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Redescription of *Xenodasys riedli* (Gastrotricha: Macrodasyida) based on SEM analysis, with first report of population density data

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Abstract During surveys of the Gastrotricha of the Tropical Northwestern Atlantic (TNWA, Caribbean Sea), we came across numerous specimens of the uncommon macrodasyidan, *Xenodasys riedli* (Xenodasyidae). Abundance data on gastrotrichs is rare and entirely absent for this species; moreover, there are no data on morphological variation of *X. riedli* outside its type locality (North Carolina, USA). Here, we provide new abundance data on specimens collected from St. John Island (US Virgin Islands), as well as new metric and morphological data from specimens collected on San Salvador Island (Bahamas), Tobago, and a sublittoral environment on the Atlantic Coast of Florida (USA). In the interstitial environments of St. John, *X. riedli* was most abundant at 0.8 m depth in moderately well-sorted sediments. It reached maximum abundance of 89.5 ± 42.7 ind./10^2 cm and made up 69.7% of the total taxocoenosis. Metric variation revealed that specimens at all sites in the TNWA and Florida had smaller body sizes than those recorded at the type locality, but showed only limited variation in the size and number of taxonomic characters. Observations of specimen from Florida using scanning electron microscopy (SEM) revealed details that were overlooked in the type description. For example, we observed 8 dorsal head plates (11 in the original description), 1 pair of anterior medial plates, and 3 ventral plates, the latter of which were not described in the type specimens. We confirm the existence of round scales on the dorsolateral margins, and note that spineless-scales are also present in between the spined scales on the lateral body wall. We also determined that the lateral spined scales possess dorsal and ventral spines instead of anterior and posterior spines, which was their original assumed position. This research reveals that SEM remains the best diagnostic tool for characterizing gastrotrich morphology, and should be part of all future studies of gastrotrich taxonomy.

Keywords Xenodasyidae · Caribbean · Meiofauna · Benthos · Biodiversity · Morphology

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Introduction

Xenodasys riedli Schöpfer-Sterrer, 1969 is a conspicuous gastrotrich described from several marine locations in the Western Atlantic. Together with three further species in two genera, *X. riedli* belongs to the family Xenodasyidae, known from across the globe (Todaro et al. 2006a).

The first described species, *Xenodasys sanctigoulveni* (original writing *sancti-goulveni*), was collected from Roscoff (northern France) by Swedmark (1967); it was later found at the same locality by Kiesielewski (1987), on the Faroe Bank by Clausen (2004), and in the "Faroe-Islandic Sill" (video database by Hummon, cited in Clausen 2004). A second species, *Chordodasys riedli* (now *X. riedli*), was found off Beaufort, North Carolina (USA) by Schöpfer-Sterrer (1969). This species was reported from several other locations including other North Carolina sites, Florida, Bermuda, U.S. Virgin Islands, Scottish Shetlands and probably South Africa (see Todaro et al. 2006a; Hochberg 2007;

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Hummon 2010). A third species, *Chordodasys antennatus*, was described from Beaufort, North Carolina (USA) (Rieger et al. 1974). Over the years, the three species have had a confounded systematic history, being placed in the families Dactylopodolidae, Turbanellidae, and Neodasyidae, based on interpretations of shared characters such as cross-striated muscles and cilia in the digestive system (d'Hondt 1970; Hummon 1974; Rieger et al. 1974). In 1982, Hummon reevaluated the morphology of the three species and stated that the differences between the two genera were not taxomomically significant, therefore, he transferred *C. riedli* and *C. antennatus* to *Xenodasys*, keeping them within the family Dactylopodolidae (Hummon 1982). Todaro et al. (2006a) described a new species, *X. eknomios*, from the sediments of a marine cave at Santa Maria di Leuca near Lecce, Italy, and once again reconsidered the systematization of the genus. They noted that *X. antennatus* lacked the dorsal cuticular plates shared by other species of *Xenodasys*, and so transferred it into a new genus, *Chordodasiopsis* (see Todaro et al. 2006a for further details). In the same paper, Todaro et al. (2006a) established the family Xenodasyidae, to include species of both *Chordodasiopsis* and *Xenodasys* .

The Xenodasyidae appear to be a well-established monophyletic taxon, defined by the presence of a pair of segmental tentacles, a pair of lateral fused adhesive tubes (Seitenfüsschen), a muscular chordoid organ in the posterior body region, and the presence of cilia in at least parts of the pharynx and intestine (see Todaro et al. 2006a for complete diagnoses of family and genera). Subsequent phylogenetic analyses based on molecular markers (e.g., Todaro et al. 2012, 2014, 2015) have always resolved Xenodasyidae as a clade separate from Dactylopodolidae, thus providing support for the systematization of Todaro et al. (2006a) based on morphological characteristics.

The unusual morphology of species of Xenodasyidae has fascinated researchers for more than half a century, which explains the constant efforts to reevaluate their systematic position. However, these efforts have largely ignored studies of phenotypic variation within species, which sheds new light on the differences among populations and is the basis for evolutionary considerations and species delimitation. Here, we provide new data on both metric and meristic variation in *X. riedli* across three Caribbean locations and a sublittoral site in the southwestern Atlantic, together with the first estimates of a single population density.

Materials and methods

Specimens of *Xenodasys riedli* were collected during a series of surveys on three Caribbean islands, i.e. St. John Island (US Virgin Islands; M.A. Todaro), San Salvador (Bahamas; A. Schmidt-Rhaesa) and Tobago (R. Hochberg), and in parallel studies conducted at Capron shoals in Florida (S. Atherton and R. Hochberg).

On St. John Island, sampling took place during February 2010. *Xenodasys riedli* was found in only one of the eight investigated locations: Little Lameshure Bay (18°19.2′N, 64°43.6′W) (see Hummon et al. 2010). Qualitative and quantitative sampling were performed at this location. For qualitative sampling, about 4 l of sediment from the littoral and shallow sublittoral areas (0.5–2.5 m water depth) were collected using 0.5-l plastic jars following a well-established procedure (see Todaro 2002; Todaro et al. 2014). In the laboratory, the specimens were extracted daily, within 1 week of collection, applying the narcotization–decantation technique using a 7% $MgCl_2$ solution; the supernatant was poured into plastic Petri dishes (3 cm diameter) and scanned for gastrotrichs at a maximum magnification of ×50 under a Wild 3 stereomicroscope (Todaro and Hummon 2008). Each gastrotrich was mounted on a glass slide and observed in vivo with Nomarski differential interference contrast optics using a Zeiss Axio Scope A1. During observation, the specimens were photographed with a DS-5 M Nikon digital camera and measured using the Nikon NIS-F v.4.0. software. Several specimens were fixed in 95% ethanol and stored for DNA analysis. Some of these specimens were used for DNA sequence data and published by Todaro et al. 2012.

For the quantitative sampling, three series of four replicate cores where obtained from a transect with collection sites located at (1) mid-water mark (littoral), (2) 0.8 m and (3) 1.5 m water depth (shallow sublittoral). Cores of 2.7 cm diameter × 10 cm height were sampled by hand using 50-ml syringes with the tip cut off. Each replicated core was extruded into a plastic jar, after the fauna was first anesthetized using a 7% $MgCl_2$ solution for 10 min then fixed with a borax-buffered 5% formalin. Some days later, the fauna was extracted by the flotation method and multiple decantations with a 30-μm-mesh sieve were used to concentrate the meiobenthos (Todaro et al. 2006b). The latter were subsequently stored in jars with 5% formalin and brought to the laboratory in Modena (Italy) for identification and counting. Meiofaunal organisms were transferred into Petri dishes and, with the aid of a stereomicroscope, subdivided into major taxonomic groups, counted, and preserved in formalin for future checks. The gastrotrichs were identified to species directly using the stereomicroscope or with the aid of DIC optics on a compound microscope. Some specimens of *X. riedli* were prepared for scanning electron microscopy (SEM) according to Todaro et al. (2015).

