Convolutidae (Acoela) from Tanzania

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Abstract

Four species of Convolutidae, including a new genus and species, from shallow marine sediments in Tanzania are described: Convoluta enelitta, Convoluta thela comb. nov., Heterochaerus australis and Picola gen. nov. renei sp. nov. The species Conaperta krana and Conaperta thela are reassigned to the genus Convoluta.

Key words: Confocal microscopy, turbellarians, meiofauna, Rupert Riedl

Introduction

Except for mention of the occurrence of Heterochaerus australis Haswell, 1905, on the coast of Kenya by Dörjes & Young (1973), no information on distribution of Acoela on the eastern shore of Africa is available. The closest body of water investigated is the Red Sea, where Antonius (1968) paid special attention to the Convolutidae. His work resulted in the erection of three new genera and the description of six new species, two of which we found in our samples from Tanzania.

Our investigation of Acoela from the beaches of Dar es Salaam and Zanzibar, Tanzania, yielded numerous species from several families. We present here descriptions of four species belonging to the family Convolutidae, including one new to science. All species were examined using classical methods (examination of live animals and histological sections) as well as with the modern techniques of confocal laser scanning microscopy (CLSM) and transmission electron microscopy (TEM), thereby providing us with an abundance of morphological data, including some new information on the previously described species.

Material and methods

Sediment samples were collected in November, 2004, from various locations in Dar es Salaam and Zanzibar, Tanzania. Geographic coordinates for sites were obtained using a Garmin Gecko 110 GPS portable receiver. Samples from Kunduchi Beach, Dar es Salaam, were taken to the laboratory of the Faculty of Aquatic Sciences and Technology, University Dar es Salaam at Kunduchi Beach for extraction and observation of living animals. Samples collected from Zanzibar beaches were similarly processed at the Institute of Marine Sciences, University of Dar es Salaam, Zanzibar. Specimens were extracted from sediment using magnesium-chloride anesthetization (Sterrer 1971). Live animals were viewed by light microscopy in squeeze preparations using an Olympus CX41 microscope and photographed...
with an Olympus C-5050 digital camera.

For histological and ultrastructural study, specimens were relaxed in isotonic magnesium chloride and fixed in warm Stefanini’s fixative (Stefanini et al. 1967) some specimens of Convoluta thela comb. nov. and Heterochaerus australis were fixed in 2.5% (v/v) phosphate-buffered glutaraldehyde. Stefanini’s and glutaraldehyde-fixed specimens were washed in phosphate buffer (Millonig’s, 0.1 M), postfixed in phosphate-buffered 1% (v/v) osmium tetroxide, dehydrated in acetone, and embedded in EMBed/Araldite epoxy resin. Dehydration was quickened by microwave radiation (Samsung oven, two 7-sec irradiations at 650 W separated by 20-sec interim, with specimen-vial on ice and with water ballast of two filled 300-ml beakers (Giberson & Demaree 1995). Serial thick sections of 1.5 µm were made according to Smith and Tyler (1984) using a diamond knife mounted in a Butler trough (Butler 1979) and stained with toluidine blue. Ultrathin sections were cut using a Sorvall Porter-Blum MT2-B ultramicrotome equipped with glass knives, stained with an aqueous solution of uranyl acetate and lead citrate and examined with a Philips electron microscope 201. Digital images were acquired with a CCD Camera (BioScan, Gatan).

Musculature was revealed through F-actin staining of whole mounts with fluorescently labeled phalloidin (Alexa 488; Molecular probes, Eugene, OR) according to Hooge (2001) and examination with a Leica TCS SP2 confocal laser scanning microscope. Histological sections, including Type material, have been deposited in the collection of the Naturhistorisches Museum Wien (NHMW), Vienna, Austria. We compared our newly collected material with conspecific type material from Prof. Rupert Riedl’s private collection, which is now held at the NHMW.

List of abbreviations

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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>afg</td>
<td>accessory frontal gland cell;</td>
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<td>b</td>
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<td>bursal nozzle;</td>
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<td>cop</td>
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<td>diatoms in digestive syncytium;</td>
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<td>evc</td>
<td>gland cell containing erythrophilic vesicles;</td>
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<td>fg</td>
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<td>female gonopore;</td>
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<td>lpo</td>
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<td>vacuolated parenchymal cell;</td>
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Results

Family Convolutidae Graff, 1905

Genus Convoluta Ørsted, 1843

Convoluta enelitta Antonius, 1968

(Figs. 1–2)

Material. 3ZooEVV 4646 and 3ZooEVV 4647, two sets of 1.5-µm-thick serial sagittal sections, living specimens in squeeze preparations, eight complete and four partially complete sets of serial histological sections, and twenty-one whole mount specimens for fluorescence microscopy.

