these remarkable animals.

## Material and methods

We explored 15 freshwater habitats in 12 provinces of Thailand for Collothecidae during the present study (Fig. 1). Submerged parts of different species of aquatic plant were collected qualitatively to search for sessile rotifers. Collecting and observation methods are detailed in Meksuwan et al. (2011). Searching and identifying rotifers



**Figure 1.** Sampling sites in Thailand. S, C, N and NE represent sampling sites in the Southern, Central, North and Northeast part of Thailand, respectively. Map from GIS center, PSU.

was performed under an Olympus SZ 51 stereo microscope and an Olympus CX 21 compound microscope. Drawings are based on photographs and observations of living animals. Trophi were prepared for scanning electron microscopy (SEM) following the method of Segers (1993), SEM photographs were taken using a FEI Quanta 400 SEM at the Scientific Equipment Center, Prince of Songkla University, Hatyai campus.

## **Results and discussion**

## Diversity of family Collothecidae in Thailand

The samples examined contained 13 species and two infraspecific variants of Collothecidae (Table 1). This corresponds with ca. 28% of the world fauna of *Collotheca* species and all *Stephanoceros* species known to date (Segers 2007). Two of the species identified could not be ascribed to any known species and we conclude that the specimens pertain to new species, one of which is described below. Of the second possibly new species we opine that insufficient material is at present available to warrant a full description, hence we only provide a brief illustration to enable future recognition. One more species, *C. ferox* (Penard) is new to the Oriental region and *C. ornata* f. *cornuta* (Dobie) is new to Thailand. These results indicate a relatively diverse Collothecidae fauna in the studied region of Thailand, and the record of one, and possibly two new species leads us to surmise that an even higher and incompletely documented diversity can be expected to occur in Southeast Asia.

## Taxonomy

## Genus Collotheca Harring

#### Collotheca ferox (Penard)

http://species-id.net/wiki/Collotheca\_ferox

**Remarks.** The morphological characters of our specimens agree closely with the description of the species by Penard (1914): the corona of the specimens is more than twice as broad as its trunk and bears five broad lobes (Fig. 2A, B, 4J). The dorsal lobe tip is relatively large and rounded anteriorly; the lateral lobes are intermediate in size whereas the triangular ventral lobes are relatively small and are set close together. The features of the ventral lobe are unique to this species and prevent confusion with other five-lobed species of the genus. Our photographs of living specimens and trophi of *C. ferox* confirm, in particular, the unique features of the ventral corona lobes illustrated by Penard (1914).

*Collotheca orchidacea* Meksuwan, Pholpunthin & Segers, sp. n. urn:lsid:zoobank.org:act:E7CA6ECF-175D-4E46-BCA8-970FA4F5C9CC http://species-id.net/wiki/Collotheca\_orchidacea Figs 2C-F, 5E

**Type locality.** Thale Noi Lake, Phatthalung Province, Thailand: 7°47.378'N, 100°8.969'E, on *Utricularia* sp., mostly on the surface of the bladder traps, 18 March 2012, P. Meksuwan leg.

Table 1. List of Collothecidae species recorded from Thailand

(\* = new to Oriental region and Thailand; \*\* = new to Thailand).

<sup>1</sup>recorded by Koste (1975), not seen during this study

**Type specimens**: Holotype female mounted in permanent microscope slide, in Princess Maha Chakri Sirindhorn Natural History Museum, Prince of Songkla University, Songkhla, Thailand, PSUZC-PK5PM2-1. Original label: "Rotifera, Family Collothecidae, *Collotheca orchidacea* Meksuwan & Segers, Locality: Thale Noi Lake, Phattalung Province, Thailand, Collected by P. Meksuwan 18-3-2012, Holotype"; two paratype females in permanent microscope slides, in Royal Belgian Institute of Natural Sciences, Brussels, Belgium, IG 32158 RIR 204-205. Original label: "Rotifera, Family Collothecidae, *Collotheca orchidacea* Meksuwan & Segers, Locality: Thale Noi Lake, Phattalung Province, Thailand, Collected by P. Meksuwan 18-3-2012, Paratype".

**Differential diagnosis.** The presence of a five-lobed corona separates the new species from most of the known members of genus *Collotheca*. In comparison with other *Collotheca* species having a five-lobed corona (*C. algicola* (Hudson), *C. ambigua* (Hudson), *C. annulata* (Hood), *C. bilfingeri* Bērziņš, *C. ferox* and *C. campanulata* (Dobie)), *C. orchidacea* sp. n. can be distinguished by its uniquely well-developed thumb-shaped lateral and semi-circular ventral corona lobes. It has a relatively broad infundibulum, and short foot and trunk, similar only to *C. ambigua* and *C. ferox*. In addition, *C. orchidacea* sp. n. and *C. ferox* hold their infundibulum and corona towards the substratum, whereas most other species including *C. ambigua* and *C. campanulata* normally hold their body and corona upright.

**Description.** Habitus (Fig. 2C–F): infundibulum funnel-shaped, trunk and corona opening held horizontally. Infundibulum and proventriculus about twice as long as the trunk. Infundibulum large, more than twice as wide as trunk. Foot short, length



**Figure 2. A, B** *Collotheca ferox* (**A** dorsal view **B** ventral view) **C–F** *Collotheca orchidacea* sp. n. (**C, E** frontal **D, F** dorsal). Scale bars:  $A-F = 100 \ \mu m$  (**A, B** by Rapeepan Jaturapruek).



**Figure 3.** Collotheca and Stephanoceros species. **A** Collotheca stephanochaeta, lateral **B** C. campanulata f. longicaudata, ventral **C** C. ornata, dorsal **D** Collotheca spec., lateral **E** C. trilobata, lateral **F** C. tenuilobata, ventral **G** Stephanoceros millsii, lateral **H** S. fimbriatus, lateral. Scale bars: **B**–**D** = 50  $\mu$ m, **A**, **E**, **F** = 100  $\mu$ m, **G**, **H** = 250  $\mu$ m.

about half of trunk, contractile, with a short peduncle. Corona five-lobed: single dorsal, and a pair of well-developed lateral and of ventral lobes. Infundibulum with a weak line running parallel to the edge of the corona, and at least four ring-shaped structures (circular muscles?). Dorsal lobe large, elongate, basally with straight and converging lateral margins; parallel-sided medially, with smoothly curved antero-lateral corners. Tip of dorsal lobe transversally sinuate. Lateral lobes relatively the smallest, thumb-shaped, about half as wide as the dorsal lobe. Ventral lobes broadest, smoothly



**Figure 4.** A *Collotheca heptabrachiata*, lateral **B** *C. ornata*, ventral **C** *Stephanoceros fimbriatus*, lateral **D** *Collotheca stephanochaeta*, lateral **E** *C. ambigua*, ventral **F** *C. algicola*, ventral **G**, **H** *C. ornata* f. *cornuta* (**G** dorsal **H** lateral) **I** *C. campanulata* f. *longicaudata*, attachment stalk **J** *C. ferox*, ventral corona margin. Scale bars: **A**, **B**, **D–H**, **J** = 50 μm, **C**, **I** = 100 μm.

rounded, separated by a large and deep sinus. A group of setae present on the tip of all corona lobes.

Trophi (Fig. 5E) uncinate. Two pairs of subequal unci teeth relatively equal in length. All arrow head unci with middle groove.



**Figure 5.** The uncinate trophi of Collothecidae species. **A** *Collotheca ferox* **B** *C. campanulata* **C** *C. trilobata* **D** *C. tenuilobata* **E** *C. orchidacea* sp. n. **F** *Stephanoceros millsii*. Scale bars: **A**, **B**, **D**, **E** = 5 µm, **C**, **F** = 10 µm.

**Measurements.** Females total length ca. 340  $\mu$ m. Length of infundibulum plus proventriculus ca. 190  $\mu$ m, trunk ca. 100  $\mu$ m, foot ca. 50  $\mu$ m. Trunk width ca. 70  $\mu$ m. Infundibulum width ca. 180; dorsal lobe length ca. 75  $\mu$ m, width ca. 30  $\mu$ m; ventral lobe width ca. 120  $\mu$ m, ventral sinus depth ca. 30  $\mu$ m.

**Etymology.** The species name – *orchidacea* is a noun in apposition, and refers to the shape of the new species' corona, which is reminiscent of the flower of certain orchid species. As such, the name of the species also refers to the biodiversity of Thailand, characterized by an abundance of orchid species.

**Distribution**. The species is known from its type locality only.

## Collotheca ornata f. cornuta (Dobie)

**Note.** This taxon (Figs 4G, H) is differentiated from the nominal form by the corona bearing an elongate projection dorsally to the dorsal lobe. The presence/absence of

this projection has classically been interpreted as of infrasubspecific relevance only (see Edmondson 1940, Koste 1978). In the absence of additional data (morphological, molecular or behavioural), we prefer to be cautious and record the taxon separately.

Specimens were found in Khlong Lam Chan Non-Hunting Area, Trang province (Fig. 1: S3); the present is the first Thai record of the taxon.

## Collotheca sp.?

**Remarks.** We found a single specimen of a species that we could not identify (Fig. 3D). Its corona consists of two lobes, one large dorsal lobe and one minute ventral lobe, which is similar to *Collotheca calva* (Hudson). The specimen, however, exhibits a unique cluster of long setae dorsally on the tip of the dorsal lobe and, in addition, shows two ring-shaped structures in the infundibulum. The presence of an egg in its gelatinous case indicates that the specimen was mature and not some incompletely developed juvenile. We believe that it represents an undescribed species but refrain from describing and naming it due to the lack of a sufficient number of specimens. The animal occurred in Khlong Lam Chan Non-Hunting Area, Trang province (Fig. 1: S3).

## Genus Stephanoceros Ehrenberg

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

**Remarks.** Genus *Stephanoceros* is diagnosed (Koste 1978) by having extraordinarily long extensions of the corona (tentacles) bearing transversally implanted rows of medium-long cilia, in addition to short mobile cilia. Following this diagnosis he suggests that *C. stephanochaeta* (Edmondson), which has short corona lobes bearing similarly inserted rows of cilia, might be better placed in *Stephanoceros* rather than *Collotheca*, while he discards the relevance of the absence of such transverse rows of cilia in *Stephanoceros millsii* (Kellicott) by considering the latter a mere infrasubspecific ecotype of *S. fimbriatus* (Goldfusz).

We believe that the diagnosis of *Stephanoceros* is questionable, considering that neither the presence of long corona lobes (also in *Collotheca judayi* Edmondson and *C. tenuilobata* (Anderson)) nor the presence of transverse rows of cilia on the corona lobes (present in *Collotheca stephanochaeta*, absent in *Stephanoceros millsii*, see below) can serve as synapomorphic diagnostic feature for the genus. To the contrary, we hypothesize that the two species now attributed to *Stephanoceros* are merely species in which the prolongation of corona lobes already present in many species of *Collotheca* has evolved to its greatest extent. We look forward to a more complete phylogenetic analysis of the taxa, knowing that a molecular phylogenetic study of the group is ongoing. A synonymy between *Collotheca* and *Stephanoceros* would have to result in the reallocation of all taxa of the junior *Collotheca* to the senior *Stephanoceros*.

# Stephanoceros fimbriatus (Goldfusz) versus Stephanoceros millsii (Kellicott) (revised status)

We found specimens matching the descriptions of two taxa in *Stephanoceros, Stephanoceros fimbriatus* (Fig. 3H) and *S. millsii* (Fig. 3G, 5F) (see Kellicott 1887, Koste 1978). *S. fimbriatus* has five very long, stout corona lobes carrying transverse rows of robust setae along their length, while *S. millsii* has five relatively slender corona lobes carrying longitudinal rows of long, fine setae. The corona lobes of *S. fimbriatus* are relatively shorter than those of *S. millsii*, when compared to their trunk length. Regarding trophi, the unci tips of *S. millsii* are acutely pointed whereas those of *S. fimbriatus* have arrow-shaped tips, and the unci are more strongly curved in *S. millsii* (compare Fig. 5F with Fig. 1B in Sørensen and Giribet 2006).

Because the morphological characters of these two taxa enable a reliable diagnosis and because the two have wide and overlapping distribution ranges, we argue that these two taxa are distinct species, in contrast to Koste (1978) who considered *S. millsii* an infrasubspecific variant ("Anscheinend Ökotyp") of *S. fimbriatus*. Ours are the first photographs of living animals and trophi of *S. millsii*.

*S. millsii* is common in Thailand whereas *S. fimbriatus* is quite rare in our survey. Both species are cosmopolitan (Koste 1978).

## The uncinate trophi of Collothecidae

The uncinate trophi type is one of nine trophi types recognized in phylum Rotifera (Wallace et al. 2006). This trophi type is characterised by unci possessing few teeth and by weakly developed manubria and fulcrum (Koste 1978) and has hardly been considered in the taxonomic analysis of Collothecacea (Families Collothecidae and Atrochidae). We examined the uncinate trophi of 6 species of Collothecidae to evaluate whether morphological differences, which might be taxonomically relevant, exist.

We found that, in all species examined, the uncinate trophi are composed of two pairs of large and sturdy unci teeth, whereas manubria, rami and fulcrum are less developed components (Figs 5A–F). Of the unci, the distal tips can be gradually sharpened (5C–D), stout (5B), or with set-off tips (5A), and the tips may carry a terminal, median groove (e.g., 5E–F). The unci are mostly strongly curved, either more or less evenly (e.g., 5A, E) or in their proximal third (5B), or terminally (5C), and the terminal tips may be slightly incurved (5A), straight (5E), or outcurved (5B). The unci pairs can be relatively equal (5A, D–F) or strongly unequal (5B, C) in length. The unci teeth are quite sturdy, as they are not easily dissolved by low concentration of commercial bleach (lower than 5% final concentration). The manubria, rami and fulcrum, on the other hand, are very weak and dissolve easily in bleach making it particularly hard to reliably compare their morphology. Nevertheless, the rami scleropilli usually remain after treatment (5E–F).

As illustrated here, the uncinate trophi, in particular the unci, do exhibit features that might be useful for taxonomic analysis. We suggest that 1) shape of the head of the unci; 2) shape of the unci teeth; and 3) relative size of the two pairs of unci teeth might be registered in future studies of *Collotheca* rotifers. Of course, the inclusion of these features in taxonomic analysis requires addition of information on more species of *Collotheca*, and evaluation of the intraspecific variability by comparing different populations of *Collotheca* species.

## Feeding in Collotheca

As mentioned above, Collothecidae species are essentially ambush predators. They remain immobile until a prey organism, guided by their long cilia and infundibulum that forms a fyke, and water current created by the beating of short cilia, comes in range of a sensory organ situated dorsally on the inner side of the infundibulum. When this organ is triggered, the cilia, corona lobes and infundibulum contract which restrains the prey organism within the infundibulum, and the prey is finally ingested whole. We observed that some species of *Collotheca*, and these appear to be species that have an enlarged funnel-shaped infundibulum, arrange their corona near the surface of the substrate they are attached to (e.g., Collotheca sp., Figs 3D; C. ferox, Fig. 2A, B - note that the specimen in Figs 2A, B was not in normal position; C. orchidacea sp. n., Figs 2C, D). Other species, mostly those that have a relatively smaller infundibulum but well-developed bands of cilia along the corona or on knobs, and a long foot, expose their expanded corona in the water column (e.g., C. campanulata f. longicaudata, Fig. 3B; C. ornata, Fig. 3C; C. tenuilobata, Fig. 3F). We hypothesize that the two groups may have different diets. The latter group probably feeds on free-swimming, planktonic/periphytic organisms, while species of the former group may target browsing animals, in a way that is strikingly similar to Cupelopagis vorax (Leidy, 1857) (Bevington et al. 1995).

#### Identification key to the Collothecidae of Southeast Asia

The keys presented here include all species recorded hitherto from Southeast Asia (Brunei, Cambodia, Indonesia, Malaysia, Myanmar, Philippines, Singapore, Thailand, Timor Leste, Vietnam), as included in De Ridder and Segers (1997) and more recent publications. To facilitate identification and discovery of species not included in the key, we provide both a dichotomous as well as a formula key to the Southeast Asian Collothecidae.

## Dichotomous key

	( ) · · · · · · · · · · · · · · · · · ·
	(Figs 3G, H)(genus Stephanoceros), 16
_	Length of corona lobes in adult specimens as long as, or longer than trunk
1	Length of corona lobe(s) shorter than trunk (Figs 3A–F) (genus Collotheca), 2

2(1)	Animals free-living (planktonic)
_	Animals fixosessile, permanently attached to a substratum
3(2)	Corona edge circular, smooth; inner side of infundibulum with five rudimen-
	tary lobes
_	Corona with projections bearing groups of cilia
4(3)	Corona with a single dorsal lobe carrying one group of long cilia <i>C. libera</i>
_	Corona with five knob-shaped projections, the dorsal one on a triangular
	lobe: all bearing a group of long cilia (Fig. 3C, 4B) <i>C. ornata</i> f. <i>natans</i>
5(2)	Corona with well-defined, rounded or club-shaped knobs (Figs 3C, F)6
_	Corona circular or with broad lobes, no knob(s) (Figs 3B, D, E)
6(5)	Corona with seven knobs, the dorsal on a small, triangular lobe (Fig. $4A$ )
0())	C. hentabrachiata
_	Corona five projections (Fig. 4B) 7
7(6)	Corona with equal elongated lobes terminating in club-shaped knobs (Fig
/(0)	3E) <i>C tomuilobata</i>
	Corona lobes unequal and/or less than three times their width (Figs 3C /B)
_	Corona robes unequal and/or ress than three times then width (Figs 5C, 4D)
	Within this approximation the second days have been recorded from
	South and Aria One (Commented for a starter) is galaxie (as (2)), solville Commented
	Southeast Asia. One (C. ornata i. natans) is pelagic (see (5)), while C. ornata
	f. cornuta is diagnosed by the presence of an elongate projection on the dorsal
0(5)	corona lobe (Figs 4G, H).
8(5)	Corona circular, smooth, bearing only short cilia C. edentata
-	Corona with broad lobes
9(8)	Corona with one large dorsal and one smaller ventral lobe, dorsal lobe with a
	group of elongate, parallel cilia (Fig. 3D) Collotheca sp.
_	Corona with a dorsal lobe and a ventral sinus (Fig. 3B)10
10(9)	Corona with three lobes separated by clear smoothly concave sinuses be
	Corona with three lobes separated by clear, smoothly concave shruses be-
	tween the dorsal and ventral lobes (Fig. 3E)
_	tween the dorsal and ventral lobes (Fig. 3E)
- 11(10)	tween the dorsal and ventral lobes (Fig. 3E)
_ 11(10) _	tween the dorsal and ventral lobes (Fig. 3E)
- 11(10) -	tween the dorsal and ventral lobes (Fig. 3E)
- 11(10) - 12(9)	tween the dorsal and ventral lobes (Fig. 3E)
- 11(10) - 12(9)	tween the dorsal and ventral lobes (Fig. 3E)
- 11(10) - 12(9)	tween the dorsal and ventral lobes (Fig. 3E)
- 11(10) - 12(9) - 13(12)	tween the dorsal and ventral lobes (Fig. 3E)
- 11(10) - 12(9) - 13(12)	<ul> <li>Corona with three lobes separated by clear, shoothly concave sindses between the dorsal and ventral lobes (Fig. 3E)</li></ul>
- 11(10) - 12(9) - 13(12) -	<ul> <li>corona with three lobes separated by clear, shoothly concave sindses between the dorsal and ventral lobes (Fig. 3E)</li></ul>
- 11(10) - 12(9) - 13(12) - 14(13)	<ul> <li>Corona with three lobes separated by clear, shoothly concave sindses between the dorsal and ventral lobes (Fig. 3E)</li></ul>
- 11(10) - 12(9) - 13(12) - 14(13)	<ul> <li>Corona with three lobes separated by clear, shooting concave sindses between the dorsal and ventral lobes (Fig. 3E)</li></ul>
- 11(10) - 12(9) - 13(12) - 14(13) -	<ul> <li>Corona with three lobes separated by clear, shoothly concave sindses between the dorsal and ventral lobes (Fig. 3E)</li></ul>
- 11(10) - 12(9) - 13(12) - 14(13) - 15(14)	<ul> <li>Corona with three fobes separated by creat, shoothly concave sindses between the dorsal and ventral lobes (Fig. 3E)</li></ul>

_	Ventral lobes rounded, dorsal lobe broad (Fig. 3B) C. campanulata
	Within this species one infrasubspecific variant has been recorded from South-
	east Asia. Collotheca campanulata f. longicaudata is characterised by the pres-
	ence of an extraordinary long peduncle (secreted attachment stalk: Fig. 4I).
16(1)	Corona lobes stout and robust, carrying parallel, transversal sets of robust
	cilia (Figs 3H, 4C)S. fimbriatus
_	Corona lobes slender, carrying dense, longitudinal rows of fine, cilia (Fig. 3G).