At San Salvador (June 2011), samples were collected with plastic jars by skin diving at 3 m depth in front of a lagoon near a location called "Sandy Hook" (23°57.828′N, 74°29.220′W) in the southeast of the island. The sediment consisted of medium to fine sand. The anterior fragment of a single specimen was found in a sample collected at about 3 m

depth in Rice Bay (24°07.289′N, 74°27.038′W) in the northeast of San Salvador. At Tobago, samples were taken with SCUBA from three sublittoral locations at Goat Island (20 m depth: 11°18.023′N, 60° 31.028′W), Japanese Garden (22.5 m depth: 11°17.863′N, 60°31.135′W), and Highway to Heaven (30 m depth: 11°20.154′N, 60°38.539′W). Specimens from Tobago and San Salvador were extracted, processed, and studied at makeshift laboratories on their respective islands. Sorting was carried out with a Leica S6E stereomicroscope and pictures and videos were recorded with a Sony HDR-XR 550 VE camera on a Zeiss A1 compound microscope (see, e.g., Kieneke et al. 2013). Specimens were otherwise fixed as process following the protocol for St. John.

In Florida, specimens were collected with a small anchor dredge (29 × 12 cm opening) from a sublittoral site, Capron Shoal (3–5 m depth: 27°29.78′ N, 80°13.73′ W), off Fort Pierce. The dredge was generally deployed for 6–8 min along the shoals. Sampling with dredge took place over the course of several summer campaigns (July–August) from 2005 to 2014. Dredge samples were placed in buckets and the animals were extracted within 3 days at the Smithsonian Marine Station at Fort Pierce. Extracted animals were measured with an ocular micrometer on a Zeiss A1 compound microscope equipped with DIC, and then photographed and digitally recorded with a Sony Handycam. Animals were prepared for SEM following the protocol of Hochberg (2007) and examined with a Philips JEOL JSM 6390 SEM at the University of Massachusetts Lowell.

Results

Findings and abundance

With the exception of samples from St. John, abundance estimations were derived from purely qualitative sampling efforts. Therefore, our quantitative estimates of population densities of *X. riedli* were based on only a single sampling location.

At St. John, *X. riedli* was present exclusively in sublittoral sediments; in general, the animals were more abundant in shallower sediments made up of medium, moderately well-sorted sand compared to deeper substrata composed of fine, moderately well-sorted sand. In quantitative samples collected at 0.8 m depth, we found a total of 212 specimens of *X. riedli*, corresponding to a population density of 89.5 ± 42.7 ind./10 cm^2 (mean ± SD), whereas in samples collected at 1.5 m depth, we found 47 individuals, corresponding to a population density of 19.8 ± 30.4 ind./10 cm^2. *Xenodasys riedli* was the dominant gastrotrich at both sites making up 69.7% and 33.5% of the total taxocoenosis at 0.8 m and 1.5 m depths, respectively. Further details on gastrotrich species richness and distribution along the St. John Island coast will be provided in a forthcoming paper. However, we would like to emphasize that the abundance of *X. riedli* present at Little Lameshure Bay ranks among the highest population densities ever recorded for a marine gastrotrich. The highest recorded densities were those of *Turbanella hyalina* with 1718 ind./10 cm^2 recorded from North Gare Sand (more precisely the value refers to the entire gastrotrich fauna, being composed almost exclusively of *T. hyalina*; Gray 1971) and *Oregodasys ocellatus* (reported as *Platydasys ocellatus*) with 139 ± 69 ind./10 cm^2 recorded in January 1997 at the Meloria shoals, Italy (Todaro 1998).

Morphology

Our studies on the morphology of *X. riedli* from three Caribbean locations and Florida largely agree with the type description provided by Schöpfer-Sterrer (1969). However, we note that several morphological differences were found in populations across the species' geographic range. All specimens were dorsoventrally flattened and elongate with a bifid caudal end (Fig. 1a–c). The body length indicated by Schöpfer-Sterrer (1969) is 490–603 μm; specimens from Florida were 230–355 μm long, from St. John 395–405 μm, and from San Salvador 335–350 μm, while those from Tobago displayed a broader range of body size variation (350–515 μm), though never reaching the total length of the type specimens (see Table 1 for measurements).

Locomotory cilia were organized in a pair of ventral longitudinal columns (Figs. 2d, f, 4b) that begin just posterior of the mouth and extend to the chordoid organ at the caudal end just beyond the anus. The ciliary columns were approximately 5–6 μm wide. According to Schöpfer-Sterrer (1969), the bands connect at the level of the anus. This connection was not observed in any of our specimens from across the Caribbean or Florida (not shown). In our SEM prepared specimens, cilia were present as "paddle cilia", which is an artifact of fixation (Short and Tamm 1991).

Putative sensory cilia were present along the whole body but were not distributed evenly from anterior to posterior. In the head region, cilia were distributed loosely around the mouth opening and ventrolaterally at the border of the cuticular head plates (Fig. 2e). In the dorsal head region, cilia formed a fringe at the anterior margin of the broad cuticular plate D2 (see below) (Fig. 4c, d). These cilia were approximately 5–7 μm long. Additional cilia extended onto the lateral head tentacles and projected from their base as well as along its length and tip (Figs. 2b, 3d, 4a–c); there were approximately 7–10 cilia along each tentacle.

In the dorsal trunk region, individual cilia were sparse but present, generally positioned lateral to the numerous dorsal cuticular knobs ("cone-like structures" of Schöpfer-Sterrer 1969) that extended down the length of the trunk. There were also individual cilia that appeared to project from the dorsal and dorsolateral spines. Each cilium was 2–3 μm long and in