Collection Locality. Kunduchi Beach (6°39'51.3" S, 39°48'22.6" E), Dar es Salaam, Tanzania. Numerous specimens collected from medium-grained sediment and green sheet-like algae in small pools from high and mid intertidal.

Description. Mature specimens are droplet shaped, 600–850 µm long and 340 µm wide, with ventrally enfolded sides (Figs. 1A, 2A). The epidermis is entirely ciliated, the
cilia are 5–6 µm long. The nuclei of the epidermal cells are sunk beneath the body-wall musculature.

The body-wall musculature is stronger on the ventral than on the dorsal side, and consists of outer circular muscles, inner longitudinal muscles running parallel to the body margin, and pore muscles originating at the mouth (Fig. 2A). The body-wall musculature is not continuous with that of the penis or vagina.

A statocyst, 25 µm in diameter, is present 100 µm behind the anterior tip. Green coloration is conferred by dense zoochlorellae, which are scattered throughout the parenchyma but are absent from the tissue of the seminal vesicle, seminal bursa, and bursal nozzle (Figs. 1A, B). Approximately 150 rhabdoid gland cells are scattered over the dorsal surface, each containing 25 orange rhabdoids that are 10 µm long and 1 µm wide. Mucus gland cells, 13 µm long and 8 µm wide, with homogenous cyanophilic content, occur on the dorsal side most numerous in the anterior third of the animal and at the posterior tip. Smaller mucus gland cells with cyanophilic granules are distributed ventrally at the lateral margins. At the anterior tip, a small frontal organ is present. Refractive concretions are scattered in the epidermis on the dorsal surface, most abundant behind the eyes and over the seminal bursa.

**FIGURE 1.** *Convoluta enelitta*; photomicrographs of living specimen. A. Dorsal view of whole specimen. Eyes are not seen in this focal plane. B. Dorsal view of male and female copulatory organs. Arrowheads point to the gland cells in the lateral extensions of the vagina.
**FIGURE 2.** *Convoluta enelitta*; whole mounts stained with Alexa-488-labeled phalloidin and viewed with confocal microscopy. A. Projection of ventral body-wall musculature. Asterisk marks the mouth. B. Projection of male and female copulatory organs. Arrows point to unspecific stained lateral extensions of vagina. C. Projection of male copulatory organ (in line with, but at a shallower depth than B). D. Lateral projection of male copulatory organ. Arrowhead points to erythrophilic cells in proximo-ventral part of penis.
The nervous system consists of a pair of ganglia lying anterior to the statocyst and one ventral and two dorsal longitudinal nerve cords. Two rectangular or star-shaped ocelli composed of orange-colored granules are located lateral to and slightly in front of the statocyst (data not shown).

The mouth is situated 250–300 µm behind the anterior tip. The margin of the mouth is strengthened by parenchymal muscles, which are connected to the body wall. The digestive central syncytium extends from behind the statocyst to the seminal vesicle and often contains small crustaceans.

The diffuse paired testes originate at the level of the statocyst and lie dorsal and lateral to the paired ovaries, which originate slightly behind the statocyst. At the level of the bursal nozzle mature sperm migrate to the ventral side of the body, reaching the paired antero-lateral openings of the seminal vesicle, which are 20 µm long canals surrounded by glandular tissue. Sperm are stored at the periphery of the seminal vesicle.

The female gonopore is positioned anterior to the male gonopore. The unciliated vagina is 130 µm long, lined with cyanophilic gland cells, is weakly muscular and connects to the seminal bursa caudally. The bursal nozzle is 30 µm long, directed ventrally, and has a "sorting apparatus" consisting of 15 cells. In a third of the specimens we examined, the distal end of the vagina possessed paired lateral extensions (Figs. 1B, 2B).