......S. millsii

## Formula key

## Characters

- 1. Species (a) free-living (pelagic); (b) living attached to a substratum (fixosessile)
- 2. Corona edge: (a) circular, smooth; (b) with well-defined knobs (Figs 3C, F); (c) with lobes (Figs 3B, E)
- 3. Number of corona projections: (a) one dorsal; (b) two: one dorsal, one ventral (Fig. 3D); (c) three: one dorsal, two lateral (Fig. 3E); (d) five: one dorsal, two lateral, two ventral (Figs 3B, 4F); (f) seven (Fig. 4A)
- 4. Length of corona projections: (a) much shorter than trunk (Figs 3C, D) ; (b) strongly elongated and parallel sided (Figs 3G, H)
- Diversification of corona projections: (a) none, all projections more or less equal (Figs 3A, F–H); (b) differentiated (Figs 3B–E)
- 6. Lateral corona lobes: (a) absent (3E); (b) indicated (sinus between dorsal and ventral lobe is not smoothly concave or indicated by presence of a distinct group of particularly long cilae: Figs 4E, F); (c) well-developed (Figs 2A–F)
- 7. Ventral corona projections: (a) with one midventral lobe, (b) with two knobs (Fig. 4B); (c) two rounded triangular lobes (Fig. 4F); (d) two semicircular lobes (Figs 3B, E)
- 8. Ventral corona sinus: (a) shallow, narrow (Fig. 4J); (b) shallow, broad (Figs 3B, 4F); (c) deep, broad, U-shaped (Fig. 4E)
- Special features: (a) elongate projection dorsally on dorsal corona lobe (Figs 4G, H); (b) peduncle (attachment stalk) longer three times diameter of foot than foot (Fig. 4I); (c) cilia inserted in parallel sets of transverse rows (Figs 4C, D); (e) group of elongate cilia dorsally on dorsal corona lobe (Fig. 3D)

## Species

*Collotheca algicola*: 1b, 2c, 3d, 4a, 5b, 6b, 7c, 8b *Collotheca ambigua*: 1b, 2c, 3d, 4a, 5b, 6b, 7d, 8c *Collotheca campanulata*: 1b, 2c, 3d, 4a, 5b, 6b, 7d, 8b (+9b: f. *longicaudata*) Collotheca edentata: 1b, 2a Collotheca ferox: 1b, 2c, 3d, 4a, 5b, 6c, 7c, 8a Collotheca heptabrachiata: 1b, 2b, 2c, 3f, 4a, 5b, 6a, 7b Collotheca libera: 1a, 2c, 3a, 4a Collotheca orchidacea sp. n.: 1b, 2c, 3d, 4a, 5b, 6c, 7d, 8c Collotheca ornata: (1b), 2b, 2c, 3d, 4a, 5b, 6b, 7b (+9a: f. cornuta; 1a: f. natans) Collotheca pelagica: 1a, 2a Collotheca stephanochaeta: 1b, 2c, 3c, 4a, 5a, (6b), 7d, 8b, 9c Collotheca tenuilobata: 1b, 2b, 2c, 3d, 4b, 5a Collotheca sp.: 1b, 2c, 3b, 4a, 5b, 6a, 7a, 9e Stephanoceros fimbriatus: 1b, 2c, 3d, 4b, 5a

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RESEARCH ARTICLE



# Western Hemisphere Zuphiini: descriptions of Coarazuphium whiteheadi, new species, and Zuphioides, new genus, and classification of the genera (Coleoptera, Carabidae)

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## Abstract

Based on small samples (exemplars) analyzed with morphological methods, including detailed descriptions and illustrations, this study treats primarily the *Zuphium* genus-group in the Western Hemisphere, which comprises two precinctive genera: *Coarazuphium* Gnaspini, Vanin & Godoy, 1998 (type species *Parazuphium tessai* Godoy & Vanin, 1990) and *Zuphioides* gen. n. (type species *Zuphium mexicanum* Chaudoir, 1863). The genus *Coarazuphium* includes six troglobitic species from Brazilian caves, and one probably hypogaeic (troglophilic) species from the mountains of Oaxaca, in Mexico (*C. whiteheadi*, sp. n., type locality, ridge top, in western Oaxaca, Mexico, at 2164 m, 35 km north of San Pedro Juchatengo, 16.462N, 97.010W). The epigaeic genus *Zuphioides* includes 23 species, with its geographical range extended from Neotropical temperate Argentina in southern South America, northward through the tropics to north temperate southeastern Canada, in the Nearctic Region. Keys are provided to the species of *Coarazuphium* and to the genera of Western Hemisphere Zuphiini.

#### Keywords

Taxonomy, Zuphiitae, classification, key, anophthalmy, phylogenetic relationships, brachyptery, cave inhabitants, laboulbenealian fungi

## Introduction

The taxonomic investigation reported herein, began with discovery of an undescribed Mexican species of *Coarazuphium* Gnaspini, Vanin & Godoy. Considering that *Coarazuphium* was known previously only from caves in eastern Brazil, its known range extension into the northern part of Middle America (Map 1) was reason to examine it closely, with the expectation of broadening our concept of the genus, and perhaps even of its tribe, the Zuphiini.

Although the six known Brazilian species had been clearly described and well illustrated, a more detailed comparison of *Coarazuphium* with other zuphiines seemed desirable. Closer comparisons with the geographically widespread, morphologically similar *Zuphium* of authors were undertaken, beginning with a sampling of the Western Hemisphere species, and extending into the Eastern Hemisphere taxa, principally those from Western Europe and eastern Africa, with emphasis on the wide-ranging *olens* species group.

We broadened our study by placing *Coarazuphium* and *Zuphioides* (see below) in the context of a short review, based on the literature, of the Western Hemisphere zuphiine genera. We also took into account the classification of the Zuphiini (see Appendix I).

## Material, methods and terms

## Material

This study is based on examination of 94 specimens of Zuphiina, from the Western Hemisphere Nearctic and Neotropical Regions, and Eastern Hemisphere Palaearctic, Afrotropical, and Oriental Regions. Much of the material was in the Strickland Museum, Department of Biological Sciences, University of Alberta (UASM). Through the generosity of Terry L. Erwin, a sampling of zuphiines was received from the Department of Entomology, United States National Museum of Natural History, Smithsonian Institution, Washington, D. C., U.S.A. 20560 (USNM).

## Methods

Measurements. Measurements were made with an ocular micrometer in a Wild M5 stereoscopic microscope, at 50×. Measurements of external body parts and abbreviations used for them in the text are:

- **HL** Length of head linear distance from apex of extended left mandible to posterior margin of the postocciput;
- **HW** Width of head maximum distance across head, including eyes;
- A1L Length of antennomere 1 linear distance from base of antennomere 1 to apex of antennomere 1;
- A2-4L Length of antennomeres 2–4 linear distance from base of antennomere 2 to apex of antennomere 4;
- PL Length of pronotum linear distance from anterior to posterior margin, measured along the midline;
- PWM Maximum width of pronotum greatest linear transverse distance;
- **EL** Length of elytra linear distance from humerus to apex;
- **EW** Width of elytra maximum distance across the elytra.
- **OBL** Overall Body Length is the sum of HL, PL, and EL. Values for ratios for species were computed, using the measurements above: A1L/A2-4L; HW/PW. These numerical data are illustrative rather than definitive.

To express quantitatively proportions of the phallus, three measurements were made, using left lateral and dorsal aspects as illustrated in Figs 10 and 13:

- **PL** Length of phallus measured in a straight line from basal to apical margin;
- **PAL** Length of apical portion measured in a straight line from apical margin of periostial area to apical margin;
- **PSW** Width of phallus maximum transverse distance across the shaft, in ventral aspect.

These measurements were combined as two ratios PAL/PL; and PSW/PL. These numerical data are illustrative rather than definitive.

Preparation of material. Dissections were made by using standard techniques. Genitalia and other small structures were preserved in glycerine in microvials and pinned beneath the specimens from which they were removed. Larger structures and those that were gold-coated for study with the SEM were glued to cards pinned beneath the specimens from which they were removed.

Micrographs of isolated structures were taken with a JEOL JSM 6301 FXV field emission SEM. Line drawings of selected body parts were prepared by using a camera lucida on a Wild W5 stereoscopic microscope. Plates were prepared by using Adobe Photoshop CS 4.

Citation of figures. Figures included in the present publication are cited in the text as "Fig." Those previously published are cited "fig."

Label data. For type material, the information on each label is reproduced as exactly as is possible using ordinary type. Information on each label is enclosed in quotation marks; as well, a semicolon marks the end of a label. A slash mark (/) indicates the end of each line of text.

## Terms

## Structural features

Most of the terms used to designate details of structures are found in textbooks of general entomology, or are used by coleopterists, generally. Other words, used to designate particular structures or parts thereof, are not in general use, though they have been used by us in previous publications. We provide information about these words here, as well as names that have been changed for certain structural features.

Microsculpture. A "sculpticell" is the space on the surface of the cuticle enclosed by adjacent microlines of the integumental system of microsculpture (Allen and Ball 1980: 485-486). In most groups, overall, microsculpture is varied. In contrast, in the *Zuphium* species group, it is absent (surface smooth) or simply isodiametric, with microlines very fine and sculpticells flat to slightly granulate. Emphasis is placed on description of microsculpture of the sclerites of the dorsal surface, which is adequate for characterization of the taxa of the *Zuphium* genus-group.

Chaetotaxy. This term refers to the so-called "fixed setae", which are the long, evidently tactile, commonly encountered setae on carabids: dorsal labral; clypeal; supraorbital; stipital; submental; mental; glossal; palpigeral; pronotal; elytral parascutellar, discal, and umbilicate (or lateral); coxal, trochanteral, femoral, and tarsomeral; abdominal sternal ambulatory (sterna IV, V, VI); and abdominal sternal terminal (sternum VII, near posterior margin). Standard leg setae were not included because of difficulty in distinguishing them from the general body setation; but see Pellegrini and Ferreira (2011a, 2011b).

Body parts. The term "segment" is restricted for use to those body parts that reflect embryonic somites; thus, somite-like portions of the abdomen are referred to as segments. Abdominal segments are designated by Roman numerals corresponding to their respective somites. The first complete sternum is III, and the last one normally exposed is VII. For numbering the genital somites, we follow Bils (1976).

Head. The term "head capsule" in the *Zuphium* genus-group is restricted to that portion of the head that extends from the anterior margin of the clypeus to the broad part of the occiput that precedes the more or markedly narrowed posterior part of occiput + postocciput.

Eyes. Three conditions are recognized in the *Zuphium* genus-group: normal size (macrophthalmous), readily seen, convex, with distinct ommatidia (Fig. 3B); small (microphthalmous), virtually flat, without ommatidia, not readily seen (Fig. 3A); and absent (anophthalmous).

Antennae. Antennomere 1 (Figs 4B, 4D) exhibits two or three types of trichoid setae, based on size and declination: **as1**, single, erect, long; **as2**, row of several, erect, moderately long; and **as3**, more or less numerous, short, decumbent.

Mandibles. Shpeley and Ball (2001: 9–21, figs 3A–E) characterized the mandibles of the lebiine subtribe Pericalina, and illustrated the major features with SEM figures. We use the same system here. See Figs 6A–F and 7A–F.

Labium. The labium of the *Zuphium* genus-group is standard for Carabidae. For the combined glossae and paraglossae, we use the standard term ligula. The central sclerotized, apically setigerous, structure is the glossal sclerite. See Figs 8C and 8F.

Male tarsal vestiture. Two types of adhesive vestiture on the ventral surface of the fore tarsi are exhibited by males of the *Zuphium* genus-group: articulo-setae (Fig. 9A, **as**) and biseriate squamo-setae (See Stork 1980, for a thorough discussion of this topic).

Male genitalia. Based on economy of expression, in preference to the widely used terms "median lobe" and "internal sac", we use "phallus" and "endophallus", respectively. Such usage is also well established in the entomological literature treating the male reproductive system. The surface of the phallus treated by convention as dorsal is really the ventral surface, and vice versa (Deuve 1993: 88). As in our previous publications, we have chosen to remain with the conventional usage. Phalli were classified as anopic, with the ostium dorso-medial. The phallus (e.g., Figs 10A-10F, and 13A-13C), illustrated in left lateral, ventral and right lateral aspects, with base toward the bottom of the page, exhibits interspecific differences in form. These differences are seen readily as overall patterns ('Gestalt') but are not so easily described, except with notation of differences in size and shape of the apical area. To provide the basis for verbal description, two principal regions are distinguished-- the shaft  $(\mathbf{s})$ , and basal bulb  $(\mathbf{bb})$ , or lobe, or phallobase. The latter is the swollen area set at an angle to the ventrally curved shaft, surrounding the basal opening; the periostial membrane (om), which surrounds the ostium, marks the place of egress of the endophallus during copulation; an apical portion (**ap**) or phalloapex, extends distally from the apex of the periostial membrane to the apex of the phallus. The phalli in lateral aspect exhibit curvature ventrally. A pair of slender paraostial sclerites (**ps**) is evident (Figs 13A, 13C) or not (Fig. 10A).

Ovipositor (Figs 11A–11F) and female genital tract (Figs 12A, 12B and 13D). For naming the sclerites of the adephagan ovipositor and units of the female genital tract, we follow the system used by Liebherr and Will (1998; see figures therein). For the gonocoxites (**gc1**, **gc2**), the surfaces that are ventral in the infolded position are lateral when the ovipositor is extended; thus such surfaces are designated as lateral, and the other surfaces are designated accordingly.

## **Systematics**

Some of the morphological features that distinguish *Coarazuphium* from the Eastern Hemisphere widespread *Z. olens* species group (includes *Z. olens* (Rossi, 1790), type species of the genus) are shared with the Western Hemisphere species of *Zuphium* of authors (Table 1). From that distribution of character states, we conclude that the Western Hemisphere *Zuphium* of authors forms an assemblage different from *Coarazuphium* and from the *Z. olens* species group, and this bridges the morphological gap between those two taxa. A reasonable taxonomic expression of this situation seems to us is to recognize three groups of generic rank: *Zuphium* (*sensu stricto*), *Zuphioides* **new genus** (= *Zuphium* of auothors), and *Coarazuphium*.

		Taxa and character states		
No.	Characters	Zuphium (s. str.) ustum	Zuphioides mexicanum	Coarazuphium whiteheadi
01	A . 1	character state 01.1	character state 01.2	character state 01.2
	row of erect cetee	absent	present	present
	10w 01 elect setae		Fig. 4D	Fig. 4B
02	Postarior	character state 02.1	character state 02.2	character state 02.2
	supraorbital seta	absent	present	present
			Fig. 4C	Fig. 4A
03	Prosternal setae	character state 03.1	character state 03.2	character state 03.2
		absent	present	present
				Fig. 9B
		character state 04.1	character state 04.2	character state 04.2
04	Male adhesive setae	biseriate	pad-like,	pad-like
			Fig. 9A	
	Mala conitalia	character state 05.1	character state 05.2	character state 05.2
05	nonaccetial colonitor	present	absent	absent
	paraostiai scientes	Figs. 13A, 13C		
06	Female genital tract, bursal sac	character state 06.1	character state 06.2	character state 06.2
		absent	present	present
			Fig. 12A	Fig. 12B
	Female genital tract,	character state 07.1	character state 07.2	character state 07.2
07	sec. spermathecal	present	absent	absent
	gland	Fig. 13D		
08	Integument color	character state 08.1	character state 08.1	character state 08.2
		rufous to piceous	rufous	testaceous
	Head capsule form	character state 09.1	character state 09.1	character state 09.2
09		trapezoidal	trapezoidal	oviform
			Fig. 4C	Fig. 4A
		character state 10.1	character state 10.1	character state 10.2
10	Eyes	macrophthalmous	macrophthalmous	microphthalmous
10			Fig. 3B	Fig. 3A
				or anophthalmous
11	Metepisternum	character state 11.1	character state 11.1	character state 11.2
		elongate	elongate	quadrate
12	Hind wings	character state 12.1	character state 12.1	character state 12.2
		macropterous	macropterous	brachypterous-apterous
	Ovipositor:	character state 13.1	character state 13.1	character state 13.2
13	gonocoxite 2	distal margin evenly rounded	distal margin evenly rounded	distal margin deeply notched
			Fig. 11F	Fig. 11C

**Table 1.** Comparison of diagnostic character state combinations exhibited by exemplars of *Zuphium* (*sensu stricto*), *Zuphioides*, new genus, and *Coarazuphium* Gnaspini, Vanin, and Godoy.

*Zuphioides mexicanum* shares character states 01.2, 02.2, 03.2, 04.2, 05.2, 06.2, and 07.2 with *C. whiteheadi*; *Zuphioides mexicanum* shares character states 08.1, 09.1, 10.1, 11.1, 12.1, and 13.1 with *Z. ustum*; *Zuphium ustum* exhibits uniquely character states 01.1, 02.1, 03.1, 04.1, 05.1, 06.1, and 07.1; *Coarazuphium whiteheadi* exhibits uniquely character states 08.2, 09.2, 10.2, 11.2, 12.2, and 13.2.

## Zuphium genus-group in the Western Hemisphere

Included are two precinctive genera of Zuphiini: *Coarazuphium* Gnaspini et al., and *Zuphioides*, **new genus**. They share the following features.

**Recognition.** With features of Western Hemisphere Zuphiini (see "Recognition", Appendix I) and head markedly constricted posteriorly, a narrow neck formed by posterior part of occiput and postocciput; maxillary and labial palpi of about equal size and

form, terminal palpomeres slightly widened apicad, apical margin obliquely truncate; antennomere 1 with short row of erect setae among the decumbent vestitural setae; prosternum with pair of long erect setae; male fore-tarsomeres 1-3 ventrally with adhesive vestiture of articulo-setae, in form of a broad pad; phallus dorsally without paraostial sclerites; endophallus with various microtrichial patches, without sclerites; ovipositor gonocoxite 2 very broad in dorso-ventral aspect without ensiform setae; female reproductive tract with terminal bursal sac, and without bulbous secondary spermathecal gland.

**Description.** *Size and proportions.* Small, OBL 4.13–4.40 mm; EW 1.36–1.48 mm; A1L/A2-4L 1.03-1.11; HW/PW 1.08.

*Color.* Body various, piceous to testaceous, or in life almost white; appendages slightly paler; vestiture flavous.

*Habitus* (Figs 1A, 1B). Flat, overall. Head (dorsal aspect) trapezoidal or oviform, subangulate or broadly rounded posteriolaterally anteriad markedly narrowed neck. Eyes (Figs 3A, 3B) convex, flat, or lacking. Pronotum narrow. Elytra parallel-sided or narrowed both anteriorly and posteriorly, lateral margins distinctly bowed.

*Microsculpture.* Dorsal surface of head (including clypeus) and pronotum smooth, microlines not evident; labrum and elytra with microlines fine (not easily seen at 50× or lower magnification), mesh pattern isodiametric. Ventral surface with microlines fine, generally transverse, sculpticells rather short.

Luster. Dorsal surface of head and pronotum shiny, elytra somewhat duller.

*Body vestiture and punctation*. Dorsal surface of head and pronotum sparsely punctate, vestiture sparse. Elytra with punctation and vestiture dense, vestitural setae decumbent.