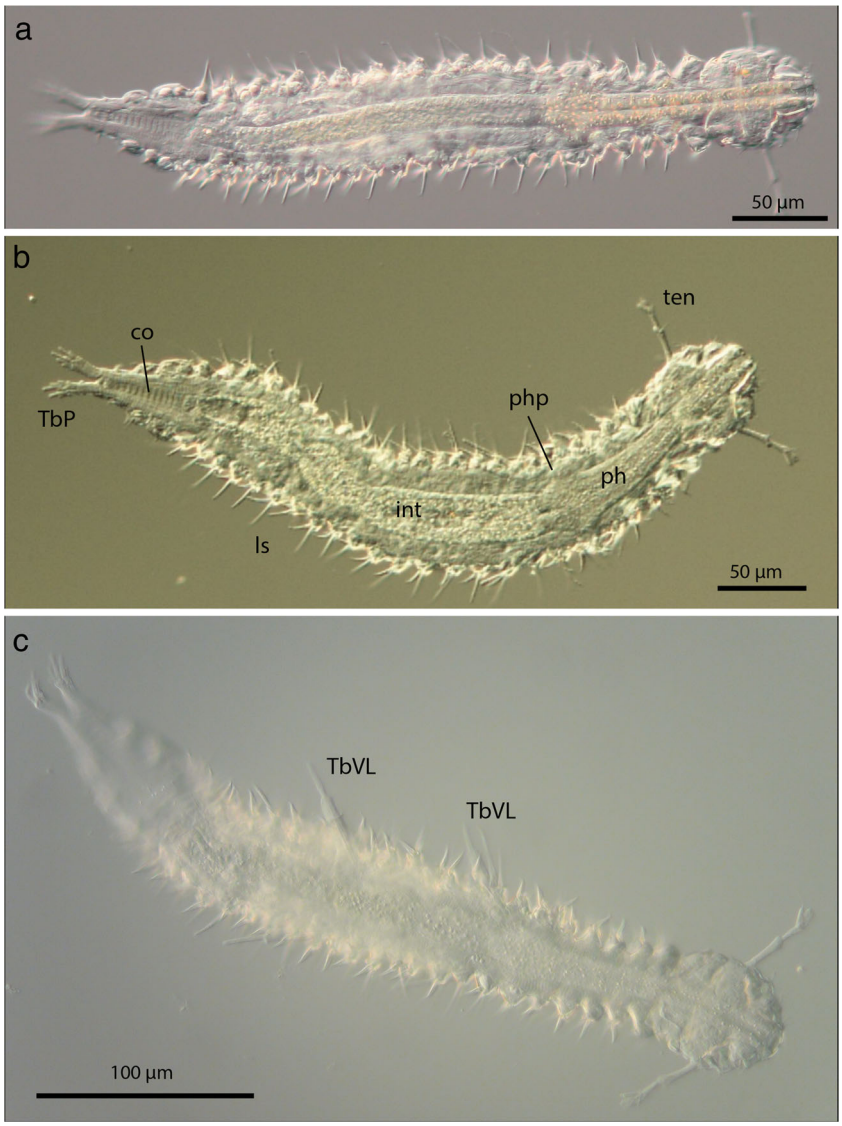

Fig. 1 *Xenodasys riedli*, DIC images from different locations in the TNWA **a**. Tobago specimen. **b**. St. John specimen. **c**. San Salvador specimen. co, chordoid organ; int, intestine; lsp, lateral spines; ph, pharynx; php, pharyngeal pores; TbVL, ventrolateral adhesive tubes; TbP, posterior adhesive tubes; ten, head tentacles

many cases appeared to project from the tip of the spine, but this could not be verified in all specimens.

The lateral head tentacles correspond to the description by Schöpfer-Sterrer (1969) (Fig. 3a, d, e). There was both a median and terminal swelling; the median swelling appeared to be joint-like, while the terminal swelling ends in a trifurcation. As reported above, single cilia were present on the swellings. The head tentacles varyied in size among specimens and were generally smaller than those reported by Schöpfer-Sterrer (1969; length 45–48 µm). For example, specimens from Florida had 31–45 µm long tentacles; specimens from Tobago 35–42 µm long (see Table 1).

Schöpfer-Sterrer (1969) described 11 cuticular plates that cover the head, although she acknowledged that the borders of many plates were difficult to discern. Our observations confirm many of her findings but also modify her descriptions and therefore require an alternative interpretation. While specimens from all locations appeared to show similar plate sizes and configurations based on DIC, we relied on SEM observations of Florida specimens to verify plate shapes and generate accurate measurements. We note that the plates were unlike scales, i.e., they were mostly fused to the general body cuticle, and while being well delimited had few free edges. In total, we observed eight dorsal plates (D1–D6, plates D4 and D6 are paired), two anteromedial plates (A), and three ventral plates (V). The plates that could be viewed from the dorsal side (both with SEM and DIC) were dorsal and anteromedial in position. The dorsal plates were arranged as follows: four single, median plates from anterior to posterior along the dorsal side of the head (D1, D2, D3 and D5); a pair of dorsolateral plates at the posterior margin of the head (D4); and a pair of dorsolateral plates (D6) that projected beneath D4 (Figs. 3b, c, 4a, c, d). The anteriormost dorsal plate (D1) was elliptical in shape and up to 30 µm wide and 12–15 µm long (Fig. 4a, c, d). Posterior to this plate was the much larger plate D2 (ca. 45 µm wide × 18–20 µm long) with a crescent-shaped anterior

Table 1 Measurements of specimens from the four investigated locations

	St. John	Bahamas	Tobago	Florida
Mean body length	399.2 μm (395–405 μm); $n = 4$	342.5 μm (335–350 μm); $n = 2$	475 μm (350–515 μm); $n = 8$	300 μm (230–355 μm); $n = 10$
Mean pharynx length	124 μm (123–127 μm); $n = 3$	112.4 μm (100–135 μm); $n = 3$	138 μm (120–162 μm); $n = 8$	96 μm (76–114 μm); $n = 8$
Position of pharyngeal pores at base	U29.5; $n = 1$	U30.2 (U26–U34.5); $n = 2$	U29–U31; $n = 8$	U28–U30; $n = 4$
Anus	79.5 μm (U79–U80); $n = 2$	U83.3 (82.9–83.6); $n = 2$	U78–U80; $n = 8$	U81; $n = 2$
Maximal width of head (minus antennae)	50.5 μm (48–53 μm); $n = 2$	35.0 μm (31.7–36.7 μm); $n = 3$	61 μm; $n = 8$	46 μm
Maximal width of body between spines	58.5 μm (57–60 μm); $n = 4$	33.3 μm (30.0–36.7 μm); $n = 3$	59 μm; $n = 8$	NM
Width at base of furca	16.7 μm (16–17.5 μm); $n = 4$	10.9 μm (10.0–11.7 μm); $n = 2$	17 μm; $n = 8$	NM
Mean antenna length	35.3 μm (34–37 μm); $n = 4$	28.3 μm; $n = 2$	40 μm; $n = 4$	31–45 μm
Length of TbA (medial to lateral)	7.1, 7.1, 12.5, 12.5, 6, 5.7 μm; $n = 1$	6.4, 12.4, 11.2, 6, 6, 6 μm; $n = 1$	7,7,13,13–14,6–7 μm; consistent for 23 adults	4 tubes ($n = 3$): 3–4, 6–7, 5–6, 3–4; 6 tubes ($n = 5$): 4–5.5, 7–11, 5.5–8, 6–7, 4–5,3–5.
Maximal length and position of first (lateral) TbVL (from "group 1")	30 μm; U38; $n = 1$	34.8 μm; U37; $n = 1$	33–35 μm; NM; $n = 8$	26–28 μm; U35–37; $n = 2$
Length and position of second (median) TbVL (from "group 1")	33 μm; U38; $n = 1$	36.1 μm; U37; $n = 1$	26–30 μm; NM; $n = 8$	30 μm; U35–37; $n = 2$
Length and position of third TbVL (from "group 2")	37 μm; U56; $n = 1$	30 μm; NM; $n = 1$	26–30 μm; NM; $n = 8$	22–25 μm; U58; $n = 2$
Length of TbP (medial to lateral)	5, 6, 12, 12, 6, 13 μm; $n = 1$	NM	6,7–8,6,14,10,7 μm); $n = 8$	4–5, 7–8, 6–6.5, 6.5, 5.5–5.9,4.4–5 μm; $n = 4$

When two or more specimens were measured, the mean value, range and number of analyzed specimens are given. Reliable measurements could not be made for each available specimen

NM not measured, TbA anterior adhesive tubes, *TbP* posterior adhesive tubes, *TbVL* ventrolateral adhesive tubes, *U* position in relation to whole body length (=100%), measured from anterior