The male gonopore opens directly to the male copulatory organ, which consists of a muscular seminal vesicle with an invaginated penis. The seminal vesicle is connected to the body wall by parenchymal muscles. The penis is 170 µm long, curled backwards, and composed of outer circular, intermediate longitudinal, and thin inner circular muscles. The distal end of the penis is lined with the same cyanophilic gland cells as the vagina. Gland cells, also visible in live observation (Fig. 1B), fill the lumen of the penis. They have a polygonal surface, and their nuclei are sunken beneath the penis musculature into the vesicle. Caudally, within the seminal vesicle, these cells form a cellular bladder with extrusions positioned beneath the penis. At its proximal end, the penis is broader, its ventral epithelium is thicker, and its inner circular muscles terminate (Figs. 2C, D). Necks of cyanophilic gland cells penetrate the ventral epithelium of the penis, their cell bodies lie within the parenchyma outside the seminal vesicle. Few of these cyanophilic cells protrude into the distal part of the penis.

Remarks. We confirmed the conspecificity of our specimens with Convoluta enelitta through comparison of the type material and found only minor differences with Antonius's (1968) description. The "high-prismatic erythrophilic cells" in the ventro-proximal penis epithelium described by Antonius (1968) are the distal necks of gland cells that lie outside the seminal vesicle, the paired sperm openings of the seminal vesicle are situated ventro-frontally, the penis has an additional inner layer of circular muscles, and no body-wall musculature is continuous with that of the penis.

While most of the specimens we examined had a straight vagina, approximately one third of the mature animals possessed a T-shaped vagina. The variation in shape could be a temporary change caused by recent copulation. This explanation is supported by the apparent lock-and-key correspondence between the shape of the penis of C. enelitta and the T-shaped vagina. The differences mentioned above between specimens from the Red Sea and from Tanzania could indicate the existence of subpopulations of C. enelitta.
Genus *Convoluta* Ørsted, 1843

*Convoluta thela* comb. nov. (Antonius, 1968)  
(Figs. 3–5)


**Material.** 3ZooEVV 4648, one set of 1.5-µm-thick serial sagittal sections, and 3ZooEVV 4649, one set of 1.5-µm-thick frontal sagittal sections, living specimens in squeeze preparations, seven sets of serial histological sections, and 10 whole-mount specimens for fluorescence microscopy.

**Collection Locality.** Bawi Island, Zanzibar, Tanzania (6°8′48.6″ S, 39°7′57.1″ E), from fine-grained sand in tide pools at the low intertidal level, and Bawi Sandbank, Zanzibar, Tanzania (6°8′9.6″ S, 39°7′50.6″ E), from east side of sandbank, fine-grained sand near corals and *Thalassia* sp. at 2 m water depth.

**Description.** Mature animals are 700 to 900 µm long, 375 µm wide, and lack enfolded sides (Figs. 3A, 4A, 5A). The epidermis is entirely ciliated, the cilia are 5–6 µm long. The epidermal nuclei lie beneath the body-wall musculature, which consists of outer circular, longitudinal cross-over, and inner longitudinal muscles.

A statocyst, 25 µm in diameter, lies 100 µm behind the anterior tip. Yellow-brown coloration is conferred by zooxanthellae, which are scattered throughout the parenchyma. The number of symbiotic algae varies among specimens. Rhabdoid gland cells are scattered across the entire surface, with especially high concentrations at the anterior end of the body. The refractive needle-shaped rhabdoids are 1 µm thick and up to 28 µm long, and tightly packed in the rhabdoid gland cells (Fig. 3C). The formation of rhabdoids appears to start with cyanophilic vesicles, which fuse progressively. When fixed with glutaraldehyde and stained with toluidine blue, the rhabdoids are preserved and purple, but when fixed with Stefanini’s or Bouin, they dissolve. Greater abundance of the rhabdoid gland cells causes replacement of the zooxanthellae and the margin of the body to look translucent. A well-developed frontal organ composed of cyanophilic mucus gland cells is present anterior to the mouth (Fig. 5B).

The nervous system consists of a mass of nerve tissue in front of and lateral to the statocyst, and four pairs of longitudinal nerve cords (ventral, ventro-lateral, dorso-lateral, and dorsal). Two ellipsoid ocelli composed of brownish granules lie lateral to the statocyst (Fig. 3D).

The mouth is situated slightly in front of the middle of the body. The digestive central syncytium extends from behind the statocyst to the seminal vesicle and sometimes contains crustaceans.

The paired testes originate behind the statocyst and mature toward the seminal vesicle where sperm sometimes form false seminal vesicles. Mature sperm enter the seminal vesicle through lateral openings (Figs. 3B, 5A). The paired ovaries are positioned ventrally near the median line.