*Fixed setae.* Head (Figs 4A, 4C): clypeus one pair; head capsule with anterior pair of supraorbitals (**asos**) above eyes; posterior pair of supraorbitals (**psos**) posteriad eyes; one pair of postocular setae (**pos**) immediately behind eyes, laterally; one pair of occipital setae (**ocs**) posteriorly and mediad eyes, or lacking; also one pair posterior supernumerary setae (**psus**) laterad and close to posterior supraorbital setae, or lacking. Antennomere 1 (Figs 4B, 4D, **as1**) with single long seta distally, in addition to decumbent vestiture and row of small erect setae. Pronotum with two pairs of lateral marginal setae, anterior pair in anterior 1/8, posterior pair at posteriorly with parascutellar seta; lateral setae about 21 (one group anteriorly, one group posteriorly, and single seta medially. Abdominal sterna IV-VI with or without ambulatory setae, sternum VII with pair of long setae near posterior margin.

*Head, dorsal aspect* (Figs 2A, 2B). Occiput posteriorly and postocciput markedly constricted, in form of narrow neck, postoccipital suture evident. Frontoclypeal suture present (most species) or absent, with frons and clypeus fused (*C. pains*, Álvares and Ferreira 2002: 41). Genal sulcus prominent.

*Eyes.* Small (microphthalmous), flat (Fig. 3A), ommatidia not evident at  $50 \times$  or lower magnification; or, eyes normally convex (macrophthalmous, Fig. 3B), ommatidia evident at  $50 \times$ .

*Antennae* (Fig. 1A). Filiform, extended about body length. Antennomere 1 (Figs 4B, 4D, **a1**) rather slender, slightly longer than antennomeres a2-a4; antennomere 2 short, about half length of a3; antennomeres 3-11 narrow, cylindrical, distinctly longer than wide.



**Figure 1.** Habitus, dorsal aspect, of: **A** *Coarazuphium whiteheadi*, new species **B** *Zuphioides mexicanum* (Chaudoir). Scale bars = 5 mm.

*Labrum* (Figs 5A, 5C). Rectangular, lateral margins rounded; anterior margin concave or irregularly convex; six dorsal setae, lateral setae longer than four medials. Epipharynx (Figs 5B, 5D) with pedium (**ped**) rather broad; parts standard (crepis **cr**, parapedial projection (**pp**), and parapedial setae (**ps**).

*Mandibles* (Figs 6A–6F and 7A–7F). Trigonal in form, with long rather slender terebra (**T**) and short basal area (**B**) with condyles; occlusal margin with long terebral ridge (**tr**), prominent incisor tooth (**it**), blunt terebral tooth (**tt**), small premolar (**pmt**) and molar (**mt**) teeth, and short basal brush (**bb**); ventrally with long ventral groove (**vg**) extended apically almost to mandibular margin, with moderately long and dense microtrichia (**vm**); lateral margin basally with distinct trianguloid scrobe (**s**), dorsal and ventral margins each with three moderately long setae (**ss**). Left mandible in dorsal aspect (Fig. 6A) apically with rather broad incisor tooth (**it**), short supraterebral ridge (**str**), shallowly concave terebral ridge (**tr**), moderately prominent terebral tooth



Figure 2. Head, dorsal aspect, of: A *Coarazuphium whiteheadi*, new species B *Zuphioides mexicanum* (Chaudoir). Scale bars = 1 mm.

(tt), and distinct premolar and molar teeth (**pmt** and **mt**); ventral surface (Figs 6B) with blunt retinacular tooth (**rt**), and short retinacular ridge (**rr**); other features as noted above. Right mandible, dorsal aspect (Figs 7A) with incisor tooth (**it**) shorter and less curved than in left mandible, terebral tooth (**tt**) not prominent; retinaculum prominent, with anterior and posterior teeth (**art** and **prt**, respectively), and intervening retinacular ridge (**rr**); terebral ridge (**tr**); premolar and molar teeth (**pmt** and **mt**, respectively) evident.

*Maxillae* (Figs 8A, 8B, 8D, 8E; left maxilla illustrated). Standard sclerites: cardo (c), stipes (basistipes (bs), dististipes (ds) and palpifer (pl)), galea (galeomere 1 (g1) and galeomere 2 (g2)); palpus with four palpomeres (mp1-mp4). Marginal setae: basistipital (bss) and palpiferal (pls). Lacinial occlusal margin densely setose, setae thick, moderately long. Galeomere 2 with numerous short erect setae; palpomeres 2 and 3 (mp2, mp3) sparsely setose; palpomere 4 (mp4) moderately densely setose. Palpomere 4 widened slightly distally, apical margin subtruncate.

*Labium, ventral aspect* (Figs 8C, 8F). Standard sclerites: submentum (**sm**), mentum, and prementum; prementum comprised of a central glossal sclerite (**gls**), pair of lateral paraglossae (**pgl**), and pair of palpi, each of three articles (**lp1-lp3**), borne on a short broad palpiger (**pgr**). Submentum (**sm**) narrow rectangular sclerite, with pair of lateral setae (**sms**). Mentum transverse, with broad lateral lobes (**mll**), broad, blunt tooth (**mt**), broad epilobes (**el**); one pair of long paramedial setae (**pms**). Glossal sclerite (**gls**), fused laterally each side with basal part of narrow paraglossae (**pgl**), latter membranous, densely setose, extended distally beyond broad distal margin of glossal sclerite. Glossal sclerite with pair of longer ventral setae (**vgs**), and four shorter



**Figure 3.** Head and prothorax, lateral aspect, of: **A** *Coarazuphium whiteheadi*, new species **B** *Zuphioides mexicanum* (Chaudoir). Legend: arrow indicates eye. Scale bars = 1 mm.

terminal marginal setae (**tgs**). Palpigers (**pgr**) cup-like, glabrous. Palpi each of three palpomeres (**lp1-lp3**), palpomere 1 short, glabrous; palpomere 2 long, slender, subcy-lindrical, and moderately densely setose, most setae erect, one pair on anterior margin (**lp2s**) long; palpomere 3 widened slightly distally, apical margin subtruncate.

Coarazuphium bezerra Gnaspini, Vanin & Godoy 1998
Coarazuphium cessaima Gnaspini, Vanin & Godoy 1998
Coarazuphium formoso Pellegrini & Ferreira 2011
Coarazuphium pains Alvarez & Ferreiera 2002
Coarazuphium tapiaguassu Pellegrini & Ferreira 2011
Coarazuphium tessai (Godoy & Vanin) 1990
Coarazuphium whiteheadi Ball & Shpeley, sp. n.
Zuphioides aequinoctiale (Chaudoir, 1862)
Zuphioides americanum (Dejean, 1831)
Zuphioides argentinicum (Liebke, 1933)
Zuphioides batesi (Chaudoir, 1862)
Zuphioides bierigi (Liebke, 1933)
Zuphioides brasiliense (Chaudoir, 1872)
Zuphioides bruchi (Liebke, 1933)
Zuphioides capitum (Liebke, 1933)
Zuphioides columbianum (Chaudoir, 1872)
Zuphioides cubanum (Liebke, 1933)
Zuphioides delectum (Liebke, 1933)
Zuphioides exiguum (Putzeys, 1878)
Zuphioides exquisitum (Liebke, 1933)
Zuphioides flohri (Liebke, 1933)
Zuphioides haitianum (Darlington, 1935)
Zuphioides lizeri (Liebke, 1933)
Zuphioides longicolle (LeConte, 1879)
Zuphioides magnum (Schaeffer, 1910)
Zuphioides mexicanum (Chaudoir, 1863)
Zuphium vivinum (Liebke, 1933)
Zuphioides pseudamericanum (Mateu, 1981)
Zuphioides punctipenne (Bates, 1891)
Zuphioides pusillum (Chaudoir, 1862)
Zuphioides salivanum (Liebke, 1933)

Table 2. List of names of species of *Coarazuphium* Gnaspini, Vanin & Godoy and *Zuphioides*, new genus.

*Pronotum* (Figs 1A, 1B). Anterior margin truncate, lateral margins markedly sinuate posteriorly, posteriolateral angles prominent, dentiform, slightly anteriad posterior margin; surface impressions (anterior and posterior transverse and median longitudinal) shallow; lateral grooves and posteriolateral impressions moderately deep.

*Pterothorax.* Metasternum moderately long or short; metepisternum longer than wide or quadrate.

*Elytra* (Figs 1A, 1B). Separate, not fused along suture. Each elytron more or less rectangular, lateral margin straight or bowed, humerus projected anteriorly, basal ridge sinuate; apical margin truncate, with narrow band of membrane. surface with striae very shallow, intervals almost flat.

Hind wings. Macropterous, or brachypterous, with each wing represented by short stub.

Abdomen. Sternum VII with apical margin truncate.

*Male genitalia* (Figs 10A–10F). Anopic. Phallus slightly curved ventrally, narrowed apically, apical margin rounded, apical portion very short; without paraostial sclerites. Left paramere ( $\mathbf{lp}$ ) conchoid or styliform. Right paramere ( $\mathbf{rp}$ ) short, broad, or styliform, relatively long.

*Female genitalia: ovipositor* (Figs 11A–11F). Gonocoxite 1 (gcl) short, thick, with or without row of long trichoid setae distally on ventral and lateral surface. Gonocoxite 2 (gc2) short, thick; in lateral aspect falciform, apex pointed, variously provided with long trichoid setae dorso- and ventrolaterally; in dorsoventral aspect, broad, paddle-like, apex deeply notched or not; dorsal surface glabrous except for dorsolateral and ventrolateral trichoid setae (ts); ventral surface toward margins with row of pit pegs (mpp), preapical setose organ (pso) circuloid, with two furrow pegs (fp) and two very short nematiform setae (ns).

*Female genital tract* (Figs 12A, 12B). Bursa copulatrix (**bc**) ended in an expanded bulbous anterior extension (**bs**). Common oviduct (**co**) inserted in bursa copulatrix at base of its anterior extension. Spermatheca (**sp**) slender, long, inserted on or at base of bursal sac, beside insertion point of spermathecal gland duct (**spgd**); latter with swelling proximad spermathecal gland (**spg**). With or without helminthoid sclerite. Without secondary spermathecal gland.

**Geographical distribution.** The range of this genus-group in the Western Hemisphere extends from Neotropical Argentina (Reichardt 1977: 449) northward through the American tropics and subtropics to temperate eastern and central U.S.A., and to southeastern Canada (Lindroth 1969: 1090), with an isolated population of *Z. americanum* in western Oregon (Bousquet, 2012: 1356).

**Included taxa.** The *Zuphium* genus-group in the Western Hemisphere includes 30 species arranged in two genera: *Coarazuphium* Gnaspini et al. and *Zuphioides* new genus (Table 2).

## Coarazuphium Gnaspini, Vanin & Godoy, 1998

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

Parazuphium; Godoy and Vanin 1990: 795 (not Jeannel 1942).

Zuphium; Mateu 1993: 486 (not Latreille 1805).

Coarazuphium Gnaspini, Vanin & Godoy, 1998: 298. Álvares and Ferreira 2002:
41. Lorenz 2005: 507. Pellegrini and Ferreira 2011a: 39. Pellegrini and Ferreira 2011b: 47.

**Type species.** *Parazuphium tessai* Godoy & Vanin, 1990 (designated by Gnaspini et al. 1998).

Generic name. As explained by Gnaspini et al. (1998: 299), the name *Coarazuphium* is a compound Latinized neuter noun derived from "coara", meaning hole or cave in the Tupi language (Brazilian, native tongue), plus *Zuphium*. The name refers to the troglophilic or troglobitic habits of the species of this genus, and their basic nominotypical *Zuphium*-like features.

**Recognition.** With character states of Western Hemisphere *Zuphium* genusgroup, restricted as follows: size small; pronotum and dorsal surface of elytra moderately densely setose, setae decumbent; body markedly depressed; integument pale; head narrow, oviform, with posteriolateral margins broadly rounded; antennae elongate, antennomere 1 as long or longer than antennomeres 2-4; humeri narrowed, slightly or markedly so; metasternum short, metepisternum quadrate. Brachypterous or apterous. Male genitalia (Figs 10A–10C): phallus without dorsal paraostial sclerites. Female genital tract (Fig. 12A, 12B): without secondary spermathecal gland.

**Description.** None required here. See Gnaspini et al. (1998: 298-299), Pellegrini and Ferreira (2011b), and description of *C. whiteheadi* below.

**Geographical distribution.** Confined to the Neotropical Region, the seven species of this genus are known only from southeastern Brazil (Pellegrini and Ferreria 2011b: 57, fig. 10) and southern Mexico (Map.1). This is an example of the "Paleo-American distribution pattern" (see Halffter (1987) and Liebherr (1994: 845)).

Way of life. The previously described species of genus *Coarazuphium* inhabit caves in one of two types of substrate (Pellegrini and Ferreira 2011b: 55): limestone, occupied by *C. bezerra* Gnaspini, Vanin & Godoy; *C. cessaima* Gnaspini, Vanin & Godoy; *C. formoso* Pellegrini & Ferreira; *C. pains* Álvares & Ferreira; and *C. tessai* (Godoy & Vanin); and iron ore, occupied by *C. tapiaguassu* Pellegrini & Ferreira. The caves in iron ore are described as "shallow". Members of those species occupying the caves in limestone substrate are described as freely walking over the soil, and presumably resting exposed, rather than resting (hiding?) under rock cover. In contrast, all of the specimens collected of *C. tapiaguassu* were found under rocks, on the cave floor. In contrast, known members of the Mexican species, *C. whiteheadi*, new species, seem to be surface inhabitants (see below)

**Parasites.** Some specimens of *C. tapiaguassu* were infested by laboulbenelian fungi. The fungi were not identified further. See this topic for *C. whiteheadi*, below.

**Evolutionary considerations.** The ultrastructural features (i.e., principally sensillar) observed with scanning electron microscopy by Pellegrini and Ferreria (2011a, 2011b) differ only slightly between the two species of *Coarazuphium* that they studied, and between that genus and *Zuphioides* (*cf.* accompanying SEM illustrations of *Z. mexicanum*). So, it seems to us unlikely that such features are or will be evolutionarily informative.

In contrast, the standard structural troglobitic features of *Coarazuphium* (lengthening of antennae and legs, depigmentation, micro- or anophthalmy, and reduction of elytral length, elytral humeri, metathorax and metathoracic wings) plus details of male and female genitalia are evolutionarily informative. Based on eye loss and more elongate appendages, Gnaspini et al. (1998: 303) proposed that *C. cessaima* showed the more modified features, compared to *C. tessai* and *C. bezerra*, the only other species of *Coarazuphium* known at that time. Álvares and Ferreira (2002: 43) proposed that, based on the features noted above, their newly described *C. pains* would occupy a position intermediate between *C. cessaima* and *C. tessai* + *C. bezerra*.



**Figure 4.** SEM micrographs of head and antennomere 1, dorsal aspect. **A** and **B** *Coarazuphium white-headi*, new species **C** and **D** *Zuphioides mexicanum* (Chaudoir). Legend: **a1**, antennomere 1; **as1**, **as2**, **as3**, erect setae on antennomere 1; **asos**, anterior supraorbital seta; **cl**, clypeus; **e**, eye; **gs**, genal sulcus; **n**, neck; **ocs**, occipital seta; **pocs**, postoccipital suture; **pos**, postocular seta; **psos**, posterior supraorbital seta; **ss**, posterior supraorbital seta; **ss**, posterior supernumerary seta. Scale bars: **A** and **C** = 500 µm; **B** and **D** = 200 µm.

The external features of *Coarazuphium* evidently evolved in parallel with, and independent of, four other zuphiine troglobite taxa: the remarkable Spanish *Ildobates neboti* Español, 1966; the Canary Islands *Parazuphium feloi* Machado (1997: 163); the Austral-



**Figure 5.** SEM micrographs of labra. **A** and **B** dorsal and ventral aspects, respectively, of *Coarazuphium whiteheadi*, new species **C** and **D** dorsal and ventral aspects, respectively, of *Zuphioides mexicanum* (Chaudoir). Legend: **cr**, crepis; **ls**, lateral seta; **ped**, pedium; **pp**, parapedial projection; **ps**, parapedial seta. Scale bars = 100 µm.

ian Nullarbor *Speozuphium poulteri* Moore (1995: 159) and *Speothalpius grayi* Moore (1995: 160). Another Canary Islands *Parazuphium (P. damascenum canariense* Machado, 1992: 580) exhibits these same reductive (though less developed) features, but it is evidently conspecific with the continental Palaearctic *P. damascenum damascenum* (Fairmaire, 1897). For a more detailed discussion of the matter see Machado (1992: 581–582).

Gnaspini et al. (1998: 308–309) proposed for the Brazilian species of *Coarazuphi-um* that "..... these highly derived troglobitic features are due to a long-term isolation



**Figure 6.** SEM micrographs of mandibles of *Coarazuphium whiteheadi*, new species. **A** and **D** dorsal aspect **B** and **E** ventral aspect **C** and **F** lateral aspect. Legend: **art**, anterior retinacular tooth; **B**, basal area; **bb**, basal brush; **it**, incisor tooth; **mt**, molar tooth; **pmt**, premolar tooth; **prt**, posterior retinacular ridge; **rr**, retinacular ridge; **s**, scrobe; **ss**, scrobal seta; **str**, supraterebral ridge; **T**, terebra; **tr**, terebral ridge; **tt**, terebral tooth; **vg**, ventral groove; **vm**, ventral microtrichia. Scale bars = 100 µm.



**Figure 7.** SEM micrographs of mandibles of *Zuphioides mexicanum* (Chaudoir). **A** and **D** dorsal aspect **B** and **E** ventral aspect **C** and **F** lateral aspect. Legend: **art**, anterior retinacular tooth; **bb**, basal brush; **it**, incisor tooth; **mt**, molar tooth; **pmt**, premolar tooth; **prt**, posterior retinacular ridge; **rr**, retinacular ridge; **s**, scrobe; **ss**, scrobal seta; **tr**, terebral ridge; **tt**, terebral tooth; **vg**, ventral groove; **vm**, ventral microtrichia. Scale bars = 100 μm.

inside the subterranean environments, which took place under the drier climate to which the region was in the past and is still submitted in the present. It is largely accepted in the literature that cave arthropods are related to litter epigean and/or endogean ancestors, which already inhabited humid habitats. Therefore, ..... the ancestral species [of *Coarazuphium*] should have been epigean and lived in forested (or at least humid) areas, and occurred at least in part of the region where the genus occurs nowadays. From [such ancestral stock] several lineages invaded the caves from the northern Bambui Speleological Province, where they became isolated with the progressive shrinkage of humid environments. Thence, the origin of the genus takes back to a time when the area was not drying yet, which is probably the Tertiary".

Discovery of the Mexican species adds important details to the story of evolution of *Coarazuphium*. First, the marked geographical range extension (from southeastern South America to the southern part of the North American continent) shows that this genus was not confined to eastern Brazil. Further, the distribution pattern lends support to the hypothesis of Gnaspini et. al. (see above) that *Coarazuphium* originated in early Tertiary time (i.e., before the beginning of the drying trend that extended through much of the Cenozoic era). Second, the extra-speleal humid forest existence of *C. whiteheadi* suggests that the basic troglobitic features of *Coarazuphium* (microphthalmy, brachyptery, depigmentation, etc.) evolved in surface habitats, though probably in forested deep leaf litter locations, and were in effect preadaptations for cave life.

An additional observation relating to evolutionary history of the *Zuphium* genus group is that shared features of *Zuphioides* and *Coarazuphium* indicate that these two genera may be adelphotaxa, with *Zuphioides* retaining mostly ancestral features including life in lowland hygrophilous or mesophilous situations.