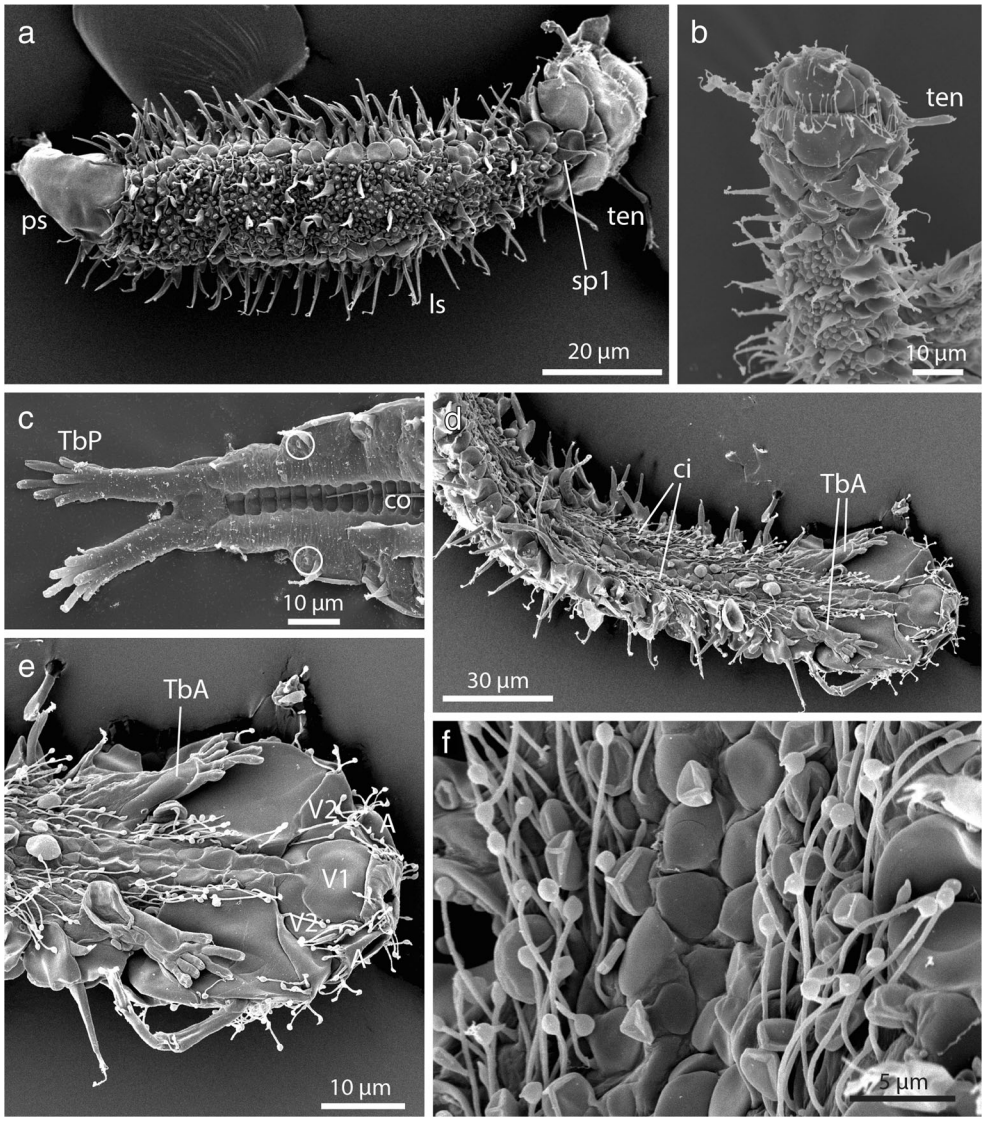

Fig. 2 *Xenodasys riedli* from Florida, SEM micrographs. **a** Dorsal view of the body divided into head region with tentacles, trunk with lateral spines (*ls*) and posterior region with smooth cuticle. Note the anteriorly-directed median first spine (*sp1*). **b** Dorsal view of head region with tentacles. **c** Ventral view of posterior end with TbP. The muscular chordoid organ is delineated by the step-like indentations of the ventral cuticle; the *circles* denote gland openings next to a pair of spines. **d** Ventral view of anterior region showing the position of TbA and longitudinal ciliary columns. **e** Closeup of ventral head showing three sets of cuticular plates. **f** Closeup of ventral ciliary region of trunk; the swelling of the cilia and the scale-like cuticle between the cilia are interpreted as fixation artifacts (see text for details). *A* anteromedial plate in head that projects from beneath D1 and D2, *ci* columns of cilia, *co* chordoid organ, *ps* posterior end with smooth cuticle, *ten* tentacles, *TbA* anterior adhesive tubes, *TbP* posterior adhesive tubes, *V1* singular ventral plate posterior of the mouth, *V2* paired ventral plates of the head

edge (Fig. 4a, c, d) that possessed two spines that projected anteriorly, each ca. 6–7 µm long (Fig. 4a). The lateral margins of the plate bent towards the side of the body and appeared to fuse with the body cuticle (not shown). The posterior edge of D2 appeared straight and bordered plate D3; it was covered along its posterolateral edges by a pair of bilateral plates, D4 (Fig. D4). Plate D3 was a small median plate with a slightly rounded, triangular shape (max. 15 µm wide at anterior edge × 10 µm long) (Fig. 4a, c, d). The paired lateral plates D4 appeared somewhat different with DIC and SEM. With DIC, each plate appeared trapezoid in shape and angled ca. 30–35° from the midline (Fig. 3b, c); the median and lateral borders and the anterior and posterior borders were mostly parallel to each other. With SEM, the trapezoid shape was observed to be artificial because the lateral edges bent ventrolaterally, meaning that these plates are more appropriately described as ovoid (see Fig. 4c, d). Each plate had a small hook-like protrusion at its posterior edge (Fig. 4d). Another median plate, D5, was oval in shape, approximately 10–13 µm wide and 8–10 µm long, and positioned immediately posterior to D3. D6 was a bilateral pair of plates positioned at the posterior border of D4 and appeared to extend