The female gonopore lies anterior to the male gonopore, separated from it by a short length of body wall. The female gonopore opens to an unciliated vagina that is surrounded by a sphincter, 35 µm wide, 12 µm thick, and composed of 8 distinct muscle fibers. The vagina leads to a seminal bursa with a single bursal nozzle that is 60 µm long, directed antero-ventrally and curved dorsally (Figs. 4B, 5B).
A well-developed sphincter consisting of body-wall musculature and penis musculature surrounds the male gonopore. The straight, muscular penis is 35 \( \mu \text{m} \) long, 25 \( \mu \text{m} \) wide, and has a lumen 13 \( \mu \text{m} \) wide. It is inserted into a muscular seminal vesicle. The penis musculature consists of longitudinal muscles and an irregular meshwork of circular fibers surrounding them (Fig. 4C). Toward the proximal end of the penis the longitudinal muscles branch repeatedly. The branched muscles appear to attach to the cell borders of gland cells described below (Fig. 4B). The muscular, spherical seminal vesicle is 100 \( \mu \text{m} \) high and 75 \( \mu \text{m} \) wide when filled with sperm and glandular secretions. At the distal end it is filled with gland cells with a homogeneous content. The nuclei of these cells lie on the outer wall of the penis, or within the parenchyma. In this case the cells extend through the distal wall of the seminal vesicle. A second type of gland cell, producing erythrophilic vesicles, fills the region of the seminal vesicle that is proximal to the cyanophilic gland cells. The nuclei of

these cells lie within the parenchyma, scattered among the muscles of the seminal vesicle or sometimes within the seminal vesicle. Erythrophilic vesicles can also be found free within the seminal vesicle.

Two prostatoid organs, 38 μm long and 20 μm in diameter, are present, mostly positioned at the body midline posterior to the male copulatory organ (Figs. 3B, 4A, 5). A prostatoid organ consists of a muscular vesicle surrounding a cone of circular muscles. Several muscle fibers connect the proximal end of the cone to the vesicle. The body-wall musculature
is not continuous with the musculature of the prostatoid organs. Gland cells producing erythrophilic vesicles surround and protrude with their distal necks into the prostatoid organs (Fig. 5C). These gland cells are identical to those associated with the seminal vesicle. The number of erythrophilic vesicles stored in the prostatoid organs is always positively correlated with the number present in the seminal vesicle—i.e., full before and empty after copulation. We examined the arrangement of prostatoid organs in 19 specimens of Conaperta thela comb. nov. and found the following patterns: in one specimen, one prostatoid organ was positioned at the body midline, anterior to the female gonopore and a second prostatoid organ was present at the body midline posterior to the male copulatory organ; in one specimen, one prostatoid organ was positioned at the body midline anterior to the female gonopore, and a second at the body midline posterior to the female gonopore; in

FIGURE 5. Convolvula thela comb. nov.; reconstructions to show arrangement of organs. A. Dorsal view showing the two most abundant distributions of prostatoid organs. The gonads are paired but for clarity just the right testes and left ovaries are shown. B. Sagittal reconstruction of whole specimen. C. Sagittal reconstruction of male copulatory organ and prostatoid organ. Insunk nuclei of the epidermis are not illustrated.
4 specimens (2 immature) two prostatoid organs were present lateral to the female opening (Fig. 5A right); in 11 specimens 2 prostatoid organs were positioned at the body midline posterior to the male copulatory organ (Fig. 5A left); in two immature specimens they were entirely absent.

**Remarks.** On the basis of the new material we could verify the validity of *Convoluta thela* comb. nov. (see Antonius 1968, page 342), which was described with a single specimen. Antonius (1968) placed the species *Convoluta krana* comb. nov. and *Convoluta thela* in the genus *Conaperta* on the basis of having a common gonopore or better the female and male copulatory organs to open out into a unciliated common genital atrium (Antonius 1968, page 358). However, our investigation of the holotype of *C. thela* and two out of four serial sections from the type material of *C. krana*, revealed that the pores are separated by a short length of ciliated body wall. In freshly collected specimens of *C. thela* examined by confocal microscopy the pores can be seen to be set apart by circular body- wall muscles and in histological sections the pores are separated and surrounded by a ciliated body wall. Consequently, we transfer both species to the genus *Convoluta*.