## Key to species of genus Coarazuphium Gnaspini, Vanin & Godoy

1	Anophthalmous (Gnaspini et al. 1998: 307, fig. 6). Maximum width of elytra
	near middle. Male genitalia: right paramere (Gnaspini et al. 1998: 306, fig.
	10) styliform, about as long as left paramere
	<i>C. cessaima</i> Gnaspini, Vanin & Godoy
1'	Microphthalmous. Maximum width of elytra near middle, or posteriad mid-
	dle. Male genitalia: right paramere styliform or not, distinctly shorter than
	left paramere
2(1')	Elytron with apical margin truncate, not sinuate. Male right paramere styli-
	form or broad
2'	Elytron with apical margin sinuate. Male right paramere broad, not styli-
	form, distinctly shorter than left paramere
3(2)	Head dorsally without setae posteriad the anterior supraorbital setae (Pel-
	legrini and Ferreira 2011: 49, fig. 2A) C. tapiaguassu Pellegrini and Ferreira

Head dorsally with one to three pairs of setae posteriad the anterior supraor-
bital setae
Labrum with anterior margin broadly concave. Prosternal setae two pair ( $\Delta$ human and Farming 2002; (2, for 3). Maximum width (of olympi) prosteriod
transverse midline. Male right paramere (Álvares and Ferreira 2002: 42, fig.
6) broad, not styliform, distinctly shorter than left paramere. Brazil
<i>C. pains</i> Álvares & Ferreira
Labrum (Fig. 5A) with anterior margin irregularly convex. Prosternal setae
one pair (Fig. 9B). Male right paramere (Fig. 10C, rp) styliform, more than
half length of left paramere. Mexico
Head dorsally with three pairs of setae posteriad the anterior supraorbital
setae (Pellegrini and Ferreira, 2011a: figs 1A, 1B)
Head dorsally with one or two pairs of setae posteriad the anterior supraorbi-
tal setae
Head dorsally with two pairs of setae (posterior supraorbitals and occipitals,)
at posterior border of head capsule (Gnaspini et al.1998: 304, fig. 1). Male
left paramere broad, conchoid (Gnaspini et al. 1998: 305, fig. 3)
<i>C. bezerra</i> Gnaspini, Vanin & Godoy
Single pair of setae (posterior supraorbitals) at posterior border of head cap-
sule (Godoy and Vanin 1990: 796, fig. 1). Male left paramere styliform (Go-
doy and Vanin 1990: 798, fig. 2) C. tessai (Godoy & Vanin)

## Species treatment

Only the following new species is treated here.

## Coarazuphium whiteheadi, sp. n.

urn:lsid:zoobank.org:act:1CC227B6-FA52-40DC-8FC4-C25A060CE300 http://species-id.net/wiki/Coarazuphium\_whiteheadi Figs 1A, 2A, 3A, 4A, 4C, 5A, 5B, 6A–6F, 8A–8C, 9B, 10A–10C, 11A–11C, 12A, Map 1

**Type material.** Three specimens, as follows. HOLOTYPE female, labeled: "MEXI-CO, Oaxaca/7100' 21.8 mi./ n. Juchatengo/ VII.18-19.1966"; "George E. Ball/ D. R. Whitehead/ collectors" (USNM). PARATYPES two: female, labeled same as holotype (UASM); male, labeled: "MEXICO, Oaxaca/ Mt. Alban, near ruins/ Acacia scrub 6000 ft/ VI-3/4-82/ Rolf L. Aalbu, col." (USNM).

**Type locality.** Ridge top, Sierra de Miahuatlan, in western Oaxaca, Mexico, at 2164 m, 35 km north of San Pedro Juchatengo, 16.462N, 97.010W.



Figure 8. SEM micrographs of left maxillae and labia. A–C *Coarazuphium whiteheadi*, new species D–F *Zuphioides mexicanum* (Chaudoir). Legend: bs, basistipes; bss, basistipital seta; c, cardo; ds, distitipes; el, epilobe; g1, galeomere 1; g2, galeomere 2; gls, glossal sclerite; l, lacinia; lp1, labial palpomere 1; lp2, labial palpomere 2; lp2s, labial palpomere 2; seta; lp3, labial palpomere 3; lt, lacinial tooth; mll, mental lateral lobe; mp1, maxillary palpomere 1; mp2, maxillary palpomere 2; mp3, maxillary palpomere 3; mp4, maxillary palpomere 4; mt, mental tooth; pgl, paraglossa; pgr, palpiger; pl, palpifer; pls, palpifer seta; pms, paramedial mental seta; sm, submentum; sms, submental seta; tgs, terminal glossal seta; vgs, ventral glossal seta. Scale bars = 100 μm.


**Figure 9. A** SEM micrograph of fore tarsus of *Zuphioides mexicanum* (Chaudoir). Legend: **as**, articuloseta; **ts**, trichoid seta. Scale bar = 100  $\mu$ m. **B** Line drawing of prothorax and base of head, ventral aspect, of *Coarazuphium whiteheadi*, new species. Only medial pair of prominent prosternal setae illustrated, vestiture omitted. Scale bar = 1 mm.

**Specific epithet.** A Latinized eponym, masculine gender, genitive case, based on the surname of Donald R. Whitehead, now deceased, one of the collectors of the type series of this species.

Recognition. See key, above.

**Description.** *Size and proportions.* Small, OBL 4.13–4.40 mm; EW 1.36–1.48 mm A1L/A2-4L 1.03–1.11; HW/PW 1.08.

Color. Body testaceous, in life almost white; appendages slightly paler; vestiture golden.

*Habitus* (Fig. 1A). Flat, overall. Head capsule (dorsal aspect, Fig. 2A) oviform, broadly rounded posteriolaterally. Eyes (Fig. 3A) flat, hardly perceptible, ommatidia not evident at 50×. Pronotum narrow. Elytra narrowed anteriorly and posteriorly, lateral margins distinctly bowed.

*Microsculpture.* Dorsal surface of head capsule (including clypeus) and pronotum smooth, microlines not evident; labrum and elytra with microlines fine (not easily seen at 50× or lower magnification), mesh pattern isodiametric. Ventral surface with microlines fine, generally transverse, sculpticells rather short.

Luster. Dorsal surface of head and pronotum shiny, elytra somewhat duller.

*Body vestiture and punctation.* Dorsal surface of head and pronotum sparsely punctate, vestiture sparse. Elytra with punctation and vestiture dense, vestitural setae decumbent. *Abdomen.* Abdominal sterna IV-VI with punctation rather sparse, vestitural setae decumbent.

*Fixed setae.* Head (Fig. 4A, 4C): clypeus one pair; head capsule with anterior pair of supraorbitals (**asos**) above eyes; posterior pair of supraorbitals (**psos**) posteriad eyes; one pair of postocular setae (**pos**) immediately behind eyes, laterally; one pair of occipital setae (**ocs**) posteriorly and mediad eyes; also one pair posterior supernumerary setae (**psus**) laterad and close to posterior supraorbital setae. Antennomere 1 (Fig. 4B, **as1**) with single long seta distally, and row of several semi-erect setae more proximally (Fig. 4B, **as2**, **as3**). Pronotum with two pairs of lateral marginal setae, anterior pair in anterior 1/8, posterior pair at posteriolateral angles. Prosternum with one pair paramedial setae anterioventrally (cf. Fig. 9B). Elytra: each elytron anteriorly with parascutellar seta; lateral setae about 21 (one group anteriorly, one group posteriorly, and single seta medially). Abdominal sterna IV–VI each with one pair of ambulatory setae, sternum VII with one pair of long setae near posterior margin.

*Head, dorsal aspect* (Fig. 1A). Postoccipital suture evident. Frontoclypeal suture present, with frons and clypeus separate. Genal sulcus (Fig. 4A, **gs**) broad, ventral margin sinuous.

Eyes. (Fig. 3A) Small, flat, ommatidia not evident at 50×, or lower magnification.

*Antennae*. (Fig. 4A). Filiform, extended about body length. Antennomere 1 (**a1**) rather slender, slightly longer than antennomeres **a2-a4**; antennomere 2 short, about half length of **a3**; antennomeres 3–11 narrow, cylindrical, distinctly longer than wide.

Mouthparts. As described for Zuphium genus-group, above. Labrum (Fig. 5A).

Rectangular, lateral margins rounded; anterior margin irregularly convex; six dorsal setae, lateral setae longer than four medials. Epipharynx (Fig. 5B).

Mandibles (Figs 6A–6F) Maxillae (Figs 8A–8B). Labium, ventral aspect (Fig. 8C).

*Pronotum* (Fig. 1A). Anterior margin truncate, lateral margins markedly sinuate posteriorly, posteriolateral angles prominent, dentiform, slightly anteriad posterior margin; surface impressions (anterior and posterior transverse and median longitudinal) shallow; lateral grooves and posteriolateral impressions moderately deep.

Pterothorax. Metasternum short; metepisternum quadrate.

*Elytra* (Fig. 1A). Separate, not fused along suture. Each elytron more or less rectangular, but narrowed anteriorly and posteriorly, lateral margin thus distinctly bowed; humerus projected anteriorly, basal ridge sinuate; apical margin truncate, with narrow band of membrane; surface with striae very shallow, intervals almost flat.

Hind wings. Short stubs, brachypterous.

Abdomen. Sternum VII with apical margin truncate.

*Male genitalia* (Figs 10A–10C). Anopic. Phallus narrow (PW/PL 0.238), slightly curved ventrally, narrowed apically, apical margin rounded, apical portion very short (AL/PL 0.063), ostial membrane extensive (OM/PL 0.317). Left paramere (**lp**) conchoid. Right paramere (**rp**) styliform, relatively long (RP/LP 0.317).

*Female genitalia*: *ovipositor* (Figs 11A–11C). Gonocoxite 1 (**gc1**) with patch of long trichoid setae distally on ventral surface. Gonocoxite 2 (**gc2**) short, thick; in lateral aspect falciform, apex pointed, row of four long trichoid setae dorso- and ventro-

laterally; in dorso-ventral aspect, broad, paddle-like, apex deeply notched; dorsal surface glabrous except for dorso-lateral and ventro-lateral trichoid setae; ventral surface toward margins with row of pit pegs (**mpp**), preapical setose organ (**pso**) circuloid, with two furrow pegs (**fp**) and two very short nematiform setae (**ns**).

*Female genital tract* (Fig. 12A). Bursa copulatrix (**bc**) ended in an expanded bulbous anterior extension (**bs**). Common oviduct (**co**) inserted in bursa copulatrix at base of the anterior extension. Spermatheca (**sp**, broken) slender, inserted on bursal sac beside insertion point of spermathecal gland duct (**spgd**); latter with swelling proximad spermathecal gland (**spg**). Without helminthoid sclerite. Without secondary spermathecal gland.

**Collecting notes and habitat.** The specimens from the type locality were collected in the remains of a cloud forest. One was on the ground, in leaf litter; using a Bowie knife, the other was dug out of wet wood of a pine log, near the ground surface. The Monte Alban specimen was likely taken from a dark crevice, "where the temperature was much cooler than outside " (Rolf Aalbu, personal communication). Judging from the structural features of the type material, this species may be troglophilic, but not troglobitic, as are its Brazilian congeners (Gnaspini et al. 1998; Pellegrini and Ferreira 2011b).

**Parasites.** Attached to the holotype was a fungus of the species *Rhachomyces zuphii* Thaxter (Laboulbeniales: Laboulbeniaceae) (determiner of fungus not indicated on label), which is now attached to a pinned rectangular piece of clear plastic by a drop of balsam.

**Geographical distribution** (Map 1). This species is known only from two montane localities in the Mexican state of Oaxaca.

Material examined. Type specimens, only. For label details, see above.

#### Zuphioides, gen. n.

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Zuphium of authors; (not Latreille 1805). Dejean 1831: 298. Chaudoir 1863: 313. Chaudoir 1872: 103. Putzeys 1878: 55. LeConte 1879: 62. Horn 1881: 149. LeConte and Horn 1883: 141. Bates 1883: 166. Bates 1891: 266. Blatchley 1910:139, 140. Schaeffer 1910: 396. Leng 1920: 65. Csiki 1932: 1562. Liebke 1933: 461. Darlington 1934: 128. Darlington 1935: 213. Blackwelder 1944: 70. Hatch 1953: 150. Ball 1960: 162. Reichardt 1977: 449. Lindroth 1969: 1089. Erwin 1979a: 360. Erwin 1979b: 564. Kirk 1969: 17. Erwin 1981: 189, 205. Mateu 1981: 111. Erwin 1991: 42. Mateu 1993: 490. Bousquet and Larochelle 1993: 283. Downie and Arnett 1996: 193. Lorenz 1998: 479 (in part). Peck and Thomas 1998: 25. Ciegler 2000: 127. Ball and Shpeley 2000: 397. Ball and Bousquet 2001: 40, 61, 115. Larochelle and Lariviére 2003: 514 515. Lorenz 2005: 506 (in part). Erwin et al. 2012: 32. Bousquet 2012: 1355.

Type species. Zuphium mexicanum Chaudoir, 1863 (here designated).

Generic name. A compound Latinized noun, treated as neuter, from the generic name *Zuphium* and *oides*, resembling; hence meaning "resembling *Zuphium*".

**Recognition.** With character states of Western Hemisphere *Zuphium* genus-group, restricted as follows: size small, pronotum and dorsal surface of elytra densely setose, setae decumbent, body depressed, integument piceous to rufotestaceous, head capsule posteriorly relatively broad, laterally broadly rounded, antennae elongate, antennomere 1 as long or longer than antennomeres 2–4. Humeri broadly rounded. Metasternum long, metepisternum longer than wide at base. Macropterous. Male genitalia: phallus without dorsal paraostial sclerites (Fig. 10D–10F; cf. Fig. 13A–C, **ps**). Ovipositor: Gonocoxite 2 (Figs 11D–F; cf. Figs 11A–C, **gc2**) short, thick; in lateral aspect falciform, apex pointed; in dorso-ventral aspect, broad, paddle-like, apex broadly rounded, not notched. Female genital tract: without secondary spermathecal gland (Fig. 12B; cf. Fig. 13D, **ssg**).

Description. None required here. See description of Z. mexicanum, below.

**Habitat.** Members of this genus are mesophilous to hygrophilous, occupying wet meadows and flood plains principally in open sites, but also in shaded areas along streams, in tropical gallery forest. For more details, see Erwin (1991: 42), Erwin et al. (2012: 32), and Larochelle and Lariviere (2003: 514–515). Ball and Shpeley (2000: 397) mistakenly classified *Zuphium* as "xerophilous", in part.

#### Zuphioides mexicanum (Chaudoir)

http://species-id.net/wiki/Zuphioides\_mexicanum Figs 1B, 2B, 3B, 4C, 4D, 5C, 5D, 7A–7F, 8D–8F, 9A, 10D–10F, 11D–11F, 12B.

*Zuphium mexicanum* Chaudoir, 1862: 314. Bates 1883: 166. Mateu 1981: 125. *Zuphium vicinum* Liebke, 1933: 471. Mroczkowski 1960: 400. Mateu 1985: 329.

**Type material.** For *Z. mexicanum*, two females, Oberthür-Chaudoir Collection, in front of the following box label: "mexicanum/ Chaud/ Mexique/ 57. Sallé". LECTOTYPE female, labeled as follows: "33"/ "Ex Musaeo/Chaudoir" [red print]; "Lecto/type" [circular, ringed with red]; in Museum National d'Histoire Naturelle, Paris; PARALECTOTYPE labeled " Ex Musaeo/Chaudoir" [red print]. Designated by Mateu (1985: 330). For *Z. vicinum*, holotype, in Institute of Zoology, Polish Academy of Sciences, Warsawa, #1711.

**Type area.** Indicated by Mateu (1985: 330) as Veracruz, for *Z. mexicanum*; for *Z. vicinum*, "Mexico".

**Specific epithet.** A Latinized eponym, nominative case, based on the name of the country in which the type locality is located.

**Description.** *Size and proportions.* Small, OBL 4.88–4.96 mm; EW 1.60–1.62 mm A1L/A2–4L 0.90–1.00; HW/PW 0.92–0.97. (For seven species of *Zuphioides:* OBL 4.64–6.586 mm; EW 1.60–1.84 mm A1L/A2–4L 0.88–1.12; HW/PW 0.91–0.97).

Color. Body rufotestaceous; appendages slightly paler; vestiture testaceous.

*Habitus* (Fig. 1B). Flat, overall. Head capsule (dorsal aspect, Fig. 2B) trapezoidal, obtusely angulate posteriolaterally.

*Eyes* (Figs 3B). Convex, easily seen, ommatidia clearly evident at 50× (about 16 ommatidia are crossed on a horizontal diameter).

Pronotum. Narrow.

Elytra (Fig. 1B). Lateral margins straight, not bowed, parallel to one another.

*Microsculpture.* Dorsal surface of head capsule (including clypeus) and pronotum smooth, microlines not evident; labrum and elytra with microlines fine (not easily seen at 50× or lower magnification), mesh pattern isodiametric. Ventral surface with microlines fine, generally transverse.

Luster. Dorsal surface of head and pronotum shiny, elytra somewhat duller.

*Body vestiture and punctation.* Dorsal surface of head and pronotum sparsely punctate, vestiture sparse. Elytra and abdominal sterna III-VII with punctation and vestiture dense, vestitural setae decumbent.

*Fixed setae.* Head (Fig. 4C): clypeus one pair; head capsule with anterior pair of supraorbitals (**asos**) above eyes; posterior pair of supraorbitals (**psos**) posteriad eyes; one pair of postocular setae (**pos**) immediately behind eyes, laterally; one pair of occipital setae (**ocs**) posteriorly and mediad eyes; posterior supernumerary setae lacking. Antennomere 1 (Figs 4B, 4D, **as1**) with single long seta distally, in addition to decumbent vestiture and row of small erect setae (**as2**, **as3**). Pronotum with two pairs of lateral marginal setae, anterior pair in anterior 1/8, posterior pair at posteriolateral angles. Prosternum with one pair setae anterioventrally (Fig. 9B). Elytra: each elytron anteriorly with parascutellar seta; lateral setae about 21 (one group anteriorly, one group posteriorly, and single seta medially. Abdominal sterna IV-VI without evident ambulatory setae, sternum VII with pair of long setae near posterior margin.

*Head, dorsal aspect* (Fig. 4C). Occiput posteriorly and postocciput markedly constricted, in form of narrow neck, postoccipital suture evident. Frontoclypeal suture present. Genal sulcus (**gs**) prominent, but less so than in *Coarazuphium* (cf. Fig. 4A).

*Eyes* (Fig. 4B). Macrophthalmous, markedly convex, readily seen, ommatidia evident at 50×, or lower magnification.

*Antennae* (Fig. 4D). Antennae filiform, extended about body length. Antennomere 1 rather slender, slightly longer than antennomeres a2-a4; antennomere 2 short, about half length of a3; antennomeres 3-11 narrow, cylindrical, distinctly longer than wide.

*Mouthparts.* As described for *Zuphium* genus-group, above. *Labrum* (Fig.5C). Anterior margin concave. Epipharynx (Fig. 5D). *Mandibles* (Figs 7A–7F). *Maxillae* (Figs 8D, 8E). *Labium, ventral aspect* (Fig. 8F).

*Pronotum* (Fig. 1B). Anterior margin truncate, lateral margins markedly sinuate posteriorly, posteriolateral angles prominent, dentiform, slightly anteriad posterior margin; surface impressions (anterior and posterior transverse and median longitudinal) shallow; lateral grooves and posteriolateral impressions moderately deep.



**Figure 10.** Digital images of male genitalia. **A–C** *Coarazuphium whiteheadi*, new species **D–F** *Zuphioides mexicanum* (Chaudoir). **A**, **D** left lateral aspect **B**, **E** dorsal aspect **C**, **F** right lateral aspect. Legend: **ap**, apical portion of phallus; **APL** length of apical portion; **bb**, basal bulb of phallus; **emf**, endophallic terminal microtrichial field; **lp**, left paramere; **LPL**, left paramere length; **om**, ostial membrane; **OML**, periostial membrane length; **pj**, parameral juxta; **PL**, phallus length; **PW**, phallus width; **rp**, right paramere; **RPL**, right paramere length; **s**, shaft of phallus. Scale bars = 0.5 mm.



