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Diplodasys rothei n. sp. (Gastrotricha, Macrodasyida), a new marine gastrotrich species from the Bahamas

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Abstract

We here describe a new species from the genus *Diplodasys* (Gastrotricha, Macrodasyida), collected at different shallow sublittoral sites of Lee Stocking Island and San Salvador, Bahamas. *Diplodasys rothei* n. sp. resembles *D. pacificus* and *D. ankeli* in some respects, but shows a new character combination in the distribution and abundance of anterior adhesive tubes, which justifies its description as new species. Hence, a clear morphological difference is present that distinguishes the new species from its congeners. *Diplodasys rothei* n. sp. is the third known species of *Diplodasys* from the Caribbean and the tenth described species in the genus.

Key words: Caribbean, Meiofauna, species description, taxonomy

Introduction

The Caribbean region is a major global marine biodiversity hot spot (Miloslavich et al. 2010). Nevertheless, knowledge on biodiversity is very heterogeneous and meiofaunal taxa are generally not very well investigated. This is particularly true for Gastrotricha. In interstitial systems, gastrotrichs can be quite abundant and are therefore a major element of the meiofaunal community. There are very few investigations of the gastrotrich fauna of particular Caribbean islands (e.g., Hochberg 2008, Hummon 2010), but recent efforts to characterize the gastrotrichs from several Caribbean island nations have made some inroads.

In keeping with these efforts to better understand the diversity and distribution of gastrotrichs of the Caribbean, we made collections during trips to two islands in the Bahamas, Lee Stocking Island (in 2010) and San Salvador (in 2011). Lee Stocking Island is within the chain of the Exumas, slightly north of Great Exuma, and it is host to the Perry Institute for Marine Science. San Salvador is the most eastward island of the Bahamas, host to the Gerace Research Center. While Lee Stocking Island is situated on the margin of the Bahamas Bank, San Salvador is more isolated and surrounded by deeper waters.

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Within Gastrotricha, the genus *Diplodasys* is part of the diverse family Thaumastodermatidae within the order Macrodasyida (Todaro et al. 2011). At present, there are 9 described species: *D. platydasyoides* Remane, 1927, *D. minor* Remane, 1936, *D. ankeli* Wilke, 1954, *D. remanei* Rao & Ganapati, 1968, *D. pacificus* Schmidt, 1974, *D. caudatus* Kisielewski, 1987, *D. swedmarki* Kisielewski, 1987, *D. meloriae* Todaro, Balsamo & Tongiorgi, 1992 and *D. sanctimariae* Hummon & Todaro, 2009 (Remane 1927, 1936; Wilke 1954; Rao & Ganapati 1968; Schmidt 1974; Kisielewski 1987; Todaro et al. 1992; Hummon & Todaro 2009).

It is not always clear which characters are important to distinguish species from each other. For example, the subspecies D. ankeli pacificus originally described by Schmidt (1974) was later raised to species level (Clausen 2004). Some of the older descriptions are quite sparse (see e.g. Remane 1927 for *D. platydasyoides*), and some species are distinguished by very fine differences such as the number of head spines. Several species described in the early 20th century have been redescribed, but in some cases the old and the new descriptions of the same species differ slightly (e.g., in D. ankeli, see discussion in Clausen 2004). Most descriptions are derived from few individuals and comparatively few illustrations are present in the literature. Little has been stated about the variance of characters within one species or population.

Some species of *Diplodasys*, namely *D. ankeli* and *D. minor*, have been reported as globally distributed. These two species have also been reported from the Bahamas (Renaud-Debyser 1963, Hummon 2010). This paper describes a new species of the genus *Diplodasys* that was collected during the two trips to different islands of the Bahamas archipelago. Our aim is to give a detailed description of this new species based on a maximum of material (specimens) and using different microscopic techniques.

Material and methods

Specimens were sampled on two trips to the Bahamas. The first trip was to Lee Stocking Island from April 5 to 22, 2010. With one exception, all sampling sites are from beaches on Lee Stocking Island (LSI, Table 1). One sample was taken from a close key (cay), Norman's Pond Cay (abbreviated NPC). Sampling sites are summarized in Table 1.

The second trip led to San Salvador, the easternmost of the Bahamas Islands, from June 8 to June 18, 2011. All sampling sites were on beaches on the island with the exception of one occasion, where sediment was taken from a boat approximately one kilometer north of the island. Samples from San Salvador (SS) are summarized in Table 2.