Antonius (1968) reported that his Bouin fixed specimens of *Convoluta thela* possessed mucoid gland cells throughout the body wall and rhabdoid gland cells that were present solely outside the body-wall musculature. However, the preservation of rhabdoids differs with use of various fixatives (Martin 1978, Smith *et al.* 1982, Smith & Tyler 1984). Comparing our Stefanini’s- and glutaraldehyde-fixed specimens of *C. thela*, it is likely that the rhabdoids in Antonius’s (1968) material dissolved in Bouin’s fixative and lead to a misinterpretation of mucous gland cells. The small rhabdoids described by Antonius (1968) were possibly the distal tips of larger rhabdoid gland cells.

Antonius (1968) described muscles of the male copulatory organ, which insert at the body-wall musculature, penetrate the seminal vesicle, and attach to the penis (Retraktoren des Penis, PR, sensu Antonius 1968). We found no similar muscles in the specimens investigated.

The presence of lateral prostatoid organs in immature animals, and the strict occurrence of two prostatoid organs in all specimens, points to a paired lateral origin of these organs. The variable distributions of the organs could be intermediate stages in the migration towards the median line of the tail. This interpretation is also strengthened by the fact that in all other *Convoluta*-species possessing prostatoid organs, they occur in a paired, lateral distribution.

In our examination of the type material of *C. krana* we found in one specimen one prostatoid organ lateral to the female gonopore and a second in a median, posterior position (Rupert Riedl-Collection #12.685). In three others the prostatoid organs were distributed in the common median, posterior pattern.

The differences between the specimen described from the Red Sea and the specimens from Tanzania may indicate the occurrence of subpopulations in *C. thela*.

**Genus Heterochaerus Haswell, 1905**

*Heterochaerus australis* Haswell, 1905

(Figs. 6–7)

**Material.** 3ZooEVV 4650 and 3ZooEVV 4651, two sets of 1.5-µm-thick serial sagittal sections, living specimens in squeeze preparations, two partial sets of serial histological sections, and 14 whole-mount specimens for fluorescence microscopy.

**Collection Locality.** Maruhubi Beach, Zanzibar, Tanzania (6°8’39.7” S, 39°12’29.4” E). Samples of green-colored sand were taken in high intertidal zone of a sandflat from
small pools of water with many small black snails. Thousands of *H. australis* occurred in this sediment.

**Description.** Mature animals are up to 4 mm long, 1.5 mm wide, and their lateral margins are not enfolded. The coloration is variable (Figs. 6A–C). Animals typically move...
with cilia, but also flap their lateral edges when swimming. Cilia, 8 µm long, occur on the entire body surface but are absent between the male gonopore and female gonopore. The epidermal nuclei are not sunken beneath the body-wall muscles. The body-wall musculature consists of outer circular muscles, longitudinal cross-over muscles, and longitudinal muscles.

A statocyst, 16 µm in diameter, is present 300 µm behind the anterior tip. Two dark brown ocelli lie lateral to the statocyst. Numerous zooxanthellae, 10–25 µm wide, are scattered throughout the parenchyma. The parenchyma consists of an outer dense peripheral parenchyma and an inner parenchyma with large vacuolated spaces. Cyanophilic rhabdoid gland cells with their nuclei sunken beneath the body wall and containing short, needle-shaped rhabdoids occur on the entire surface. Epidermal concrements, silver colored in reflected light, are variably distributed on the dorsal side (Figs. 6 A–C). Common mucous gland cells are absent, as is a frontal organ.

Nervous tissue surrounds the statocyst and forms a complex at the anterior tip. Three ventral and two dorsal pairs of nerve cords run caudad from it.

The mouth is positioned at about half of body length.

The paired testes lie in the dorsal peripheral parenchyma, but spermatids and sperm often migrate into the vacuolated parenchyma. Spermatozoa are 35 µm long and sometimes form false seminal vesicles before entering the seminal vesicle dorso-laterally. The paired ovaries lie in the ventral peripheral parenchyma, more medi ally than the testes, rarely extending into the spaces of the vacuolated parenchyma.