**Figure 11.** SEM micrographs of female ovipositor sclerites. **A–C** *Coarazuphium whiteheadi*, new species **D–F** *Zuphioides mexicanum* (Chaudoir). **A**, **D** left lateral aspect **B**, **E** dorsal aspect **C**, **F** ventral aspect. Legend: **fp**, furrow peg; **gc1**, gonocoxite 1; **gc2**, gonocoxite 2; **mpp**, marginal pit pegs; **ns**, nematiform seta; **pso**, preapical setose organ; **ts**, trichoid seta. Scale bars = 100 μm.



**Figure 12.** Line drawings of female reproductive tract, ventral aspect. **A** *Coarazuphium whiteheadi*, new species **B** *Zuphioides mexicanum* (Chaudoir). Legend: **bc**, bursa copulatrix; **bl**, base of laterotergite; **bs**, bursal sacculus; **bgc1**, base of gonocoxite 1; **co**, common oviduct; **hs**, helminthoid sclerite; **sp**, spermatheca; **spg**, spermathecal gland; **spgd**, spermathecal gland duct. Scale bar = 0.25 mm.

*Pterothorax.* Metasternum of average length; metepisternum elongate, lateral margin longer than anterior margin.

*Elytra* (Fig. 1B). Separate from one another, not fused along suture. Each elytron more or less rectangular, lateral margin straight; humerus projected anteriorly, basal ridge straight; apical margin truncate, with narrow band of membrane. Surface with striae very shallow, intervals almost flat.

Hind wings. Macropterous; wings folded beneath elytra at rest.

Legs. Male fore-tarsus (Fig. 9A).

Abdomen. As described for Western Hemisphere Zuphium genus-group.

*Male genitalia* (Figs 10D–10F). Phallus narrow (PW/PL 0.254), slightly curved ventrally, narrowed apically, apical margin rounded, apical portion very short (AL/ PL 0.036), ostial membrane extensive (OM/PL 0.317). Left paramere (**lp**) conchoid. Right paramere (**rp**) short, ovoid in form (RP/LP 0.317).

*Female genitalia*: *ovipositor* (Figs 11D–11F). Gonocoxite 1 (**gc1**) with patch of long trichoid setae distally on ventral surface. Gonocoxite 2 (**gc2**) short, thick; in lateral aspect falciform, apex pointed, row of four long trichoid setae dorso- and ventro-laterally; in dorso-ventral aspect, broad, paddle-like, apex broadly rounded, not notched; otherwise, as described for Western Hemisphere *Zuphium* genus-group.

*Female genital tract* (Fig. 12B). Bursa copulatrix (**bc**) bulbous (collapsed in Fig. 12B), inserted at base of common oviduct (**co**). Spermatheca (**sp**) inserted near junction of common oviduct and bursa copulatrix, markedly elongate and slender, with helminthoid sclerite (**hs**) at base. Spermathecal gland duct inserted near base of spermatheca, with distinct swelling proximad spermathecal gland (**spg**). Without secondary spermathecal gland.

**Geographical distribution.** This species ranges from northern Mexico (states of Nuevo Leon, Nayarit, and Tamaulipas) northward to southwestern U.S.A (states of Arizona, New Mexico, and Texas; Bousquet (2012: 1357).





**Figure 13.** Digital images of male genitalia and line drawing of female reproductive tract of *Zuphium ustum* Klug. **A–C** male genitalia, left lateral, dorsal and right lateral aspects, respectively **D** female reproductive tract, ventral aspect. Legend: **ap**, apical portion of phallus; **bb**, basal bulb of phallus; **bc**, bursa copulatrix; **bl**, base of laterotergite; **bgc1**, base of gonocoxite 1; **co**, common oviduct; **lp**, left paramere; **rp**, right paramere; **om**, ostial membrane; **pj**, parameral juxta; **ps**, paraostial sclerites; **rp**, right paramere; **s**, shaft of phallus; **smf**, spiny microtrichial field; **sp**, spermatheca; **spg**, spermathecal gland; **ssg**, secondary spermathecal gland. Scale bars: **A–C** = 1 mm; **D** = 0.5 mm.



**Map 1.** Outline map of southern North America, Middle America and northern South America, showing generalized geographical range of species of *Coarazuphium*. **A** *Coarazuphium whiteheadi*, new species **B** *Coarazuphium bezerra*, *cessaima*, *formoso*, *pains*, *tapiaguassu* and *tessai*.

# **Concluding remarks**

Treatment of the Western Hemisphere zuphiines remains incomplete. The large geographical gap between the Brazilian and Mexican species of *Coarazuphium* causes one to wonder just how large the gap is. Surely, considering that this genus is likely to be an old (early Tertiary) resident of the Middle American highlands, we can expect therein additional species.

At hemisphere level, *Zuphioides* must be reviewed in detail, using as the principal basis the work of Joaquin Mateu (1981). No doubt, many more species remain to be discovered and described. From a World perspective, a generic-subtribal revision of the Zuphiini is required, based primarily on structural details of the male and female reproductive organs.

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# Appendix I

# Tribe Zuphiini in the Western Hemisphere

#### Nature of the Zuphiini

Based on morphological features, most authors have treated the Zuphiini as a "natural" group, meaning in phylogenetic parlance that it is essentially monophyletic. But a molecular analysis, based on two nuclear genes (28S rDNA and *wingless*) conducted by Ober and Maddison (2008: 8, fig. 2) indicated otherwise, namely that this tribe is polyphyletic, its constituent genera having emerged on four different lines, though all of these are within the supertribe Zuphiitae. However, the Ober/Maddison sample included only a small part of zuphiine diversity, and sequences from only two genes. The limited taxon sampling is especially problematic given the large divergences between DNA sequences within Zuphiitae. In addition, the bootstrap values for this result were low (Ober and Maddison 2008: 6, fig. 1). We choose to continue treating the tribe as a practical taxonomic unit, with the hope that a more extensive sample of both zuphiite taxa and genes will give a different result, namely confirming the monophyly of the morphologically based Zuphiini.

# Recognition

Specimens of this tribe are small in size (overall length less than 7.00 mm), integument various (nearly white, testaceous to piceous), and generally pilose, head more or less constricted posteriorly, antennomere 1 (scape) elongate more than length of antennomeres 2+3 and wider than antennomeres 2-11; elytra with apical margin truncate, or subtruncate-sinuate, with a narrow membranous fringe; elytral interval 3 without fixed setae; tibiae without prominent spines. For a more detailed characterization of Zuphiini, see Jeannel (1942: 1091–1092).

# Geographical distribution

The range of this tribe in the Western Hemisphere is co-extensive with the range of the *Zuphium* genus-group, as noted above.

# Classification

Based on previous studies, Reichardt (1977: 448) included five Western Hemisphere genera in the tribe Zuphiini, which he arranged in two subtribes: Zuphiina (*Zuphium* Latreille, 1805) and Patriziina (*Pseudaptinus* Laporte de Castelnau, 1835; *Thalpius* Le-Conte, 1851; *Mischocephalus* Chaudoir, 1862; and *Metaxidius* Chaudoir, 1852). A

third subtribe (Leleupidiina), with genera known only from the Old World tropics, was recognized but not treated further. To these, Mateu (1992) added the monogeneric Mischocephalina, placing therein the Neotropical *Mischocephalus* Chaudoir. Previously, Mateu (1982) had added the Neotropical monobasic genus *Chaudoirella* Mateu, including it in the Patriziina.

Lorenz (2005: 504–507) recognized only three subtribes, namely Leleupidiina, Dicrodontina, (a monogeneric group proposed by Machado (1992: 569)), and Zuphiina, including in the last-named the Zuphiina of authors, Patriziina, and Mischocephalina. We accept provisionally the Lorenz proposal of three subtribes, but recognize genusgroups within the Zuphiina. For the Western Hemisphere, we recognize the *Zuphium* genus-group, the *Patrizia* genus-group (the latter name proposed by Alluaud 1931, a junior synonym of *Agastus* Schmidt-Goebel, 1846), and, the *Mischocephalus* genusgroup. Based on marked similarities in the female genitalia, we place *Chaudoirella* (Mateu 1992: 196, fig. 1H; cf. fig. 199, fig. 3A) in the *Mischocephalus* genus-group. We note also that the marked divergence in structure of the male genitalia and female ovipositor between the members of *Agastus* and those of *Pseudaptinus* + *Thalpius* suggest that the latter might best be placed in a supraspecific group of their own.

The following key distinguishes among the Western Hemisphere genus-groups and genera of Zuphiini. Note that among other recent authors, Messer (2011: 419-424) and Bousquet (2012: 1359) treat *Thalpius* and *Pseudaptinus* as subgenera of genus *Pseudaptinus*. Messer (2011) provides a key to the Mexican-USA species of *Thalpius*.

Martinez (2005: 426–434, figs 3.132–3.136) provides a habitus illustration for the following genera: *Metaxidius*, fig. 3.132; *Mischocephalus*, fig. 3.133; *Pseudaptinus*, fig. 3.134; *Thalpius*, fig. 3.135; and *Zuphium (auct.)*, fig. 3.136. A habitus illustration of *Chaudoirella* is found with the original description (Mateu 1982: 48). Habitus illustrations of each of the Brazilian species of *Coarazuphium* are found with their respective original descriptions.

# Key to Western Hemisphere genera of Tribe Zuphiini (modified from Reichardt (1977: 444) and Mateu (1992: 195–196))

1	Maxillary and labial palpi similar to one another in size and proportions;
	maxillary palpomere 4 (Figs 8A-8F) not markedly enlarged. Head posteriorly
	markedly constricted as a narrow neck (Figs 1A, 1B). Male fore-tarsomeres
	1-3 with adhesive vestiture pad-like, not biseriate (Fig. 9A) or with a single
	row of squamo-setae
1'	Maxillary palpi much larger than labial palpi; maxillary palpomere 4 mark-
	edly enlarged. Head posteriorly less constricted. Male fore-tarsomeres 1-3
	with adhesive vestiture biseriate, or a single row of squamo-setae
2(1')	Eyes absent or flat, ommatidia not evident. Metepisternum about quadrate.
	Elytra with humeri markedly constricted, lateral margins broadly rounded
	(Fig. 1A) Coarazuphium Gnaspini, Vanin & Godoy

2'	Eyes convex, normal size, ommatidia evident. Metepisternum elongate, long-
	er than wide at base. Elytra with numeri broadly rounded, lateral margins straight parallel to one another (Fig. 1B)
3(2')	Head posteriorly constricted as very narrow neck (more or less as wide as di-
0(-)	ameter of one eve (cf. Fig. 1A)). Gonocoxite 2 of ovipositor without ensiform
	setae, with slender setae on dorsal margin, near base (Mateu 1992: 196, fig.
	1I; 199, fig. 3A) Mischocephalus genus-group, 4
3'	Head posteriorly with neck thick (wider than diameter of one eye). Gonocox-
	ite 2 with broad ensiform setae (Mateu 1992: 201, figs 4A, 4B)
	Patrizia genus-group, 5
4(3)	Elytron with lateral margin straight for most of length. Pronotal posteriolat-
	eral angles long, very prominent spines. Labium with submentum and men-
	tum fused (Mateu, 1992: fig. 1G) Mischocephalus Chaudoir
4'	Elytron with lateral margin broadly rounded. Pronotal posteriolateral angles
	short spines. Labium with submentum and mentum separated by a suture
	<i>Chaudoirella</i> Mateu
5(3')	Pronotal posteriolateral angles not spined
	<i>Pseudaptinus</i> Laporte de Castelnau
5'	Pronotal posteriolateral angles each a small, sharp spine6
6(5')	Antennal socket limited above and below by a sharp carina, inferior carina
	better developed than superior one (clearly visible from above). Antennomere
	1 relatively shorter (slightly shorter than antennomeres 2-4). Pronotum as
	long as wide; anterior angles more or less sharp. Integument generally gla-
	brous
6'	Antennal socket with superior and inferior carinae equally developed. Anten-
	nomere 1 relatively longer (as long or longer than antennomeres 2-4). Prono-
	tum longer than wide. Pronotum and elytra setose

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DATA PAPER



# Circumpolar dataset of sequenced specimens of Promachocrinus kerguelensis (Echinodermata, Crinoidea)

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# Abstract

This circumpolar dataset of the comatulid (Echinodermata: Crinoidea) *Promachocrinus kerguelensis* (Carpenter, 1888) from the Southern Ocean, documents biodiversity associated with the specimens sequenced in Hemery et al. (2012). The aim of Hemery et al. (2012) paper was to use phylogeographic and phylogenetic tools to assess the genetic diversity, demographic history and evolutionary relationships of this very common and abundant comatulid, in the context of the glacial history of the Antarctic and Sub-Antarctic shelves (Thatje et al. 2005, 2008). Over one thousand three hundred specimens (1307) used in this study were collected during seventeen cruises from 1996 to 2010, in eight regions of the Southern Ocean: Kerguelen Plateau, Davis Sea, Dumont d'Urville Sea, Ross Sea, Amundsen Sea, West Antarctic Peninsula, East Weddell Sea and Scotia Arc including the tip of the Antarctic Peninsula and the Bransfield Strait. We give here the metadata of this dataset, which lists sampling sources (cruise ID, ship name, sampling date, sampling gear), sampling sites (station, geographic coordinates, depth) and genetic data (phylogroup, haplotype, sequence ID) for each of the 1307 specimens. The identification of the specimens was controlled by an expert taxonomist specialist of crinoids (Marc Eléaume, Muséum national d'Histoire naturelle, Paris) and all the COI sequences were matched against those available on the Barcode of Life Data System (BOLD: http://www.boldsystems.org/index.php/

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IDS\_OpenIdEngine). This dataset can be used by studies dealing with, among other interests, Antarctic and/ or crinoid diversity (species richness, distribution patterns), biogeography or habitat / ecological niche modeling. This dataset is accessible through the GBIF network at http://ipt.biodiversity.aq/resource.do?r=proke.

#### **Keywords**

Antarctica, Crinoidea, Cytochrome Oxydase subunit I, Echinodermata, Phylogeography, *Promachocrinus*, Southern Ocean, Sub-Antarctic

# **Project details**

**Project title:** Comprehensive sampling reveals circumpolarity and sympatry in seven mitochondrial lineages of the Southern Ocean crinoid species *Promachocrinus kerguelensis* (Echinodermata)

Personnel: Lenaïg G. Hemery

**Funding:** French ANR ANTFLOCKS (n° 07-BLAN-0213-01); MNHN Paris intern grants (DMPA's BQR, ATMs "Biominéralisation"; "Biodiversité actuelle et fossile; crises, stress, restaurations et panchronisme: le message systématique"; "Taxonomie moléculaire: DNA Barcode et gestion durable des collections").

**Study area descriptions/descriptor:** The 1307 specimens in this dataset were collected from the Southern Ocean, south of the Sub-Antarctic Front (SAF): Kerguelen Plateau (Kerguelen and Heard islands), Davis Sea, Dumont d'Urville Sea, Ross Sea, Amundsen Sea, West Antarctic Peninsula, East Weddell Sea and Scotia Arc (from the tip of the Antarctic Peninsula and the Bransfield Strait to the South Georgia island). The bathymetric range extended from 65 to 1162 meters deep.

Design description: This dataset was gathered to conduct a circumpolar phylogeographic study of the crinoid species Promachocrinus kerguelensis (Hemery et al. 2012) and designed to spatially improve the sampling of Wilson et al. (2007), which was limited to the Atlantic sector of the Southern Ocean. The aim of Hemery et al. (2012) was to test the circumpolarity of the genetic lineages of Wilson et al. (2007), and to test whether these lineages represented an under-sampling artifact of a large and genetically diverse metapopulation or whether they were truly representative of the Southern Ocean. The authors used a sampling strategy designed to cover the broadest possible genetic variation and to explore the evolutionary relationships among the seven lineages, in order to be able to conduct population analyses (Meyer and Paulay 2005). They also wanted to understand the distributional limits of each phylogroup in *Promachocrinus kerguelensis* to assess the connectivity displayed throughout their range, and to test the "multiple refugia" theory by studying the demographic history of each phylogroup. For this purpose, more than two thousand specimens, sampled during the most recent Antarctic cruises focused on benthic biodiversity and fixed and preserved in a way allowing for DNA extraction and amplification (fixed in ethanol or frozen), were provided by several taxonomists and benthologists from different institutions. Specimen identifications during the sampling cruises were conducted to a higher level allowed by the taxonomic skills of the collectors then checked principally at the Muséum national d'Histoire naturelle, Paris by taxonomists trained to deal with Ant-



**Figure 1.** Synoptic of the procedure used to generate the dataset. Yellow exclamatory marks show where the data quality has been checked.

arctic crinoids. The Cytochrome c Oxydase subunit I (COI) was successfully sequenced for 1307 of these specimens. Both collection data and produced sequences were digitized in appropriate databases, used or ready to be used for publishing purpose (Figure 1).

**Data published through GBIF:** http://ipt.biodiversity.aq/resource.do?r=proke as an Excel spreadsheet of the dataset, available through the Darwin Core Archive format at http://ipt.biodiversity.aq/archive.do?r=proke.

# Taxonomic coverage

**General taxonomic coverage description:** This dataset focuses on the Antarctic comatulid species *Promachocrinus kerguelensis* (Carpenter 1888), the most abundant and morphologically variable comatulid species in the Southern Ocean (Speel and Dearborn 1983). It corresponds to the 1307 specimens sequenced in Hemery et al. (2012).

# Taxonomic ranks

Phylum: Echinodermata Class: Crinoidea Order: Comatulida Family: Antedonidae Subfamily: Heliometrinae Genus: Promachocrinus Species: kerguelensis Common names: echinoderm, crinoid, comatulid, feather star

# Spatial coverage

# General spatial coverage

The specimens of *Promachocrinus kerguelensis* gathered in this dataset were collected from most of the strategic regions in the Southern Ocean (triangles in Figure 2): the Antarctic continental shelf (East Weddell Sea, Davis Sea, Dumont d'Urville Sea, Ross Sea, Amundsen Sea, West Antarctic Peninsula), the Scotia Arc islands (South Shetland, South Orkney and South Sandwich) and the Sub-Antarctic islands (South Georgia, Kerguelen and Heard). Specimens were sampled at depths ranging from 65 m to 1162 m. This covers most of the known distribution area of this species (black circles in Figure 2), but only a portion of the bathymetric range for this species, which extends from 10 m to 2100 m (Speel and Deardorn 1983).

# Coordinates

76°49'58.8"S and 47°12'14.4"S Latitude; 107°24'28.8"W and 170°23'6"E Longitude.



**Figure 2.** *Promachocrinus kerguelensis* sampling stations in the Southern Ocean. Triangles represent the sampled stations, circles represent the bibliographic data, numbers are sequenced specimens per region (modified from Hemery et al. 2012).

# General temporal coverage

The specimens were collected during one to four different cruises per sampling region for a total of 17 cruises from 1996 to 2010 (Figure 3). However, the number of specimens was too variable among cruises to be statistically compared (see details of numbers in the Methods part).

Temporal coverage: January 26, 1996 – March 16, 1996 Temporal coverage: March 18, 2000 – May 11, 2000 Temporal coverage: January 23, 2002 – May 5, 2002 Temporal coverage: November 17, 2003 – January 19, 2004 Temporal coverage: January 15, 2004 – March 15, 2004 Temporal coverage: February 9, 2004 – February 22, 2004 Temporal coverage: January 20, 2005 – April 7, 2005 Temporal coverage: February 27, 2006 – April 11, 2006 Temporal coverage: December 16, 2007 – January 27, 2008 Temporal coverage: February 18, 2008 – April 11, 2008 Temporal coverage: February 31, 2008 – March 16, 2008 Temporal coverage: February 6, 2009 – March 12, 2009 Temporal coverage: December 1, 2009 – December 11, 2009 Temporal coverage: December 29, 2009 – January 8, 2010 Temporal coverage: August 28, 2010 – September 28, 2010



**Figure 3.** Proportion of specimens per phylogroup per year and sampling region. Acronyms of regions are given in Figure 2, A - F are the names of the 7 COI haplogroups found in Hemery et al. 2012.