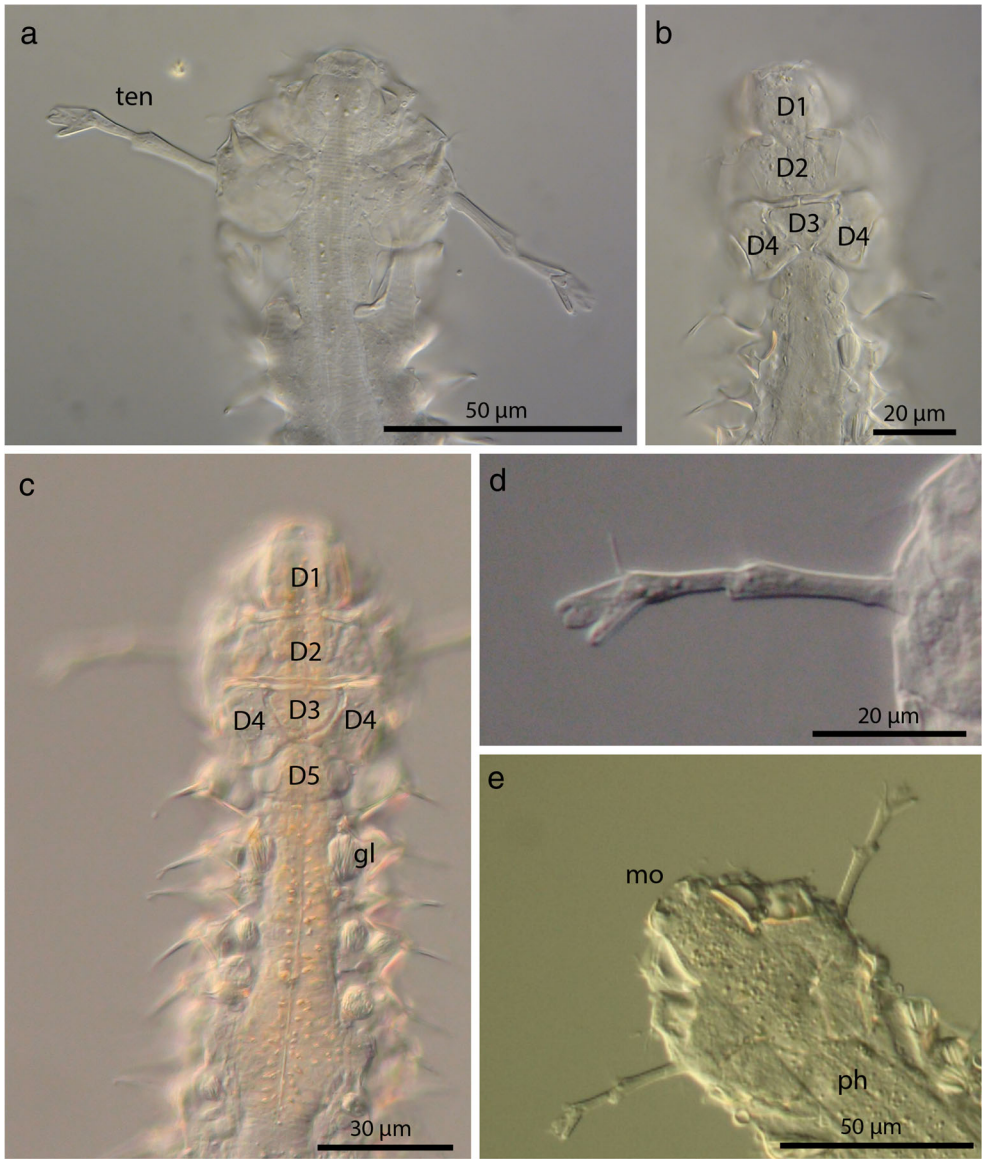

Fig. 3 *Xenodasys riedli*, views of the anterior end of specimens from different locations in the TNWA**. a** Ventral view of a specimen from San Salvador. **b** Dorsal view of a different specimen from San Salvador showing some of the head plates. **c** Dorsal view of a specimen from Tobago showing head plates. **d** Closeup of a head tentacle of a specimen from Tobago. **e** Specimen from St. John. *D1–D5* head plates, *gl* epidermal gland, *mo* mouth, *ph* pharynx, *ten* tentacle. (**a**) from Kieneke and Schmidt-Rhaesa (2015), with kind permission of De Gruyter, Berlin

beneath D4 (Fig. 4c). Tentative measurements revealed that the plates were at least 15 µm wide and ca. 11–12 µm long, with irregular lateral borders.

Anteriorly, there were two anteromedial plates (A) on either side of the mouth (Fig. 4a, b); both plates projected only 5–6 µm from beneath the two dorsal plates that covered them (D1, D2). With SEM, each plate could be seen to project at least 20–25 µm between D1 and D2.

Ventral plates are difficult to distinguish with DIC, but with SEM, we observed a single median ovoid plate (V1; ca. 13 µm wide × 10 µm long) posterior of the mouth, and a pair of crescent-shaped plates (V2) (Fig. 4b) on either side of V1. These plates were ca. 10 µm long and followed the curvature of the median plate. On either side of each curved plate was a large field of cuticle that was mostly continuous with the rest of the ventrum but displayed two lines (see arrows, Fig. 4b) that might delimit a pair of incomplete plates.

The entire body was covered with cuticle but only a few regions are devoid of any sculpture. For example, the dorsal cuticle at the posterior end (about U83–U100) had a smooth surface (Fig. 2a). As reported above, the ventrolateral cuticle of the head region was also without sculpture (Fig. 2e). The rest of the body had several types of cuticular projections including knobs, scales, spines, and spined scales (Fig. 5).

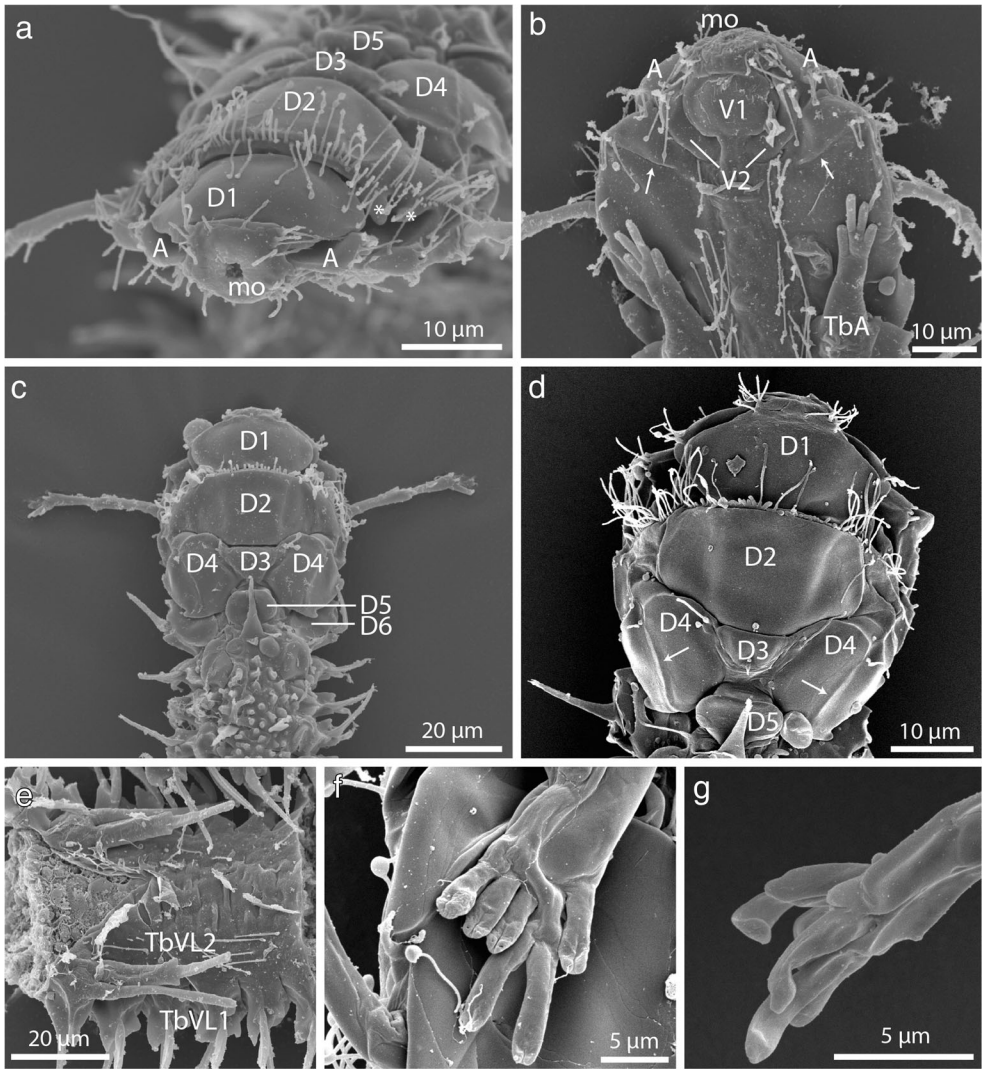

Fig. 4 *Xenodasys riedli* from Florida, SEM micrographs. **a** Anterior view of head showing the anteromedial and dorsal head plates; *asterisks* denote a pair of short spines on the free edge of plate D2. **b** Ventral view of head revealing plates V1, V2 and the anteromedian plates. **c** View of dorsal head plates. **d** View of a specimen with broken head tentacles. **e** Ventral view of a damaged specimen showing the first group of ventrolateral adhesive tubes. **f** Anterior adhesive tubes, anterior is down. **g** Single pedicle showing posterior adhesive tubes in lateral view. *A* anteromedian plates in the head, *D1–D6* dorsal plates in the head, *mo* mouth opening, *TbA* anterior adhesive tubes, *TbP* posterior adhesive tubes, *TbVL* ventrolateral adhesive tubes, *V1* and *V2* ventral plates in the head

The cuticular knobs were present along most of the dorsal cuticle and appeared to arise individually from circular scales that ranged from 2 to 4 µm in diameter; the knobs were approximately 1 µm diameter and 1 µm long (dk, Fig. 5a). Some scales had two knobs arising from a common base (black arrow, Fig. 5d) or had two knobs arising from separate scales that showed evidence of incomplete separation (white arrow, Fig. 5d). Among the knobs there were two columns of dorsal spiny projections (ds, Figs. 2a, 5d). A single 15-µm-long spine was positioned posterior to head plate D5 but projected anteriorly (Figs. 2a, 4c, d). The remaining spines, each 12–13 µm long, formed two lateral columns toward the posterior end, ending at ca. U83 where the cuticle became smooth. The columns of spines were not perfectly symmetrical, and several spines were not in line with the columns. The spine cuticle was mostly smooth and featureless, although some showd a ridge or keel along that imparted a tetramerous symmetry (Figs. 2a, 5a, b).