The female gonopore is a 110-µm-wide, transverse slit, and lies 80 µm in front of the male gonopore. The vagina is dead-ended at its proximal end and lined by high prismatic cells, cells containing cyanophilic vesicles, and erythrophilic gland cells. It opens to a muscular bursa frontally (Figs. 6D, 7). The bursa is T-shaped, with a distal unpaired part splitting proximally into two paired lobes. Each bursal lobe contains 1–11 bursal nozzles (Fig. 6E). Often the number of nozzles in the lobes is unequal. Each bursal nozzle is 70 µm long, directed antero-ventrally, and is often bent in a C- or S-shape (Figs. 6E, F). The bursal nozzle consists of a stack of short cells, which contain cyanophilic vesicles and are visible even in squeeze preparations (Fig. 6F) and some elongate cells that are directed caudally

![FIGURE 7. Heterochaerus australis; reconstruction to show arrangement of male and female copulatory organs.](image-url)
and interconnect with the bursal tissue. The lobes appear somewhat spongy in histological sections where the interconnecting bursal nozzle cells meet them. At the proximal end of the nozzle is a rosette of cyanophilic bursal nozzle cells.

The male gonopore is a 90-µm-wide transverse slit positioned 350 µm in front of the posterior end (Figs. 6D, 7). The highly glandular penis is 300 µm long, curled backwards, and invaginated into a muscular seminal vesicle. The nuclei of the glandular cells, containing cyanophilic vesicles, are sunken beneath the penis musculature, into the seminal vesicle. The size and number of vesicles varies highly within the gland cells. The penis musculature consists of inner circular muscles and outer longitudinal muscles. A glandular penis fold is invaginated into the caudal side of the seminal vesicle (Fig. 7). It is lined with the same gland cells as the penis frontally but erythrophilic gland cells caudally.

Remarks. *Heterochaerus australis* was originally described by Haswell (1905) from shallow rock-pools in Port Jackson, New South Wales, Australia. Later Winsor (1990) reported the species from subtidal sand on Orpheus Island and from Queensland, Australia. Dörjes and Young (1973) found *H. australis* in small pools on a sandy beach at Mombassa, Kenya, approximately 135 km north of our collection site in Zanzibar. The presence of *H. australis* at different sites in Australia as well as in Mombassa, Kenya, and Zanzibar, Tanzania, suggests that this species may be widely distributed throughout the Indian Ocean.

According to Haswell (1905), *H. australis* has three pairs of longitudinal nerve cords, but we found two additional pairs of ventral nerve cords. Haswell (1905) described “small” and “large” types of sperm. We found only the large type of sperm and suspect that his “small type” represents developing spermatids.

Neither Haswell (1905) nor Winsor (1990) recorded the presence of a penis fold, perhaps because, as fixation causes partial protrusion of the penis, it can be obscured.

A vagina configured like that of *H. australis* was shown in *Polychoerus carmelensis*, Costello & Costello 1938a (Costello & Costello 1938b) and *Amphiscolops japonicus* Kato, 1947.

Genus *Picola* gen. nov.

**Diagnosis.** Convolutidae with one bursal nozzle. Male copulatory organ with seminal vesicle composed of a few muscle pairs and a glandular bladder at its distal end that surrounds an actin-rich penis-like structure.

**Type species:** *Picola renei* sp. nov.

**Etymology.** Genus name is a derivation of the Italian word *piccola* (small), and refers to the species’ extraordinarily small size for a convolutid.

*Picola renei* sp. nov. (Figs. 8–12)

**Type Material.** Holotype: 3ZooEVV 4652, one set of 1.5-µm-thick serial sagittal sections of epoxy-embedded specimen stained with toluidine blue. Paratype: 3ZooEVV 4653, one set of 1.5-µm-thick serial sagittal sections of epoxy-embedded specimen stained with toluidine blue.

**Type Repository.** Natural History Museum Vienna (NHMW), Austria

**Type Locality.** Bawi Sandbank, Zanzibar, Tanzania (6°8’9.6” S, 39°7’50.6” E). Eastern side of sandbank in fine-grained sand near *Thalassia sp.* and corals at 2 m water depth.

**Other Material examined.** Living specimen in squeeze preparations, two complete sets of serial sections of epoxy-embedded specimens stained with toluidine blue, 13 wholemounts for fluorescence microscopy.
**Etymology.** Species named in honor and memory of Rene Mähr.