# Natural collections description

Parent collection identifier: Muséum national d'Histoire naturelle, Paris (MNHN) Collection name: Echinodermes (MNHN-IE) Collection identifier: Marc Eléaume Specimen preservation method: Alcohol

# **Methods**

**Method step description:** See sampling description below and graphic summary in Figure 1.

Study extent description: The specimens were collected during 17 cruises in the Southern Ocean, from 1996 to 2010 (Figures 2 and 3): 53 specimens from EASIZ I (ANT XIII/3) onboard the RV Polarstern (1996) in the East Weddell Sea, 53 from EASIZ III (ANT XVII/3) onboard the RV Polarstern (2000) in the East Weddell Sea and the Scotia Arc, 12 from ANDEEP I&II (ANT XIX/3&4) and 25 from LAMPOS (ANT XIX/5) onboard the RV Polarstern (2002) in the Scotia Arc, 43 from BEN-DEX (ANT XXI/2) onboard the RV Polarstern (2003-2004) in the East Weddell Sea, 15 from ITALICA 2004 onboard the RV Italica (2004) in the Ross Sea, 106 from TAN0402 onboard the RV Tangaroa (2004) in the Ross Sea, 12 from ANDEEP III (ANT XXII/3) onboard the RV Polarstern (2005) in the East Weddell Sea, 14 from BIOPEARL I (JR144) onboard the RV James Clark Ross (2006) in the Scotia Arc, 418 from CEAMARC (2007/08 V3) onboard the RV Aurora Australis (2007-2008) in the Dumont d'Urville Sea, 17 from BIOPEARL II (JR179) onboard the RV James Clark Ross (2008) in the Amundsen Sea, 2 from HIMI-SC50 onboard the FV Southern Champion (2008) on the Kerguelen Plateau (Heard island), 148 from TAN0802 onboard the RV Tangaroa (2008) in the Ross Sea, 68 from AMLR 2009 Leg II onboard the RV Yuzhmorgeologiya (2009) in the Scotia Arc and the West Antarctic Peninsula, 26 from BASWAP (JR230) onboard the RV James Clark Ross (2009) in the West Antarctic Peninsula, 184 from BR09 onboard the RV Aurora Australis (2009-2010) in the Davis Sea, and 111 from POKER II onboard the FV Austral (2010) on the Kerguelen Plateau (Kerguelen island).

**Sampling description:** The specimens were sampled using several sampling gears, depending on the cruise: agassiz trawls, beam trawls, bottom trawls, box corers, epibenthic sledges (Arntz and Brey 2001, 2003, 2005; Arntz and Gutt 1997; Beaman and O'Brien 2009; Duhamel et al. 2011; Fahrbach 2006; Fütterer et al. 2003; Lockhart et al. 2009). During each cruise, specimens were sorted onboard and then fixed and preserved in 70–95% ethanol or first frozen and subsequently preserved in ethanol. The specimens were curated by each institution once back from the field and digitized in their own databases before the specimens were gathered by the authors in the purpose of the molecular study. Metadata associated with each specimen were extracted from the

cruise reports. The molecular data (barcoding) were generated following the protocols described in Ivanova et al. (2006), Eléaume et al. (2011) and Hemery et al. (2012).

Quality control description: The initial geo-referencing was done by means of the vessel onboard GPS systems. Samples identification was supervised and checked by Marc Eléaume, crinoid taxonomist at the Muséum national d'Histoire naturelle, Paris, following Clark and Clark (1967) taxonomic description of the species, and matched to the World Register of Marine Species (WoRMS). The barcoding was done by Lenaïg G. Hemery at the Muséum national d'Histoire naturelle, Paris, and by the Canadian Center for DNA Barcoding, Toronto, and the Scripps Institution of Oceanography, San Diego, and matched to sequences already available on the Barcode of Life Data System (BOLD: http://www.boldsystems.org/index.php/IDS\_OpenIdEngine). All sequences, specimen occurrences and identifications are linked together through unique numbers in BOLD under the public project name PROKE.

# Datasets

**Dataset description:** This dataset has been generated for a molecular study of the Antarctic comatulid species *Promachocrinus kerguelensis*, improving the geographic coverage of the previous study by Wilson et al. (2007). All the specimens are identified by several types of numbers that are linked together: Sample ID (characteristic of each individual), BOLD ID, GenBank ID and SeqID (all three characteristic of each sequence in different databases), Field Number (when available) and Museum ID. In some cases, the two last identifiers are shared by several individuals identifiable from each other by their own Sample ID. The dataset also includes the name of the institution storing the specimens, the complete taxonomy, names of identifiers and collectors, and information on the sampling itself: cruise names, vessel names, sampling gears, dates, regions, sectors, exact sites (when available), station numbers, latitudes and longitudes in decimal degrees, and depths in meters. This dataset is suitable to be used in studies dealing with, for example, Antarctic and/or crinoid diversity (species richness, distribution patterns), biogeography or habitat / ecological niche modeling.

**Object name:** Darwin Core Archive Circumpolar dataset of sequenced specimens of *Promachocrinus kerguelensis* (Echinodermata, Crinoidea)

Character encoding: UTF-8 Format name: Darwin Core Archive format Format version: 1.0 Distribution: http://ipt.biodiversity.aq/archive.do?r=proke Publication date of data: 2012-03-01 Language: English Metadata language: English Date of metadata creation: 2012-04-25 Hierarchy level: Dataset

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# Appendix

Sampling data associated to the 1307 specimens of *Promachocrinus kerguelensis* sequenced in Hemery et al. (2012). (doi: 10.3897/zookeys.315.5673.app) File format: Comma Separated Value (csv).

**Explanation note:** Each sample is associated to three unique sequence IDs (BOLD ID, GenBank ID and SeqID); Haplotype and Clade refer back to the genetic data from Hemery et al. (2012); Latitude and Longitude are given in decimal degrees; Depth is given in meters.

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**Citation:** Hemery LG, Améziane N, Eléaume M (2013) Circumpolar dataset of sequenced specimens of *Promachocrinus kerguelensis* (Echinodermata, Crinoidea). ZooKeys 315: 55–64. doi: 10.3897/zookeys.315.5673 Sampling data associated to the 1307 specimens of Promachocrinus kerguelensis sequenced in Hemery et al. (2012). doi: 10.3897/zookeys.315.5673.app

RESEARCH ARTICLE



# Pareiorhina hyptiorhachis, a new catfish species from Rio Paraíba do Sul basin, southeastern Brazil (Siluriformes, Loricariidae)

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# Abstract

*Pareiorhina hyptiorhachis* is described from Ribeirão Fernandes and Rio Pomba, Rio Paraíba do Sul basin, Brazil. The new species is distinguished from its congeners (*P. brachyrhyncha, P. carrancas, P. cepta*, and *P. rudolphi*) by the presence of a conspicuous ridge on the trunk posterior to the dorsal fin (postdorsal ridge), simple teeth, a completely naked abdomen, a round dorsal profile of the head, greater suborbital depth and greater head width. We discuss the distributional pattern of the new species and its congeners and hypothesize that headwater capture is responsible for the distribution of *Pareiorhina* species across different watersheds in southeastern of Brazil.

# Resumo

*Pareiorhina hyptiorhachis* é descrita do ribeirão Fernandes e do rio Pomba, bacia do rio Paraíba do Sul, Brasil. A nova espécie é distinguida de suas congêneres (*P. brachyrhyncha, P. carrancas, P. cepta*, e *P. rudolphi*) por caracteres relacionados a presença de uma quilha conspícua na superficie pós-dorsal do tronco, dentes simples, abdomen completamente nú, perfil dorsal da cabeça arredondado, altura do sub-orbital e largura da cabeça. Nós discutido o padrão de distribuição da nova espécie e de seus congêneres e a hipótese de que a captura de cabeceira é responsável pela distribuição das espécies de *Pareiorhina* através das diferentes bacias hidrográficas do sudeste do Brasil.

#### Keywords

Cascudinhos, Taxonomy, Freshwater, Neoplecostominae, Neotropical Region

#### **Palavras chaves**

Cascudinhos, Taxonomia, Água Doce, Neoplecostominae, Região Neotropical

#### Introduction

The genus *Pareiorhina* was proposed by Gosline (1947) to include *Rhinelepis rudolphi* Miranda-Ribeiro, 1911 and is currently included in the subfamily Neoplecostominae (sensu Chiachio et al. 2008; Roxo et al. 2012a, 2012b). Recently, three new species have been described: *P. carrancas* by Bockmann and Ribeiro (2003); *P. brachyrhyncha* by Chamon et al. (2005); and *P. cepta* by Roxo et al. (2012c). In their description of *P. carrancas*, Bockmann and Ribeiro (2003) proposed a combination of characters to separate *Pareiorhina* from other genera of Loricariidae: the lateral borders of the head lacking hypertrophied odontodes; unicuspid teeth; a naked abdomen; dorsal plates meeting along the mid-dorsal line between the dorsal and caudal fins; adipose fin absent; ventral plates covering the mid-ventral line behind the anal-fin base; and the dorsal portion of the body behind the dorsal fin flattened. However, no exclusive synapomorphies were presented to diagnose the genus. Recently, the molecular studies of Cramer et al. (2011) and Roxo et al. (2012a, 2012b) have found that *Pareiorhina* may not be monophyletic.

An examination of the fish collections at the Laboratório de Biologia e Genética de Peixes de Botucatu (LBP) and Museu de Ciências e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul (MCP) revealed the existence of an undescribed *Pareiorhina* species from the Rio Paraíba do Sul basin, Brazil. This new species is formally described herein.

# Material and methods

All measurements were taken from point to point to the nearest 0.1 mm using digital calipers (except the postdorsal ridge depth, which was measured using a stereomicroscope and analyzed using the software Axio Vision Release 4.8.2). Counts were taken from the left side when possible. In the description, counts are followed by their frequencies in parentheses. The measurements followed Bockman and Ribeiro (2003), except for the folded dorsal-fin length and the snout-opercle length that were not included in that publication. We added the following measurements from Carvalho and Reis (2009): mandibular ramus, suborbital depth and unbranched anal-fin ray length. We also added the measurement of postdorsal ridge depth (from the base of the postdorsal ridge to its upper portion). Osteology was performed on specimens cleared and double-stained (c&s) according to the procedures of Taylor and Van Dyke (1985). The osteological and the body-plate nomenclature followed Schaefer (1997). Vertebral counts were obtained from clearedand-stained specimens and included the first five vertebrae modified into the Weberian apparatus. The compound caudal centrum (PU1 + U1; Lundberg and Baskin 1969) was counted as one vertebra. The pores nomenclature followed Arratia and Huaquin (1995). Asterisks in the text refer to the holotype. After collection the animals were anesthetized using 1% benzocaine in water and fixed in 10% formalin for at least two days, then transferred to 70% ethanol for permanent storage for morphological studies.

All examined material was housed at the following Brazilian institutions: LBP (Laboratório de Biologia e Genética de Peixes, Universidade Estadual Paulista Júlio de Mesquita Filho, Botucatu - SP); MCP (Museu de Ciências e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre - RS); MZUSP (Museu de Zoologia da Universidade de São Paulo, São Paulo - SP); and NUP (Coleção Ictiológica do Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura, Universidade Estadual de Maringá, Maringá - PR).

# Results

#### Pareiorhina hyptiorhachis, sp. n.

urn:lsid:zoobank.org:act:1D6D4D43-68CF-485B-9ABC-8FFB270E2460 http://species-id.net/wiki/Pareiorhina\_hyptiorhachis Figure 1; Table 1

"*Pareiorhina* sp. 1" - Roxo et al. 2012a:2443 [phylogenetic relationships]. - Roxo et al. 2012b:38 [phylogenetic relationships].

**Holotype.** MZUSP 111956, female, 33.6 mm SL, Brazil, Minas Gerais State, municipality of Santa Bárbara do Tugúrio, Ribeirão Fernandes, a tributary of Rio Pomba, Rio Paraíba do Sul basin, 21°14'47"S, 43°34'07"W, 19 Jun 2011, Ferreira AT, Roxo FF, Silva GSC.

**Paratypes.** Brazil, Minas Gerais State, municipality of Santa Bárbara do Tugúrio, Rio Paraíba do Sul basin. LBP 12248, 2 males, 4 females, 1 c&s, 26.6–34.8 mm SL, collected with holotype. NUP 14331, 1 female, 29.6 mm SL, collected with holotype. LBP 1093, 1 male, 33.4 mm SL, Ribeirão Fernandes, 21°14'47"S, 43°34'07"W, 12 Oct 2001, Oliveira JC, Alves AL, Sato LR. LBP 8368, 5 females, 27.9–34.4 mm SL, Rio Pomba, 21°14'07"S, 43°30'50"W, 19 May 2009, Oliveira C, Silva GJC, Roxo FF, Pereira TNA. LBP 12257, 1 female, 27.2 mm SL, Rio Pomba, 21°14'07"S, 43°30'50"W, 19 Jun 2011, Ferreira AT, Roxo FF, Silva GSC. MCP 29432, 3 male, 1 female, 2 unsexed, (1 juvenile not measured) 23.8–39.0 mm SL, Ribeirão Fernandes, 21°14'47"S, 43°34'07"W, 12 Aug 2001, Oliveira JC, Alves AL, Sato LR.

**Diagnosis.** *Pareiorhina hyptiorhachis* is distinguished from its congeners, except for *P. carrancas*, by the presence of a postdorsal ridge (vs. the absence of a postdorsal ridge). The new species differs from *P. carrancas* by having a more elevated postdorsal



**Figure 1.** *Pareiorhina hyptiorhachis*, sp. n., MZUSP 111956, 33.6 mm SL, holotype from Ribeirão Fernandes, Rio Paraíba do Sul basin, municipality of Santa Barbara do Tugúrio.

ridge, (Fig. 2; 16.7–26.8% of CP depth vs. 4.47–9.03%; table 1). Additionally, the new species can be distinguished from *P. cepta* by having a naked abdomen (vs. having small plates covered with odontodes irregularly distributed on the abdomen); from *P. brachyrhyncha* and *P. cepta* by having unicuspid teeth (vs. teeth with a minute lateral

	Pareiorhina hyptiorhachis n=21			
	Holotype	Range	Mean	SD
Standard length (SL)	33.6	26.6-38.8	31.0	3.0
Percents of Standard length (SL)				
Predorsal length	44.2	41.5-48.8	44.7	1.6
Preanal length	59.2	56.1-65.9	60.5	2.4
Head length	31.7	28.6-35.5	31.8	1.5
Cleithral width	32.8	30.4–36.9	33.5	1.8
Dorsal-fin unbranched ray length	21.2	20.3-24.1	22.2	1.1
Base of dorsal fin length	15.4	14.3–18.3	16.5	1.1
Thorax length	18.1	15.1–19.6	17.0	1.3
Pectoral-fin unbranched ray length	20.5	20.5-26.0	22.6	1.5
Abdomen length	27.0	22.6-30.1	26.2	1.6
Pelvic-fin unbranched ray length	22.5	17.7–26.6	22.9	2.1
Anal-fin length	15.3	13.7–17.8	15.6	0.9
Ventral unbranched caudal ray	24.9	20.3-30.5	25.2	2.8
Caudal-peduncle depth	9.0	8.3-11.0	9.39	0.7
Postanal length	34.9	31.6-38.1	33.9	1.4
Anal width	15.4	11.3–16.0	14.0	1.5
Percents of Head Length (HL)				
Head width	103.8	100.1-108.6	103.8	2.6
Head depth	61.7	53.5–62.8	56.9	2.3
Snout length	63.1	58.0-64.7	61.2	1.9
Interorbital width	37.7	34.8-40.7	38.0	1.4
Orbital diameter	11.4	11.1–15.5	12.7	1.6
Suborbital depth	39.2	35.0-40.5	37.3	1.6
Mandibular ramus	18.1	16.0-23.4	19.9	1.9
Other measurements (expressed as percentages)				
Anal width/cleithral width	47.1	32.0-49.7	42.0	5.1
IO/OD	29.5	21.6-42.1	33.5	4.5
IO/Mandibulary ramus	50.6	44.1-62.5	52.4	5.6
Predorsal length/first ds ray length	47.9	45.7–54.5	49.7	2.6
Postanal length/CP depth	25.8	24.8-31.1	27.6	1.9
Pelvic-fin length/CP depth	40.0	36.0-46.8	41.1	3.0
Ventral unbranched caudal ray/CP depth	36.2	32.1-46.0	37.5	4.3
Postdorsal ridge depth/CP depth	19.0	16.7–26.8	21.5	3.4

**Table 1.** Morphometric data for *Pareiorhina hyptiorhachis*. SD = Standard Deviation, IO = Interorbital, OD = Orbital Diameter, CP = Caudal Peduncle.

cusp); from *P. carrancas* and *P. rudolphi* by having the anterior profile of the head rounded in dorsal view (vs. elliptical; Fig. 3) and by having a greater suborbital depth (35.0–40.5% of HL vs. 27.4–34.2% in *P. carrancas* and 24.5–31.8% in *P. rudolphi*). Moreover *Pareiorhina hyptiorhachis* is distinguished from its congeners by having a wider head (100.1–108.6% of HL vs. 91.7–98.1% in *P. brachyrhyncha*, 82.9–96.2% in *P. carrancas*, 83.4–90.5% in *P. cepta* and 77.8–82.1% in *P. rudolphi*).

**Description.** Morphometric and meristic data are given in Table 1. In lateral view, dorsal profile of body strongly convex from snout tip to distal margin of supraoccipital;



**Figure 2.** a *Pareiorhina hyptiorhachis*, sp. n., paratype, LBP 12248, 29.2 mm SL, showing the conspicuous postdorsal ridge **b** *Pareiorhina carrancas*, LBP 8380, 38.2 mm SL, showing the poorly-developed postdorsal ridge **c** *Pareiorhina rudolphi*, LBP 8044, 40.5 mm SL, showing the absence of a postdorsal ridge.

straight from supraoccipital to dorsal-fin origin; slightly decreasing to end of caudal peduncle. Ventral surface of body, slightly concave at head portion, straight to convex from posterior end of head to pelvic-fin insertion, and straight but angled to posterior end of caudal peduncle. Snout tip rounded in dorsal view. Nostril small. Trunk and caudal peduncle rectangular in cross-section.

Greatest body depth at dorsal-fin origin. Body progressively narrowing posteriorly from cleithrum. Head flat to slightly convex between orbits; superior margin of orbits elevated. Eye small, orbital diameter 11.1–15.5% of HL, situated dorsolaterally just posterior of midpoint of head. Rostral margin of snout with minute, posteriorlydirected odontodes; numerous small odontodes on dorsal portion of head. Opening of swimbladder capsule small. Perforations of compound pterotic distributed on whole bone, greater and more concentrated on its ventral margin; its openings nearly rounded in median region, and irregular along inferior and posterior margins of bone. Lips large; oral disk rounded, papillose; premaxillary teeth 22 (1), 29 (1), 30 (1), 32 (1), 33 (1), 34 (2), 36 (1)\*, 37 (2), 38 (1), 39 (2), 40 (2), 42 (2) or 44 (1). Dentary teeth 17 (1), 21 (1), 23 (1), 28 (1), 30 (2), 32 (2)\*, 33 (2), 34 (2), 35 (1), 36 (1), 39 (1), or 45 (1). Teeth unicuspid. Maxillary barbel short and free distally.

Dorsal-fin rays ii,7; dorsal-fin originating at vertical through posterior end of pelvicfin base; distal margin slightly convex. Pectoral-fin rays i,6; distal margin slightly convex; unbranched pectoral-fin ray reaching middle of unbranched pelvic-fin ray; unbranched pectoral-fin ray covered with large and pointed odontodes. Pelvic-fin rays i,5; distal margin of fin slightly convex; tip of adpressed pelvic fin almost reaching anal-fin origin; unbranched pelvic-fin ray covered with conspicuously pointed and well-developed, and uniformly distributed odontodes which are larger at ventral portion. Anal-fin rays i,5; distal margin slightly convex. Caudal fin rays i,7-7,i. Adipose fin absent. Caudal fin truncated with ventral unbranched principal ray longer than dorsal ray.