Sediment samples were collected at the shoreline or in shallow water by snorkelling. Sediment (biogenic calcareous sand of varying grain size, see Tables 1 and 2) was collected in plastic bottles. All samples were extracted and investigated on the same day as collection. For extraction, sediment was mixed with 7 % magnesium chloride (MgCl₂) for 10 minutes. The solution was then decanted over a sieve with 63 µm mesh size and specimens were washed with sea water from the sieve into a Petri dish. Gastrotrichs were sorted out under a dissecting microscope and each specimen was mounted alive on a slide. A drop of magnesium chloride solution (7 %) was added to anaesthetize the animals. Specimens were investigated with a Zeiss Axioscope using Differential Interference Contrast (DIC). Series of images as well as videos through all focal layers were recorded with a Sony Handycam HDR-XR550VE. Length measurements were made with the open source program ImageJ using reference bars which were produced when the pictures were taken.

Some specimens were preserved for Scanning

Station	Date	Location	Coordinates	Water depth	Sediment type
LSI 2	April 8	Airport Beach	23°46.445'N, 76°05.878'W	0.3-3 m	medium fine sand
LSI 5	April 10	Charley's Beach, eastern end	23°46.758'N, 76°06.286'W	4-5 m	sand
LSI 7	April 10	Charley's Beach	23°46.755' N, 76°06.452' W	~4 m	sand
LSI 8	April 10	Northern Beach	23°46.906'N, 76°06.661'W	~4 m	sand
LSI 11	April 15	Coconut Beach	23°45.968'N, 76°05.768'W	0-0.5 m	fine sand
NPC 1	April 13	channel to lagoon	23°46.085'N, 76°07.880'W	0.5-1 m	coarse sand

Table 1. Sampling sites on Lee Stocking Island and Norman's Pond Cay in 2010.

Electron Microscopy (SEM) or for preparing permanent glycerol wholemounts. Animals for SEM were fixed in 4 % formaldehyde over night at 4 °C, then washed with 0.1 M PBS (phosphate buffered saline) and stored in PBS+NaN₃ (0.05 %, w/v). Specimens were dehydrated in an increasing ethanol series, critically point dried and coated with gold in a sputter coater. Observation and documentation took place using a LEO SEM 1524 under 10 kV.

Specimens for glycerol whole mounts were narcotised with 7 % MgCl₂ (approximately 5-10 min), then carefully fixed with 5 % formaldehyde solution in 0.1 M PBS (pH 7.4), and finally stored in small (1.5 ml) tubes filled with the fixative. For dehydration, specimens were rinsed in PBS, then transferred into a glycerol-water solution (ratio: 1:10), and kept therein for several days in order to let the water evaporate. Single specimens were next transferred into a minute drop of pure glycerol on a microscopic slide surrounded by a beeswax-paraffin mixture (ratio: 1:2) and covered with a coverslip by melting the wax at 60 °C. In order to achieve the drawings of the new species, digital image series from all focal layers of the holotype specimen were carried out with a Leica DM 2500 microscope equipped with DIC and a digital camera Leica EC3. Printouts of these digital data served as the basis for the original line drawings on parchment paper. Slight additions to the original drawings (i.e. the positions of the ventral locomotory cilia) were made using Adobe Photoshop CS5. Additional line drawings of the mid-dorsal scales of two further specimens were based on SEM images.

Positions and size ranges of internal and external structures are referred to as percentage body units (U), where the total body length represents 100 units (see Hummon 1974, Schmidt 1974). Order Macrodasyida Remane, 1925 [Rao & Clausen, 1970] Family Thaumastodermatidae Remane, 1927 Subfamily Diplodasyinae Ruppert, 1978 Genus Diplodasys Remane, 1927

Diplodasys rothei spec. nov.

Material examined. 36 individuals, examined in part by light microscopy and in part by SEM.

Collection localities. Bahamas: Lee Stocking Island, stations LSI2 (5 specimens), LSI5 (2 specimens), LSI7 (8 specimens), LSI8 (1 specimen), LSI11 (1 specimen); Norman's Pond Cay, station NPC1 (9 specimens); San Salvador Island, stations SS1, SS2, SS3 (1 specimen each), SS4 (3 specimens), SS5 (4 specimens); see Tables 1 and 2.

Holotype. The holotype is a specimen prepared for light microscopy, the slide is stored in the Zoological Museum Hamburg under the accession number ZMN V13333.

Etymology. The name honours Dr. Birgen Holger Rothe, who was a major help in collecting the specimens and is an esteemed colleague and friend of ASR and AK.