**Description.** Mature animals are 300–400 µm long and up to 190 µm wide (Figs. 8A, 9A). The epidermis is 4 µm thick, its nuclei are not sunken beneath the body-wall musculature. The epidermis is entirely ciliated, with cilia that are 6 µm long. The body-wall musculature consists of outer circular muscles, cross-over muscles, and few inner longitudinal muscles.
On the ventral side, approximately three pairs of longitudinal muscles run along the lateral margins. Two pairs of longitudinal muscles are situated centrally and extend caudally, one originating at the posterior edge of the mouth, the other between mouth and lateral margin. Longitudinal cross-over muscles are present. The outermost branch out at the posterior end. Three to five U-shaped muscles extend longitudinally from the anterior tip of the body before bending around the posterior rim of the mouth (Fig. 9A).

A statocyst, 16 µm in diameter, is present 30 µm behind the anterior tip. Numerous yellow-brown zooxanthellae are scattered throughout the parenchyma. A few rhabdoid gland cells containing tightly packed, refractive, needle-shaped rhabdoids are distributed on the dorsal surface. Numerous frontal gland cells fill the anterior third of the animal, constituting a frontal organ. They merge in front of the statocyst and form a large reservoir before they protrude through the frontal pore. A few accessory gland cells containing vesicles lie ventral to the pore. Due to a fixation artifact, rhabdoid gland cells and cyanophilic mucus glands could not be distinguished but the presence of mucous glands is presumed.

The nervous system comprises nervous tissue around the frontal organ and statocyst; nerve cords could not be distinguished.
The mouth is positioned in the anterior half of the body. The digestive syncytium is inconspicuous, but often contains diatoms.

The diffuse testes are paired, lying dorso-lateral to the ovary, and originate in an unpaired germative zone behind the statocyst. Early spermatids contain fine granules that stain metachromatically pink and are joined in later stages by larger vesicles. Later stages get stained more intensively due to higher concentration of granules and vesicles. Droplet-shaped spermatids enter the seminal vesicle dorso-ventrally. Within the seminal vesicle a few mature spermatozoa are among many spermatids (Figs. 10A, C, 12A). The spermatozoa have axial microtubules in the cytoplasm and axonemes that lack central microtubules.
Due to serial ultrathin sections we can exclude the possibility that erratically occurring electron-dense material within the axonemes, could be a central microtubule or a central pair of microtubules (data not shown).

The unpaired ovary is positioned ventral to the testes. A female gonopore and vagina are both absent. The well-developed seminal bursa has a distinct wall and a bursal nozzle that is 16 µm long and directed antero-ventrally in sectioned animals. The sperm within the seminal bursa lack metachromatic granules, but the vesicles described above are present. Within the vestibulum the sperm have a homogenously stained cytoplasm (Figs. 10A, B, 11A, B).

FIGURE 11. Picola gen. nov. renei sp. nov.; reconstructions to show arrangement of organs. A. Dorsal reconstruction of whole specimen. B. Sagittal reconstruction of whole specimen.

The male gonopore lies in a subterminal indentation of the body wall (Figs. 9B, C, 10C, 11B). The seminal vesicle consists of four pairs of bridge-like, bilateral muscle pairs that run from the ventral body wall underlying the seminal bursa to the subterminus of the body where they connect to the outermost longitudinal cross-over muscles of the ventral body wall. Muscle fibers of the seminal vesicle are connected to the dorsal body wall by other parenchymal muscles attaching mid-fiber (Figs. 9B, C). Special multilayered tissue
surrounds the seminal vesicle (Fig. 12A). A 15 µm long, sclerotized penial structure, lies in a glandular bladder at the distal end of the seminal vesicle. Extensions of the glandular bladder connect to the male gonopore (Figs. 10C, 11B).

**Remarks.** The placement of *Picola renei* in the family Convolutidae is supported by characters of sperm ultrastructure, including the presence of axial cytoplasmic microtubules and the lack of central microtubules within the axonemes (Petrov *et al.* 2004). Other features in common with species of Convolutidae include the presence of zooxanthellae, rhabdoids, a frontal organ, a seminal bursa, a single bursal nozzle as well as a seminal vesicle that surrounds the penis. Besides these characters placing *P. renei* within the Convolutidae, no synapomorphies with other described species can be identified to tie it more closely with any given convolutid taxa. During copulation, the multi-layered tissue sac of the seminal vesicle is probably squeezed by the surrounding seminal vesicle muscles to eject sperm through the penial structure into a recipient. The sclerotization of this apparatus implies it is capable of hypodermal injection of sperm, which could explain the lack of a female gonopore and a vagina.

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**References**

Antonius, A. (1968) Faunistische Studien am Roten Meer im Winter 1961/62,