The lateral margins of the sculptured dorsal cuticle had three types of scales: smooth round scales (rs), spined scales (ss), and smooth spineless scales (sl) (Figs. 2a, 5a, b, e). The smooth round scales were positioned on the dorsolateral margins of the trunk to either side of the knobby surface (ds, Fig. 5a); scales varied in size from 6 × 8 µm to 7 × 11 µm. There was very little overlap among scales along the anterior–posterior axis. Slightly ventrolateral to these scales was a series of spined and spineless scales that appeared to alternate down the anterior–posterior axis (Fig. 5b). Both scale types appeared to wrap

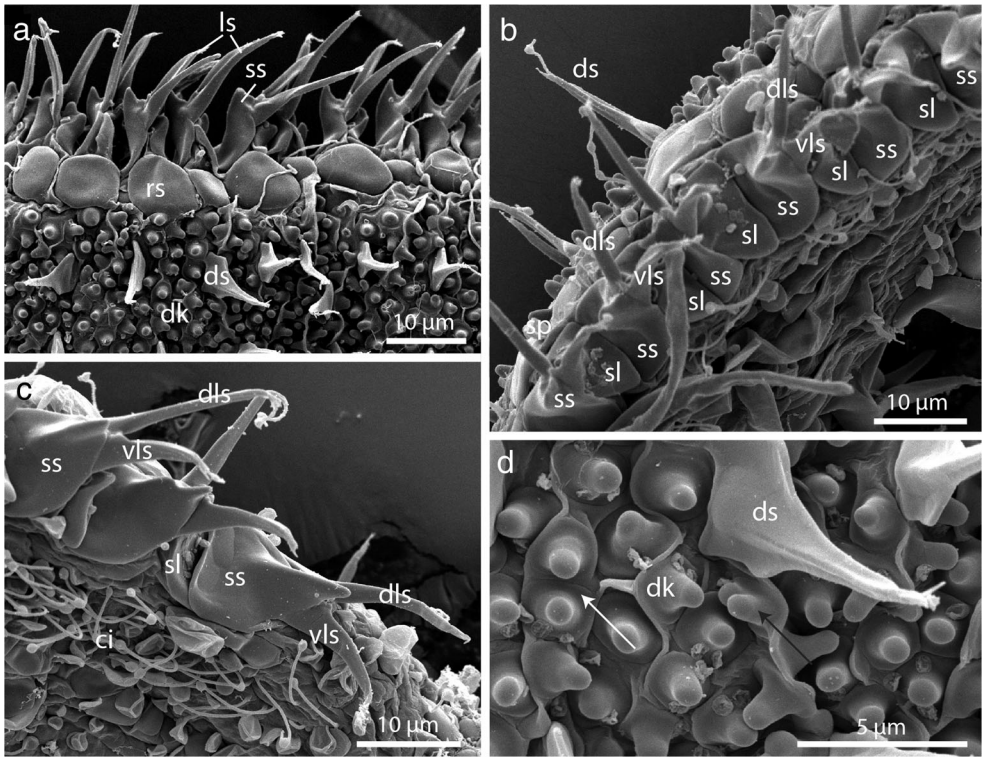

Fig. 5 *Xenodasys riedli* from Florida, SEM micrographs. **a** A portion of the dorsolateral cuticle showing the dorsal knobs, round dorsolateral scales, spined scales, and lateral spines; the spineless scales are not visible in this view. **b** Ventrolateral view of the mid-trunk region, anterior is to the *lower left*, ventral is to the *bottom*; the spined scales have both dorsolateral and ventrolateral spines, corresponding to the lateral (anterior and posterior) spines reported in Schöpfer-Sterrer (1969) and in (a); spineless scales alternate with the spined scales. **c** Ventrolateral view of a specimen showing the spined scales, anterior to the *upper left* and ventral is *down*; the swollen cilia (paddle cilia as fixation artifact) are seen on the ventral side. **d** Closeup of the dorsal cuticle revealing the dorsal knobs and dorsal scales; the *black arrow* points to two fused dorsal knobs on a single scale and the *white arrow* points to two fused dorsal scales with separate knobs. *ci* cilia, *dk* dorsal knobs, *dls* dorsolateral spines, *ds* dorsal spine, *ls* lateral spines, *rs* round dorsolateral scales, *sl* spineless scales, *ss* spined scales, *vls* ventrolateral spines

around the lateral body surface (Fig. 5c). The spined scales were fused to the general body cuticle at the anterior edge while the posterior edge projected free. The scale bodies were 8–11 µm in length. All scales had a median ridge along their anterior–posterior axis that forms a singular blunt tip at the free end of the scale (Fig. 5c). On some scales, the ridge had a notch, thereby forming a short pointed tip or spine on either side of the notch (Fig. 5c). These tips are likely to be the "thorns" described by Schöpfer-Sterrer (1969). A pair of long spines (dls, vls) projected from the dorsolateral (dls) and ventrolateral (vls) edges of the scales. These spines were 11–16 µm long (Fig. 5b, c) and often curved. Some spines appeard to articulate with the posterior face of the scale rather than the edge (Fig. 5c). The spines were also quite delicate and readily broke off the scale in some specimens (not shown). Most animals had 24–25 pairs of spined scales (Fig. 1a–c).

The ventral cuticle between the columns of cilia appeared mostly without sculpture under light microscopy but observations with SEM revealed some variation. Specifically, some areas showed evidence of a scale-like pattern (Fig. 2f), but we interpret this feature as a fixation artifact because the pattern was not consistently present in any one specimen or among specimens. We also note that many of the locomotory cilia had peculiar shapes (Fig. 2f). The swollen terminal ends of the cilia, which makes them appear as paddles, were a common fixation artifact (see Short and Tamm 1991). Posteriorly, there was a series of step-like indentations in the cuticle around the region of the chordoid organ (described below; Fig. 2c) that corresponded to its internal structure.

Anterior adhesive tubes (TbA) were borne on fleshy hand-like extensions of the cuticle as described by Schöpfer-Sterrer (1969) (Figs. 2d, e, 4b, 6a–c). In the original description, six TbA per side were described: one short, two longer and three medium-sized tubes (from medial to lateral). This pattern was present in most specimens from the Bahamas (Fig. 6a), St. John, and Florida (Fig. 2e, 4f), but other patterns were also found. For example, several specimens from Florida possessed only 4 tubes: a short medial tube (3–4 µm), two longer tubes (6 µm), and a short lateral tube (3–4 µm). Also, some specimens from Tobago (Fig. 6b) and some from St. John (Fig. 6c) had only five tubes, as one of the medial, medium-sized tubes was absent (see Tab. 1 for measurements).