Body entirely covered by bony plates, except for ventral surface of head, abdomen and region overlaying swimbladder capsule. Dorsal series of plates 24–26, mid-dorsal 17–21, median perforated plates 24–26, mid-ventral 17–22, and ventral 19–22. Trunk with conspicuous, elongated, postdorsal ridge formed by 13–15 raised, unpaired, median plates; ridge continuous posteriorly with procurrent caudal-fin rays. Head lacking crest. Head and body plates covered with minute, uniformly sized and distributed odontodes. Seven pairs of ribs associated with vertebrae 8–14. Ribs slender and poorly ossified. Total vertebrae 29.

Supraorbital sensory canal with four pores; pore s1 located on prenasal plate below nasal plate; pore s3 located on posterior portion of nasal; pore s6+s6 located between frontal plates, on horizontal line through anterior limits of eye; pore s8 located on division between frontals, sphenotic and supraoccipital plates, just above eye. Infraorbital sensory canals with six pores; pore io1 located on anterior portion of first infraorbital; pore io2 located in medial region between first and second infraorbitals; pore io3 located in medial region between second and third infraorbitals; pore io4 located in medial region between third and fourth infraorbitals; pore io5 located in medial region between fourth and fifth infraorbitals and pore io6 located between sixth infraorbital and sphe-



**Figure 3.** a *Pareiorhina carrancas*, LBP 8380, 36.5 mm SL, showing the elliptical anterior profile of the head elliptical in dorsal view **b** *Pareiorhina rudolphi*, LBP 8044, 42.0 mm SL, showing the elliptical anterior profile of the head in dorsal view **c** *Pareiorhina hyptiorhachis*, new species, holotype, MZUSP 111956, 33.6 mm SL, showing the rounded anterior profile of the head in dorsal view.



**Figure 4.** Additional coloration pattern of coloration of *Pareiorhina hyptiorhachis*, sp. n., LBP 12257, female, 27.2 mm SL.

notic. Preopercular canal with three pores; pore pm2 located on ventral portion of cheek plate, pore pm3 located between cheek plate and preopercle; pore pm4 located between preopercle and compound pterotic. Two postotic pores; pore po2 located just above of branchial slit; pore po3 located in region of overlying opening of swim-bladder capsule.

**Color in alcohol.** Two body-coloration patterns observed. First pattern (Fig. 1): Ground color of dorsal surface of head and body yellowish brown. Ventral surface of body and head lighter than dorsal with dark spots of melanophores widely separated. Three dark saddles on dorsal surface of trunk (in some specimens not present), most anterior one inconspicuous. Lateral portion of body with inconspicuous dark stripe from head to caudal fin. Pectoral, pelvic and dorsal fins with three irregular, poorly defined bands. Caudal fin with variegated blotches. Second pattern (Fig. 4): Ground color of body uniformly dark except, ventral portion of body mostly clear; Fins with inconspicuous, irregularly defined bands: one in anal fin, two in pectoral and pelvic fins. Dorsal and caudal fins entirely dark.

**Sexual dimorphism.** Males with a papilla at urogenital opening and fewer teeth in premaxillary 22–39 (*vs.* 32–44 females) and 17–32 dentary (*vs.* 30–45 females).


**Figure 5.** The Rio Paraíba do Sul basin indicating the type locality of *Pareiorhina hyptiorhachis* in Ribeirão Fernandes, a tributary of Rio Pomba, Rio Paraíba do Sul basin, 21°14'47"S, 43°34'07"W.

**Etymology.** The specific name, *hyptiorhachis* is a combination of Greek, *hyptios* = supine, lying on the back, and *rhachis* = ridge, midrib, and is in reference to the conspicuous postdorsal ridge found in this species.

**Distribution and habitat.** *Pareiorhina hyptiorhachis* is known from Rio Pomba and one of its tributaries, the Ribeirão Fernandes, in the municipality of Santa Barbara do Tugúrio, Minas Gerais State, Brazil (Fig. 5). This species inhabits moderate to fast-flowing streams, with a substrate of rocks and sand and margins covered by aquatic vegetation. Specimens were collected in association with loose stones, on the streambed. The new species is syntopic throughout its distribution with *Astyanax* sp., *Characidium* sp., *Geophagus brasiliensis, Harttia* cf. *carvalhoi, Imparfinis* sp., *Neoplecostomus microps, Trichomycterus* cf. *alternatus*, and *Trichomycterus* sp.

## Discussion

Bockmann and Ribeiro (2003) proposed seven characters to diagnose *Pareiorhina*. The new species described herein, *P. hyptiorhachis*, possesses all of these characters. On the other hand, *Pareiorhina* did not form a monophyletic group in the molecular analysis of Roxo et al. (2012a, 2012b); in that analysis, *P. hyptiorhachis*, cited as *Pareiorhina* sp. 1, appeared as the sister group of *P. carrancas*, and these two species formed the sister group of *Neoplecostomus*. Furthermore, *P. rudolphi*, the type species of *Pareiorhina* was the sister group of *Pseudotocinclus*. Considering that *P. hyptiorhachis* exhibits all of the characters listed by Bockmann and Ribeiro (2003) for *Pareiorhina*, the molecular data conflict with the available morphological data for Neoplecostominae, and new morphological studies in Neoplecostominae are in progress (Edson Henrique Lopes Pereira, pers. comm.), we prefer to include *P.* 

*hyptiorhachis* in *Pareiorhina* rather than in another Neoplecostominae genus or in a new genus.

*Pareiorhina hyptiorhachis* is similar to *P. carrancas* from the upper Rio Paraná basin. The two species share unicuspid teeth and the presence of a postdorsal ridge of unpaired plates, although the postdorsal ridge is better developed in *P. hyptiorhachis* (all female and male samples) (Fig. 2). Moreover, the new species has more raised median unpaired plates in the postdorsal ridge (13–15 vs. 10–13 in *P. carrancas*). The close relationship between *P. hyptiorhachis* and *P. carrancas* suggested by the molecular data of Roxo et al. (2012b) is thus at least superficially supported by morphology.

Pareiorhina is distributed across three hydrographic basins, with P. rudolphi, P. brachyrhyncha and P. hyptiorhachis from the Rio Paraíba do Sul basin; P. carrancas from the upper Rio Paraná basin; and *P. cepta* from the Rio São Francisco basin. Ribeiro et al. (2006) suggested that the activation of old faults in southeastern Brazil during the Miocene and Pliocene resulted in several headwater captures between adjacent drainages of the São Francisco, upper Paraná and Coastal rivers. Roxo et al. (2012a) suggested that the lineage that gave rise to P. carrancas and P. hyptiorhachis was from the upper Rio Paraná basin and that P. hyptiorhachis reached the Rio Paraíba do Sul basin about 6.2 (2.3–11.2) million years ago, probably through headwater captures between the upper Paraná and several coastal drainages (Rio Paraíba do Sul and Ribeira do Iguape basin) during the late Miocene. Chamon et al. (2005) suggested that the evolutionary history of *P. rudolphi* and *P. brachyrhyncha* was linked to Pleistocene and pre-Pleistocene climatic fluctuations that may have temporarily isolated hillside streams at or near the headwaters of the Ribeirão Grande, producing the events that subsequently led to the sympatry of P. brachyrhyncha and P. rudolphi. However, as suggested by Crammer et al. (2008, 2011), Chiachio et al. (2008) and by Roxo et al. (2012a, 2012b), P. brachyrhyncha and P. rudolphi do not share an exclusive most recent common ancestor, which negates the hypothesis of Chamon et al. (2005). Additionally, Roxo et al. (2012a) suggested that the origin of the lineages that gave rise to the species of Pareiorhina were much older, originating in the Miocene [17.87 (8.24-28.42) million years ago for Pareiorhina rudolphi and 6.27 (2.33-11.21) million years ago for Pareiorhina carrancas plus P. hyptiorhachis (Pareiorhina sp. 1 in Roxo et al. 2012a)].

#### **Comparative material**

*Isbrueckerichthys alipionis*: LBP 7373, 17, 31.7–81.6 mm SL, municipality of Iporanga, SP, Rio Ribeira de Iguape basin; LBP 2660, 1, 55.1 mm SL, municipality of Iporanga, SP, Rio Ribeira de Iguape basin. *Kronichthys subteres*: LBP 515, 31, 28.4–61.9 mm SL, municipality of Iporanga, SP, Rio Ribeira de Iguape basin. *Neoplecostomus microps*: LBP 8036, 38, 41.3–65.0 mm SL, municipality of Piquete, SP, Rio Paraíba do Sul basin. *Neoplecostomus franciscoensis*: LBP 6489, 50, 42.8–55.9 mm SL, municipality of São Bartolomeu, MG, Rio São Francisco basin. *Neoplecostomus paranensis*: holotype, MZUSP 38572, 71.4 mm SL, municipality of Cajuru, MG, Rio Grande basin. *Pareiorhaphis splendens*: LBP 1117, 20, 32.0–100.0 mm SL, municipality of Morretes, PR, Coastal Drainage. *Pareiorhaphis steindachneri*: LBP 739, 6, 33.8–49.0 mm SL, municipality of Jaraguá do Sul, SC, Coastal Drainage. *Pareiorhina brachyrhyncha*: LBP 12240, 50, 26.4–36.9 mm SL, municipality of Pindamonhangaba, SP, Rio Paraíba do Sul basin. *Pareiorhina carrancas*: LBP 8380, 24, 21.3–38.2 mm SL, municipality of Carrancas, MG, Rio Grande basin. *Pareiorhina cepta*: holotype, MZUSP 111095, 41.5 mm SL, municipality of São Roque de Minas, MG, Rio São Francisco basin, paratypes, LBP 10261, 1, 30.2 mm SL, municipality of São Roque de Minas, MG, Rio Paraíba do Sul basin, LBP 10287, 13, 21.5–43.6 mm SL, municipality of São Roque de Minas, MG, Rio Paraíba do Sul basin, LBP 10287, 13, 21.5–43.6 mm SL, municipality of São Roque de Minas, MG, Rio Paraíba do Sul basin, LBP 10287, 13, 21.5–43.6 mm SL, municipality of São Roque de Minas, MG, Rio Paraíba do Sul basin, LBP 10287, 13, 21.5–43.6 mm SL, municipality of São Roque de Minas, MG, Rio Paraíba do Sul basin, LBP 10287, 13, 21.5–43.6 mm SL, municipality of São Roque de Minas, MG, Rio Paraíba do Sul basin, LBP 11835, 19, 25.1–44.0 mm SL, municipality of São Roque de Minas, MG, Rio Paraíba do Sul basin. *Pareiorhina rudolphi*: LBP 8044, 18, 31.7–48.9 mm SL, municipality of Piquete, SP, Rio Paraíba do Sul basin. *Pseudotocinclus juquiae*: LBP1081, 2, 29.0–31.9 mm SL, municipality of Juquitiba, SP, Coastal Drainage. *Pseudotocinclus tietensis*: LBP 2931, 3, 38.6–62.3 mm SL, municipality of Salesópolis, SP, Rio Tietê basin.

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RESEARCH ARTICLE



# Neogosseidae (Gastrotricha, Chaetonotida) from the iSimangaliso Wetland Park, KwaZulu-Natal, South Africa

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## Abstract

Among the mostly benthic gastrotrichs, the Neogosseidae (Gastrotricha, Chaetonotida) are particularly interesting from an evolutionary point of view in virtue of their planktonic lifestyle; yet, they are poorly known and uncertainties concerning morphological traits hamper accurate in-group systematics. During a recent survey of meiofauna in the iSimangaliso Wetland Park, South Africa, two species of Neogosseidae were found in a freshwater pond near Charter's Creek on the Western Shores of Lake St Lucia. Based on morphological traits, one species has been identified as *Neogossea acanthocolla*, originally described from Brazil, while the other, affiliated to the genus *Kijanebalola*, is proposed as new to science. Using a combination of differential interference contrast and scanning electron microscopy, fine anatomical details were observed and are here discussed in a larger taxonomic framework, especially regarding *K. devestiva* **sp. n.** Results have also provided reasons for a revision of the diagnostic traits of *Kijanebalola, Neogossea* and the whole Family Neogosseidae. Besides expanding awareness about the biodiversity hosted by South Africa's first UNESCO World Heritage Site, our study will be beneficial to future phylogenetic studies of

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the Gastrotricha based on morphology, by allowing the selection and/or a more precise character coding of traits of phylogenetic relevance.

#### **Keywords**

Gastrotricha, meiofauna, new species, taxonomy, Kijanebalola, Neogossea, diagnoses

## Introduction

Most freshwater gastrotrichs (Chaetonotida, Paucitubulatina) have an epibenthic, periphytic or interstitial lifestyle, however, there are several species that conduct a pelagic and/or semi-planktonic existence (e.g., Balsamo and Todaro 2002, Todaro and Hummon 2008). Common features of the latter species are the absence of the furcal adhesive tubes and a re-arrangement of the locomotory ciliation, which in these forms includes discrete tufts along the trunk region and at least a band of long, strong propelling cilia that encircles more or less completely the head (e.g., Kisielewski 1991). Based on additional traits, planktonic gastrotrichs are placed into three main taxa: 1) the monotypic genus *Undula* Kisielewski, 1991, provisionally considered as a distinct subfamily of the Chaetonotidae Gosse, 1864 (see Kisielewski 1991); 2) the Family Dasydytidae Daday, 1905, which currently includes seven genera and 25 species (Todaro and Tongiorgi 2013), with most characterized by long and movable spines (e.g., Kieneke and Ostmann 2012); and 3) the Family Neogosseidae Remane, 1927, which includes two genera and eight (9?) species, all bearing a pair of peculiar, club-like cephalic tentacles.

The origin and evolution of planktonic gastrotrichs remain largely unknown (Hochberg and Litavaitis 2000, Kieneke et al. 2008b); however, the long-held hypothesis that planktonic forms derive from benthic ancestors (e.g., related to Chaetonotus (Zonochaeta) Remane, 1927; see Kisielewski 1991) has gained support from a recent phylogenetic study based on the analysis of molecular markers (Kånneby et al. 2013). This study has in fact found that the 11 Dasydytidae analysed (7 putative species) can be combined into a monophyletic group deeply nested within the Chaetonotidae as sister to a clade containing *Chaetonotus* (Z) species. While shedding light on the probable origin of the planktonic forms, at least that of the Dasydytidae, the work by Kånneby et al. (2013) has also shown phylogenetic relationships that in part contrast with the current systematization of the group, e.g., Dasydytes carvalloe Kisielewski, 1991, appears basal to a clade composed of *Dasydytes* spp. and *Stylochaeta* spp. This highlights the fact that planktonic gastrotrichs are still too poorly known for reasonable confidence in the current phylogenetic inferences based on morphological traits to prevail. Uncertainties in this regard are also shown by the unstable systematics that plague some taxa (e.g., Kisielewski 1991 vs Schwank 1990 for Dasydytidae). This situation is due to the scarcity of information available, both at the level of the number of known species included in the currently recognized genera and subgenera (generally too low to provide reliable taxonomic boundaries) and of the techniques that have been utilized in the past to investigate these animals (in many instances inadequate to current standards). The Family Neogosseidae, and particularly the genus *Kijanebalola* Beauchamp, 1932, may be considered a paradigmatic example in both regards.

*Kijanebalola* includes the type species *K. dubia* Beauchamp, 1932 from an Ugandan lake and *K. canina* Kisielewski, 1991, from a pond in the State of Parà, Brazil. The status of a third species, described as a rotifer, *Eretmia cubeutes* Gosse in Hudson and Gosse 1886, is uncertain and will not be considered further. All species have been found only once. *K. dubia* was established on the basis of two formalin-fixed, contracted specimens examined with light microscopy (very likely bright field) and, by admission of the author, the description is to be considered incomplete (Beauchamp 1932). Better is the situation for the second species, *K. canina*, the description of which benefitted from the availability of numerous specimens (10). Most of these were relaxed with novocaine and examined in vivo using a bright field microscope, with some specimens fixed later and observed further with DIC optics (Kisielewski 1991). The thorough examination of the Brazilian species allowed the improvement of the diagnosis at familial and generic level (cf. Kisielewski 1991).

We report here on two interesting Neogosseidae found during an ongoing survey of the meiofauna and macrobenthos of the iSimangaliso Wetland Park (Pillay and Perissinotto 2009, Todaro et al. 2011, Bownes and Perissinotto 2012, Daly et al. 2012, Gómez et al. 2012). One species belongs to the genus *Neogossea* Remane, 1927 and is reported here for the first time outside of its Brazilian type locality, whereas the second appears to be new to science. The details of the anatomical characteristics of the latter, observed using a variety of techniques including electron microscopy, warrant a further revision of the diagnostic traits not only of the genus *Kijanebalola*, but actually of the entire Family Neogosseidae. Thus, beside increasing our knowledge of the biodiversity hosted by South Africa's first UNESCO World Heritage Site (Taylor 2006), this study will be beneficial to future phylogenetic studies of Gastrotricha based on morphology, by allowing the selection and/or a more precise character coding of traits of phylogenetic relevance.

## Methods

Samples containing gastrotrichs were collected on 11 February 2013 from a 310 m  $\times$  70 m freshwater pond located near Charter's Creek on the Western Shores of Lake St Lucia, iSimangaliso Wetland Park, South Africa. The pond was unevenly divided in two interconnected-pools by a white road; its maximum depth was about 1-1.5 m and the water surface almost completely covered by aquatic vegetation, constituted mainly of blue waterlilies, *Nymphaea nouchali*. Collection was carried out in both pools by operating from the edge of the road with a hand-held plankton net (15 cm diameter, 25 µm mesh) used to scoop up water from the thick vegetation. The geographic coordinates of each site were recorded by means of a Garmin GPS-12 portable receiver, while the physico-chemical characteristics of the water were measured by means of a YSI 6600 multiprobe system. A list of the main characteristics of the sites surveyed is

reported in Table 1 and a comprehensive map along with photos taken at the time of sampling are provided in the Appendix: Figure 1S.

A total of four 1 L plastic jars filled with water, some debris and little vegetation from each pool (2+2 stations) were brought back to the laboratory at the University of KwaZulu-Natal (Durban) within 24 hr and analysed during a one-week period. To extract gastrotrichs, samples were stirred with a plastic pipette and aliquots of the sediment-water mixture were poured into 10 cm diameter plastic Petri dishes and scanned under a Wild M5 stereo-microscope. The animals of interest were pickedout with a micro-pipette, transferred to a slide in a drop of 1% MgCl<sub>2</sub> solution and studied live (Kånneby et al. 2009, Todaro et al. 2012). Photos and measurement were taken with a Nikon Eclipse 80i DIC microscope equipped with a Nikon Digital Sight DSFi1 digital camera and a Nikon Nis-Elements software. Some specimens were fixed in 10% borax-neutralized formalin and stored for later SEM analysis. To this end, gastrotrichs were rinsed in distilled water, dehydrated through a graded ethanol series, critical point-dried using CO<sub>2</sub>, mounted on aluminium stubs, sputter coated with gold-palladium and observed with a Philips XL 30 scanning electron microscope at the first author's institution in Modena, Italy (cf. Todaro 2013). Five additional specimens were fixed and kept in absolute ethanol for future DNA analysis. The description of the new species follows the convention of Hummon et al. (1992), whereas the position of key morphological characters is given in percentage units (U) of total body length, measured from anterior to posterior end, excluding the terminal spines.

Abbreviations used in the text are as follows: **CT** cephalic tentacles; **PhIJ**, pharyngeo-intestinal junction; **PhL**, pharynx length, **TL**, total length.