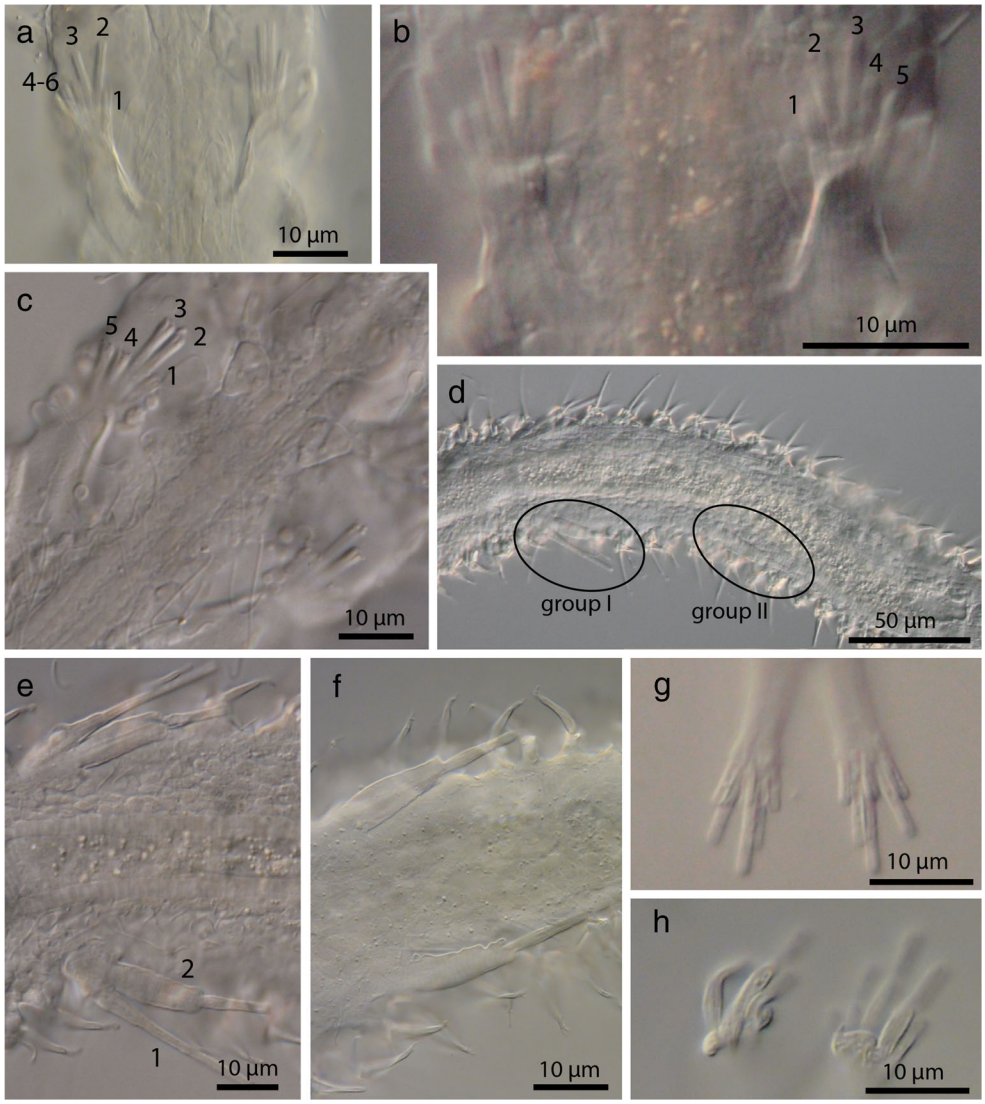

Fig. 6 *Xenodasys riedli*, number and distribution of adhesive tubes on specimens from San Salvador (**a**, **d**, **f**, **h**), Tobago (**b**, **g**), and St. John (**c**, **e**). **a**–**c** TbA numbering from medial. **d** Position of three pairs of ventrolateral adhesive tubes, grouped into an anterior (*group I*) and a posterior group (*group II*). **e** Anterior group (group I) with adhesive tubes *1* and *2*. **f** Posterior group (group II) with third pair of adhesive tubes. **g**, **h** Posterior adhesive tubes. *TbA* anterior adhesive tubes, *TbP* posterior adhesive tubes, *TbVL* ventrolateral adhesive tubes

Schöpfer-Sterrer (1969) reported a total of 8 ventrolateral adhesive tubes (TbVL), present as 4 pairs but arranged in 3 groups (Group 1: 2 pairs; Groups 2 and 3: each 1 pair). We observed a similar pattern in most of our specimens (see Table 1 for variation). In our specimens, Group I (the following refers to only one side of the body) was present at U35–38. This group consisted of two TbVL which formed a common base. The most lateral tube pair was slender, the medial tube pair had a broad basal portion and a thinner apical portion (Figs. 4e, 6d, e; Table 1). The third TbVL (group II of Schöpfer-Sterrer (1969), originating at U55–57, resembled the median tube of the first group (Fig. 6d, f). The fourth TbVL (group III) consisted of a single small TbVL, ca. 6 µm long, around U63; this TbVL was present in specimens from St. John and Florida, but was not observed in specimens from San Salvador. The tube-like structures called "Wimperzapfen" by Schöpfer-Sterrer (1969), which was present close to the posterior end, were not observed in our specimens. Instead, we observed small spine-like structures in a similar position, with a gland pore close to their base (Fig. 2c).

The posterior adhesive tubes (TbP) were borne on two pedicles ca. 26–28 µm long. Schöpfer-Sterrer (1969) described seven TbP per pedicle, with the fourth and sixth tubes (counting medial to lateral) being the longest. This pattern was present in most specimens, though there was considerable variation is tube length from most medial (4–6 µm) to most (4.4–13 µm) (see Table 1). The tubes of most of these specimens were aligned in parallel along the edge of the pedicle except for the fourth tube (from the medial); this tube was positioned ventral to the other tubes. In specimens from the