#### Taxonomic account

Order Chaetonotida Remane, 1925 [Rao & Clausen, 1970] Suborder Paucitubulatina d'Hondt, 1971 Family Neogosseidae Remane, 1927 Genus *Kijanebalola* Beauchamp, 1932

#### Kijanebalola devestiva sp. n.

urn:lsid:zoobank.org:act:53BA8B47-3BC3-4AD1-9B5F-1CBD5F9F34DB http://species-id.net/wiki/Kijanebalola\_devestiva Figs 1–6

**Type locality.** Roadside freshwater pond near Charter's Creek, Lake St Lucia, Western Shores, iSimangaliso Wetland Park, South Africa (Lat. 28°15'19"S; Long. 32°23'37"E; Table 1, Figure 1S).

**Type specimens.** Holotype: adult specimen 267  $\mu$ m long shown in Figure 2, no longer extant (International Code of Zoological Nomenclature, Articles 73.1.1 and 73.1.4).

**Table 1.** Physico-chemical data and geographic coordinates of the four sampling sites investigated at the pond near Charter's Creek on the Western Shores of Lake St Lucia, iSimangaliso Wetland Park, South Africa. Pool A represents the larger pool situated on the right side of the road, travelling from Charter's Creek towards St Lucia town. Pool B is the smaller pool on the left side of the same road (see also supplementary file Figure 1S).

V-st-Ll-	Poo	ol A	Pool B			
variable	1	2	3	4		
Temperature (°C)	24.8	24.9	22.8	22.9		
Conductivity (mS/cm)	0.24	0.25	0.25	0.40		
Salinity	0.12	0.12	0.12	0.19		
Dissolved O <sub>2</sub> (%)	9.7	15.4	5.5	6.0		
Dissolved $O_2$ (mg/L)	0.78	1.23	0.46	0.5		
pH	8.1	7.3	6.7	6.6		
Turbidity (NTU)	21.5	17.6	16.8	14.3		
Surface light intensity (µmol.cm <sup>-2</sup> .s <sup>-1</sup> )	184.9	222.4	242.6	179.1		
Bottom light intensity (µmol.cm <sup>-2</sup> .s <sup>-1</sup> )	20.97	16.73	32.48	3.93		
Depth (m)	0.5	0.7	0.60	0.80		
Canaraphic coordinates	Latitude 28°15	'19"S	Latitude 28°15'19"S			
	Longitude 32°2	23'37"E	Longitude 32°23'38"E			

**Material examined.** Thirteen specimens (including the holotype) collected by the first author (10 from pond A and 3 from pond B, see Table 1). Seven specimens were observed alive and are not longer extant, while six were prepared for SEM analysis and are kept in the meiofauna collection of the first author (Ref. n. 2013-SA-01-02).

Ecology. Above silty substratum, among vegetation.

**Diagnosis.** A *Kijanebalola* with an adult length to 310  $\mu$ m; body roughly barrelshaped with head weakly separated from trunk and the rounded-off posterior end exhibiting medially a group of five spines; head with a pair of 26  $\mu$ m long, club-like tentacles and a shallow cephalion; cuticular covering mostly smooth, except for a tiny patch of small triangular, keeled scales on the ventral side at the rear trunk; locomotor cilia arranged in tufts and interrupted bands on the head and 4 paired transverse bands along the trunk; three pairs of 14–16  $\mu$ m long sensory bristles on the dorsal side, at U12, U22 and U92; mouth 13  $\mu$ m in diameter, slightly protruding down forward and reinforced internally by 17-20 thick, longitudinal ridges; pharynx up to 67  $\mu$ m, consisting of anterior spherical and posterior nosecone-shaped bulbs; PhIJ at U27; intestine straight, with anterior portion embracing the posterior portion of the pharynx; one pair of conspicuous protonephridia located adjacent to the intestine, from the PhIJ to about mid-body; parthenogenetic.

**Etymology.** The specific name *devestiva* (from the Latin, *devestivus*, undressed), alludes to the general absence of cuticular ornamentations, such as scales and spines that cover the body of other congeneric species.

**Description.** This description is mainly based on an adult specimen,  $267 \mu m$  in total length (TL, posterior spines excluded). The body is roughly barrel-shaped with



**Figure 1.** *Kijanebalola devestiva* sp. n. schematic drawings. **A** Dorsal habitus **B** Ventral habitus, showing some internal structures. **a** anus **alt** antero-lateral tuft of cephalic cilia **asb** anterior sensory bristle **ce** cephalion **ct** cephalic tentacle **dlvb** lateral band of cephalic cilia extending dorsally and ventrally **e** egg **I-IV**, first to fourth band of trunk ciliature **mvb** median ventral band of cephalic cilia **pcl** proximal canal cell lumen **PhIJ** pharyngeo-intestinal junction **ps** patch of keeled scales **psb** posterior sensory bristle **tsp** terminal spines **vlt** ventro-lateral band of cephalic cilia.

the head weakly separated from the trunk by a slight neck constriction and the posterior trunk region rounded-off, without paired lateral projections but exhibiting medially a group of five spines. Body widths at the head/neck/trunk/caudum are 57/55/90/32  $\mu$ m, at U09/14/52/97, respectively. The head is provided with a pair of club-like tentacles projecting antero-laterally; they are 26  $\mu$ m in length and insert ventro-laterally at U07; the hypostomion is absent; a shallow cephalion (10 × 4.5  $\mu$ m) is appreciable only under SEM (Figure 5C). Under dissecting microscope, the animals appear swimming slowly in a rectilinear direction, with some following loose helicoidal trajectories. When purposely stimulated with a needle, specimens react by escaping aside, but never retracting the head inside the body; by contrast, most of the fixed specimens appear to have the head retracted to some extent (Figure 6A).



**Figure 2.** *Kijanebalola devestiva* sp. n. DIC photomicrographs. **A** habitus **B**, **C** close-up ventral view of the anterior region showing the ciliary bands **D** close-up ventral view of the mid-trunk region showing the second ciliary bands **E** close-up ventral view, of the posterior region, showing the fourth ciliary bands (arrowheads) and the residual patch of spined scales (arrow).

*Cuticular armour.* The body is covered by a smooth cuticle, except for a minute patch of keeled scales located on the ventral side of the posterior trunk region, at U93 (Figures 1B, 2E). The scales, arranged in 5–7 columns of 3–5 scales each, are very small (ca 1  $\mu$ m) and may go undetected under light microscopy (DIC); when observed with a scanning electron microscope, they appear roughly triangular in shape and their keel continues in a proportionally long spiny process (Figure 6C). Five robust terminal spines, 15–24  $\mu$ m in length, ornate the posterior end of the trunk; they are inserted dorsally to the anus (Figure 6B, D).

*Ciliation.* Locomotory cilia are arranged in tufts and interrupted bands around the head and paired transverse bands along the trunk (Figures 1, 2A–E, 5A, B). Most of the cephalic cilia have a ventral or ventro-lateral distribution; however, a precise

organization is difficult to see due to their high density and relatively long span (16-18  $\mu$ m in length). From anterior to posterior end, it is possible to discern the following groups: a pair of antero-lateral tufts, a median ventral band, a pair of lateral bands extending ventrally and dorsally, followed by a pair of ventro-lateral tufts (Figure 1). The trunk ciliature consists of four pairs of oblique short bands, with first (U32) and fourth (U94) inserted dorso-latero-ventrally, the second (U59) inserted latero-ventrally and the third (U66) latero-dorsally (Figures 1, 2D, E, 5A).

Three pairs of sensory bristles (14–16  $\mu$ m in length) are present on the dorsal side at U12, U22 and U92, respectively (Figures 1A, 4B, C). The bristles of the first two



**Figure 3.** *Kijanebalola devestiva* sp. n. DIC photomicrographs. **A** habitus of a gravid specimen **B** closeup view of the inside egg with the shell bearing spine-like ornamentation (arrowheads).

pairs emerge from round pits, while posterior bristles originate directly from the cuticle and are flanked by two anteriorly-converging keels. Presence of additional sensory bristles hidden among the cephalic locomotor ciliation cannot be excluded.

*Digestive tract:* The strong mouth ring is terminal and about 13  $\mu$ m in diameter; it appears slightly protruding down forward and is reinforced inside by 17–20 thick longitudinal cuticular ridges, which protrude externally and bend on the outer contour (Figure 5). The pharynx is 64  $\mu$ m in length and shows anterior and posterior bulbs separated by a noticeable constriction; the anterior bulb, deprived of cuticular reinforcement, is roughly spherical (28 × 24  $\mu$ m), while the posterior one (30 × 40  $\mu$ m) is more nosecone-shaped (Figures 1, 4A); PhIJ is at U27. The intestine is straight; in the adult it appears impressively filled with green material (Figures 2A, 3A) while in juveniles it is packed with translucent globules (Figure 4A). Peculiarly, the anterior portion of the intestine extends forward encircling a large part of the posterior portion of the pharynx (about half the length of the posterior bulb), resulting in this region much wider than the pharynx itself (36  $\mu$ m); at the PhIJ and for a short tract the intestine is about as wide as the pharynx (28–30  $\mu$ m), then it widens again reaching a maximum width of



**Figure 4.** *Kijanebalola devestiva* sp. n. DIC photomicrographs. **A** habitus of a subadult specimen showing developing egg (arrow) **B** close-up dorsal view of the posterior trunk region showing the two sensory bristles (arrow-heads) **C** close-up dorsal view of the anterior trunk and neck regions, showing two pairs of sensory bristles.



**Figure 5.** *Kijanebalola devestiva* sp. n. SEM photomicrographs. **A** habitus in a ventro-lateral view; note the arrangement of the first, second and third ciliary bands on the trunk region (number and arrows) **B** anterior region of a different specimen in a frontal view **C** close-up view of the mouth ring and cephalion (arrows).

45  $\mu$ m at about mid-body (U52); after this point the gut progressively narrows until it joins the 5–6  $\mu$ m sub-terminal anus at U97 (Figures 1, 6B, C).

*Nephridial system.* There is a pair of conspicuous protonephridia adjacent to the intestine; each protonephridium occupies an area extending from the PhIJ to about midbody (U27-U53) and includes a clearly visible tubular canal containing two vibrating flagella, corresponding to the proximal canal cell lumen of Kieneke et al. (2008a). This canal is about 25  $\mu$ m in length and runs almost parallel to the intestine, slightly converging towards the gut with its posterior portion (see Appendix: Figure 2S).

*Reproductive tract.* All the adult specimens were in parthenogenetic phase, with respective eggs at different stages of development.

Variability and remarks. The largest adult was about 310  $\mu$ m in total length (terminal spines excluded) and was carrying a very big egg inside (73 × 107  $\mu$ m, Figure 3A). Remarkably, the egg shell was ornated with spikes (Figure 3B); this is quite surprising because in freshwater Gastrotricha the shell ornamentation is believed to appear only after the egg has been laid, probably due to osmotic differences between the external



**Figure 6.** *Kijanebalola devestiva* sp. n. SEM photomicrographs. **A** anterior region of specimen with head partially retracted inside the body (ventral view) **B** posterior trunk region of different specimen, showing the fourth ciliary band and the anus (arrow) **C** close-up view of the posterior end showing the anus (arrow) and the residual patch of spined scales (arrows) **D** close-up view of the posterior end (dorsal view), showing the terminal spines and the sensorial bristles (arrows).

vs internal milieu (Hummon 1984). The smallest animal was about 183  $\mu$ m in total length and had no recognizable female gametes inside. Oocytes became appreciable as such in a 230  $\mu$ m long specimen (Figure 4A).

**Taxonomic affinities.** The general body appearance, the presence of club-shaped cephalic tentacles and the planktonic lifestyle that characterise *K. devestiva* sp. n. suggest that the closest affiliation of the South African specimens lies with the Neogosseidae. Notwithstanding this, autoapomorphic traits of the new species make somewhat difficult its affiliation to any of the two currently recognized genera, *Neogossea* and *Kijanebalola*, based on their current diagnosis (see Kisielewski 1991). For instance, the size of the South African worms exceed by far that of any other possible keen (max length 310 µm vs 200 µm in *Neogossea* spp., vs 210 in *Kijanebalola* spp.) and the body covering, made for most part of smooth cuticle, is a characteristic so far unknown among species of *Neogossea* or *Kijanebalola*. The presence in the new species of a single cephalic plate (i.e., the cephalion), combined with the structure of its mouth and pharynx, complicate further its taxonomic affiliation at generic level. However, in our opinion, some of the differences between the anatomical traits of the iSimangaliso gastrotrichs and those highlighted in the diagnosis: 1) is ambiguous about the presence of cephalic plates

(i.e., cephalion and hypostomion reported as undetected); and 2) includes the mouth as consisting of two-segmented units (mouth units = internal cuticular ridges). A reevaluation of these characteristics, especially in light of the new information gained by Kieneke and Riemann (2007) on German specimens of *Neogossea* (reported as *N. voigti* (Daday, 1905) allows for the amendment of the generic diagnosis of *Neogossea* in both these aspects. More specifically, the specimens studied by Kieneke and Riemann (2007) under differential interference contrast and scanning electron microscopy show: 1) the hypostomion and 2) the mouth units as unsegmented. Considering that in Neogosseidae the presence of a cephalion may be elusive (as reported above for the new species) we conclude that the presence/absence of the cephalic plates and the structure of the mouth and pharynx cannot be considered diagnostic characters at genus level (i.e., useful to distinguishing *Neogossea* from *Kijanebala* and *vice versa*); rather, peculiarities in these traits and others such as the presence/absence of cuticular reinforcements in the anterior pharyngeal bulb, may help differentiate among species (i.e., they are species specific).

Another potential difference between the two genera i.e., the presumed ability of *Kijanebalola* species to partially retract the head inside the body, can also be dismissed. Our observations with the new species demonstrate that retraction of the head is actually an artefact due to fixation (see above). Consequently, none of the above reported traits may be used to allocate our specimens to either of the two genera.

In summary, only a single character is apparently left to differentiate *Neogossea* and *Kijanebalola*; it pertains to the structure of the posterior part of the trunk, which appears truncate with a pair of postero-lateral projections, each provided with a tuft of relatively long spines in *Neogossea*, but rounded-off with a median group of spines in *Kijanebalola*. As the difference in this character between species of the two genera is consistent, it is reasonable to use it as diagnostic feature and as a solid synapomorphy for each genus.

Consequently, based on the round shape of the posterior trunk region of the iSimangaliso specimens, we propose that their closest affiliation is to the genus *Kijanebalola*. Therefore, the large size, the cuticular ornamentation reduced to an epaulet of scales on the ventral side of the trunk ending, and the number and size of the terminal spines are considered autoapomorphic characters that can easily distinguish *K. devestiva* sp. n. from *K. dubia* and *K. canina*. Emended diagnoses are reported below.

#### Genus Neogossea (Remane, 1927)

## *Neogossea acanthocolla* Kisielewski, 1991 http://species-id.net/wiki/Neogossea\_acanthocolla Fig. 7

**Material.** 2 adult specimens (1 measured and documented), South Africa, KwaZulu-Natal, roadside freshwater pond near Charter's Creek, Lake St Lucia, Western Shores, 11 February 2013, MA Todaro legit.



**Figure 7.** *Neogossea acanthocolla.* DIC photomicrographs. **A** habitus **B** anterior region showing the group of thick spines on the neck (arrow) **C** close-up of the posterior region of the trunk showing a tuft of long, barbed spines (arrow).

**Morphometry.** TL, 122  $\mu$ m (posterior spines excluded); PhL, 34.5  $\mu$ m; PhIJ at U36; CT, 24.5  $\mu$ m; dorsal cuticular covering made up of about 16 columns of 17–24 trilobed scales bearing a short, simple spine; a group of 18 densely packed spines is present on the dorsal side of the neck; spines are 10–13  $\mu$ m in length, rather thick and with a notched tip; posterior tufts of long spines made up of 7 spines each; spines are 45–50  $\mu$ m in length and barbed, with the lateral denticle positioned at about 3/4 of the spine length.

**Remarks.** Morphometry and general appearance of the specimen from the iSimangaliso Wetland Park are in general accordance with data reported for the Brazilian *N. acanthocolla*, the main peculiarity of which is the presence of the brush-like group of spines on the neck. A noticeable difference between the South American and African specimens lies in the type of scales covering the body: pedunculated and rhomboidal in the former, but ordinary (trilobed) and spined in the latter. However, as similar differences have been reported among members of the Brazilian populations (cf. Kisielewski 1991), it may be cautious not to regard this trait alone as a discriminatory character. Future studies, accounting for morphological and/or molecular marker differences on a statistical basis could support or disprove this hypothesis (e.g., Todaro et al. 1996, Leasi and Todaro 2009, Kånneby et al. 2012, Kieneke et al. 2012).

## Diagnoses

#### Family Neogosseidae Remane, 1927 (emended diagnosis)

Paucitubulatina exhibiting weakly flattened body,  $90-310 \mu m$  in length (posterior spines excluded). With a pair of club-shaped cephalic tentacles. Caudal furca and adhesive tubes absent. Posterior trunk with paired postero-lateral projection or rounded-off. Locomotor ciliature consisting of tufts and interrupted bands around the head and several pairs of tufts or short obliquely-running bands along the trunk. Cephalion and hypostomion present or absent. Body covered with scales, rarely naked with scales reduced to an epaulet on the ventral side; scales in general spined rarely pedunculated; spines simple, occasionally barbed. Trunk end with long spines distributed in paired lateral and/or unpaired median group. Mouth ring large and terminal, reinforced inside by thick longitudinal cuticular ridges, which may protrude externally. Pharynx strong with one to four bulbs. With a pair of protonephridia in the anterior trunk region. Parthenogenetic, with paired ovary. Male system unknown. Freshwater, semipelagic and/or planktonic.

#### Genus Neogossea Remane, 1927 (emended diagnosis)

Neogosseidae with body 90–200 µm in length (caudal spines excluded). Posterior end of body truncate, with a pair of postero-lateral projections, each provided with a tuft of long spines, simple or barbed, occasionally also with short claw-like structure. Cephalion and hypostomion present or absent. Body covered with fine and spined scales; scales trilobed or with edges often fused with basal cuticle; occasionally and only partially with pedunculated scales. Spines short and simple, occasionally partly long and barbed. Mouth ring large and terminal, reinforced inside by thick longitudinal cuticular ridges, protruding externally. Anterior half of pharynx consisting of terminal bulb followed by two smaller dilations; posterior half in the form of large bulb. Cuticular pharyngeal reinforcements lacking. Six species: *N. antennigera* (Gosse, 1851) - type-species, *N. acanthocolla* Kisielewski, 1991, *N. fasciculata* (Daday, 1905), *N. pauciseta* (Daday, 1905), *N. voigti* (Daday, 1905) and *N. sexiseta* Krivanek & Krivanek, 1959.

#### Genus Kijanebalola Beauchamp, 1932 (emended diagnosis)

Neogosseidae exhibiting body 135–310 µm in length (caudal spines excluded). Posterior end of trunk rounded-off, with median group of spines and without lateral protrusions. Cephalion and hypostomion present. Body covered with convex scales with median keels and rudimentary spines, the latter tend to prolong in some trunk portions; occasionally body mostly naked with scales reduced to an epaulet on the ventral side. Pharynx consisting of at most two bulbs. Anterior pharynx portion at least as thick as

posterior one and occasionally provided with strong and complex system of cuticular reinforcements. Mouth ring large and terminal, reinforced inside by thick longitudinal cuticular ridges, which may protrude externally.

Three species: *K. dubia* Beauchamp, 1932 (type-species), *K. canina* Kisielewksi, 1991 and *K. devestiva* sp. n. The status of an additional species, originally described as a rotifer, i.e., *Eretmia cubeutes* Gosse in Hudson and Gosse 1886, but potentially belonging to the genus *Kijanebalola* as fourth species (see Beauchamp 1932 and Remane 1936) should be adequately re-assessed before a formal decision is made in this regard.

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## Appendix

Comprehensive map and DIC photomicrograph. (doi: 10.3897/zookeys.315.5593. app) File format: JPEG Image (jpg).

**Explanation note:** Figure 1S. Sampling location. Comprehensive map along with photos taken at the time of sampling. Figure 2S. *Kijanebalola devestiva* sp. n. DIC photomicrograph. Close-up of the protonephridial apparatus.

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