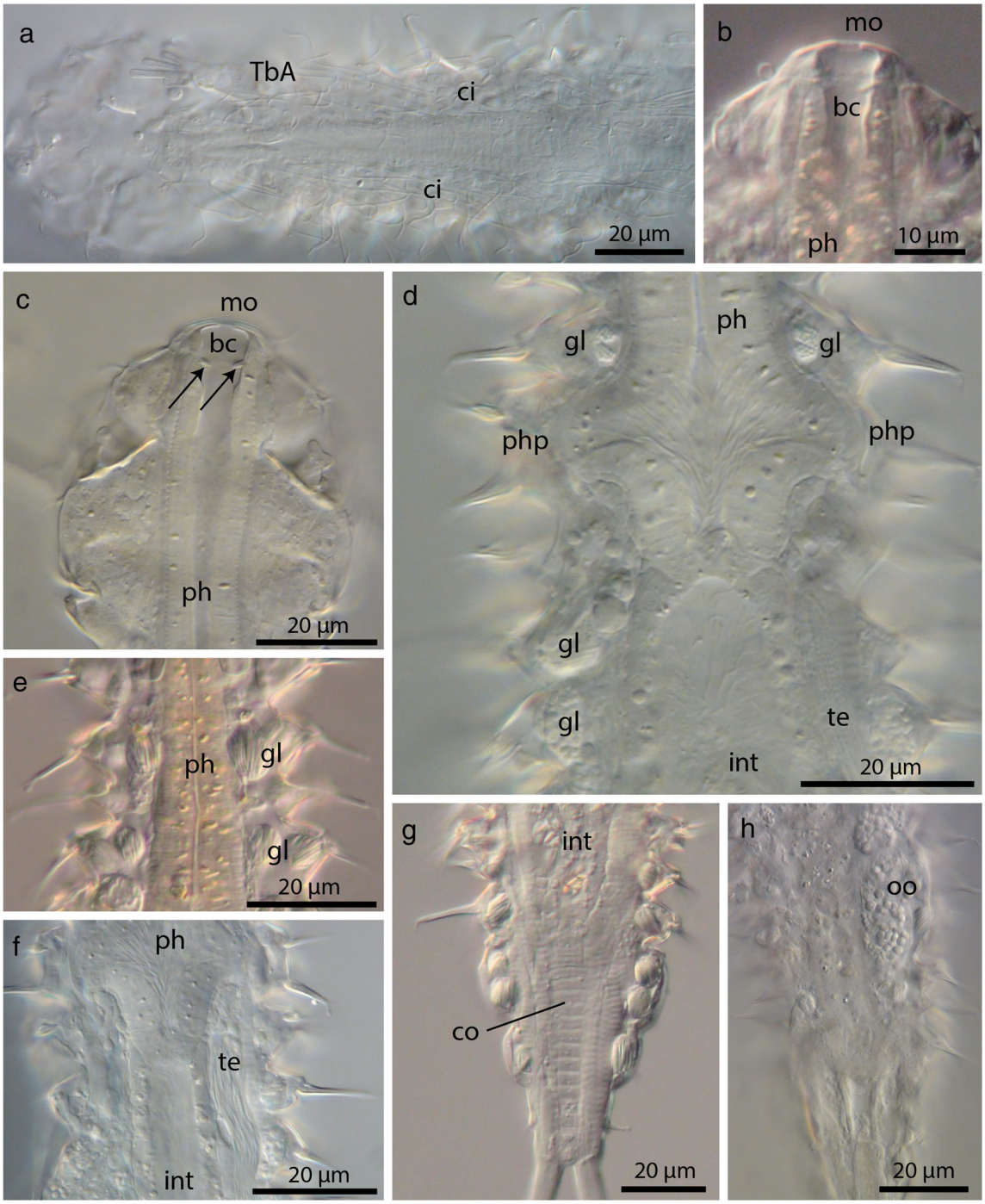

Fig. 7 *Xenodasys riedli*, additional anatomical structures of specimens from San Salvador (**a**, **c**, **d**, **f**, **h**), and Tobago (**b**, **e**, **g**). **a** Ventral ciliation in the anterior end. **b** Mouth opening and buccal cavity with transition to pharynx. **c** *Arrows* show protrusions into the buccal cavity. **d** Dense ciliation in pharynx at the pharyngeal pores. **e** Epidermal glands with rod-like inclusions along both sides of the pharynx. **f** Position of testes beginning at the transition between pharynx and intestine. **g** Chordoid organ. **h** Oocytes at posterior end. *bc* buccal cavity, *ci* cilia, *co* chordoid organ, *gl* epidermal glands, *int* intestine, *mo* mouth opening, *oo* oocytes, *ph* pharynx, *php* pharyngeal pores, *TbA* anterior adhesive tubes, *te* testes

Bahamas, Tobago, St. John, and some from Florida, only six TbP were observed (Figs. 2c, 4g, 6g, h; Table 1).

Epidermal glands (gl) were present along the anterior–posterior axis; all glands contained rod-like inclusions that formed parallel stacks (Figs. 3c, 7e). With SEM, we observed that the openings of these glands formed a ring of cuticle on some dorsal scales and even on the ventral surface (circles, Fig. 2c). The presence and position of the muscular chordoid organ from the original description was observed in all the analyzed specimens (Figs. 1a, b, 7g).

The digestive system began with a circular mouth (3 μm in diameter) that was surrounded by a cuticular ring (Fig. 4a). The mouth led to a funnel-shaped buccal cavity (Fig. 7b) and into the pharyngeal lumen. In the Bahamian specimens, we observed the presence of short cuticular protrusions projecting into the anterior portion of the buccal cavity (Fig. 7c). Both the pharynx and intestine contained cilia; ciliation around the pharyngeal pores is extremely dense (Fig. 7d).

Schöpfer-Sterrer (1969) reported paired testes with elongate spermatozoa between U37 and U58; these were also present in our specimens (Fig. 7f). Oocytes were present on the right side anterior to the chordoid organ (Fig. 7h).

Discussion

Xenodasys riedli is probably a regional species, having been reported from the southeast coast of the USA (North Carolina, Florida), Bermuda, the Virgin Islands, Scottish Shetlands, and maybe South Africa (see Todaro et al. 2006a; Hochberg 2007; Hummon 2010; this study). We now extend the distribution to the Bahamas and Tobago. As density data on this species is entirely absent, we can now report that *X. riedli* is present in relatively high abundances in shallow sublittoral waters (at least on St. John), and may represent one of the dominant meiofaunal species under certain conditions. It remains to be determined if and how the abundance of this species varies throughout the year, and on other Caribbean islands, but based on our qualitative assessments in Florida, the Bahamas, and Tobago, the species appears to be more abundant than previously imagined.

To date, the monophyly of *X. riedli* has not been verified with molecular data, so the question of cryptic speciation in this species remains open. This species does appear to have a relatively consistent morphology across the TNWA and other locales despite the differences in total body size among populations. Whether these differences reflect genetically distinct populations remains to be determined. Furthermore, compared to many macrodasyidan gastrotrichs that have smooth cuticles and relatively few external features for comparison, *X. riedli* is easily recognized due to the complex nature of its cuticle. In fact, the ornamentation is comparable to that of many thaumastodermatid gastrotrichs, which also show extreme variation in scales and spines that requires SEM for correct interpretation (Araujo and Hochberg 2016). Despite this, we can verify that much of the original type description of *X. riedli* based solely on phase contrast microscopy remains valid for specimens collected across the TNWA.

Still, it is important to note that SEM remains the best tool for describing species morphology, and this is proven by our observations on the specimens from Florida where we observed deviations from the type description. For example, we observed that the lateral cuticle is composed of two types of scales rather than the single type reported in the original description. Schöpfer-Sterrer (1969) originally described the presence of only lateral spined scales, which were scales that had spines projecting from the anterior and posterior surfaces, and with a small "thorn-like protuberance" in front of and behind each spine. However, our SEM observations revealed that the lateral spines are in fact not anterior and posterior in position but rather dorsolateral and ventrolateral in position relative to the scale body. We also observed that the thorn-like protuberances, which are in some cases paired and singular in other cases, are always positioned anterior to the spines. Lastly, we observed that there are spineless scales in between the spined scales. These scales are extremely difficult to observe with brightfield microscopy, and with SEM interpretation is not always straightforward depending on the orientation of the specimen.

In addition to these findings on the general body cuticle, we also observed new details about the head plates that require a reinterpretation of the species' morphology. Schöpfer-Sterrer (1969) reported a total of 11 dorsal head plates, but we observed only 8 plates. This difference is due to the SEM's ability to differentiate folds or crests on single plates from fissures that actually separate plates. For example, Schöpfer-Sterrer (1969) described a pair of plates lateral to D4, which we interpret as an anterior–posterior crest of D4. We also observed a pair of dorsal head plates (D6) that were not noted in the type description. At the tip of the head on either side of the mouth, we observed a pair of anteromedial plates (A) that project from beneath D2 and are not easily observed in live specimens. In addition, we discovered three ventral plates (single V1, paired V2) not described in the type specimens.

This study represents a detailed examination of a species that is widespread yet relatively rare compared to many other macrodasyidan gastrotrichs. We emphasize that high-resolution studies of morphological variation in these tiny but complex animals is necessary for an accurate description of their cuticle, and that such studies are likely to reveal characteristics that are nearly impossible to determine with brightfield microscopy (see also Araújo and Hochberg 2016). We strongly recommend that future studies of new and previously described species include SEM analysis, which is clearly necessary to reveal minute (and unforeseen) characteristics that might lead to a reevaluation of species-level taxonomy.

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