Diagnosis. *Diplodasys* with a body of 200–300 μ m length. Body divided into head, neck and trunk regions with a pair of caudal pedicles. Body width between 38 μ m (at the level of the constrictions) and 55 μ m. Pharynx from tip of oral hood to pharyngeo-intestinal junction (at U38) up to 115 μ m long. Oral hood rounded and bearing numerous sensory bristles (cilia) along its margin. Epidermal glands few to numerous along lateral margins of body. Five columns of roughly oval

Station	Date	Location	Coordinates	Water depth	Sediment type
SS1	June 10	Bonefish Bay, south of North Victoria Hill settlement	24°05.134'N, 74°31.744'W	0-5 m	coarse sand
SS2	June 12	Sandy Point	23°56.948' N, 74°33.985' W	0-5 m	fine to coarse sand
SS3	June 14	Sandy Hook	23°57.838'N, 74°29.220'W	0-4 m	coarse and medium fine sediment
SS4	June 15	Sediment from boat tour	24°08.770'N, 74°28.502'W	2-8 m	medium coarse sand
SS5	June 16	Rocky Point	24°06.425' N, 74°31.147' W	0-5 m	medium fine sand

Table 2. Sampling sites on San Salvador Island in 2011.

scales cover the entire dorsal surface. Diplodasysspines on lateral margins, and smaller oval scales on ventral surface. Scales with a roughly eveshaped depression at centre bearing a cruciform pattern. Pore-like pits surround the eye-shaped central depression. Adhesive tubes arranged in anterior (TbA), ventrolateral (TbVL) and posterior series (TbP). TbA arranged as two paired median groups of 6 tubes anteriorly and 2-4 tubes posteriorly; one additional tube at each side of the anterior median cluster. TbVL arranged as a column of 18-21 tubes on either side of locomotory cilia, roughly between U30 and U90, occurring in groups of 1-3 tubes. One isolated pair of TbVL present slightly posterior to the level of the first constriction between head and neck (U22). TbP 5-7 per side, three of which located on each of the paired caudal pedicles, 2-4 additional TbP per side anterior to each pedicle. Ventral locomotor cilia arranged in two longitudinal columns of separated patches of cilia, median to the rows of TbVL. Additional unpaired median patches of cilia present in the pharyngeal region and posterior to the anus. Paired, tube-shaped testes along the first three quarters of the intestine, narrowing into caudally projecting sperm ducts that possibly open into the caudal organ. Mature oocyte present at around U80. Spherical frontal sac and three-lobed caudal organ present.

Description

Size and shape. Specimens were on average 242 μ m long (n=11, adults and few advanced subadult specimens), with a range of 209–306 μ m. The holotype measures 265 μ m in length and has a width of 50, 38, 45, 38, 50, 55, 42, and 20 μ m at U10, 18, 25, 32, 50, 75, 90, and U97, respectively. Video recording showed that the shape differs distinctly as the animals stretch or contract (Fig. 1A-H). Usually the body seemed to be divided into head, neck and trunk region by lateral constrictions as often described in the literature (Fig. 1A). Sometimes these constrictions were not very prominent (e.g. Fig. 1B) and sometimes additional constrictions

may be temporarily formed (see Fig. 1D,E). In the posterior end of the animals the body divides into two short branches usually referred to as caudal feet (Fig. 1A–C,G). These caudal feet are not always clearly evident, because of contraction they may be held close together, or are almost invisible when the animal bends ventrally.

Dorsal scales. The dorsal scales are slightly imbricated. They change their orientation halfway between anterior and posterior end, with scales anterior to ca. U50 having their posterior edge covered by each posteriorly following scale, while scales posterior to U50 have their anterior edge covered by each anteriorly foregoing scale, respectively (Figs. 2B, 4A). There are five columns of scales extending along the dorsum; the medial column has 22 to 30 scales depending on specimen length. All anteriorly projecting scales (U00-U50) have a more or less symmetric, arched anterior edge, while posteriorly projecting scales (U50-U90) either have a more straight (scales of median row) or acute-oval posterior edge (scales of lateral rows, see Figs. 2B, 4C). Also the dimension differs between anterior and posterior scales: Lengths (only the free part of the scales measured) of the anterior scales range between 6 and 8 µm, those of the posterior scales vary between 10 and 15 µm. Width of the anterior scales is approximately $8-10 \,\mu\text{m}$, that of the posterior scales $10-15 \,\mu\text{m}$. The precise shape and size of the scales differs slightly within and between specimens. One or two scales from the median longitudinal column may have a particular shape. Scale 4 or scales 4 plus 7 (counting posteriorly from the tip of the oral hood) have an anterior edge with an undulating appearance (Fig. 2B, 4A). This pattern is of varying distinctness among different specimens. Scales extend onto the caudal pedicles (Fig. 5A).

The four scales around the point where scales change their direction (approximately at U50) were found to vary slightly in their pattern from individual to individual. However, a common observed pattern was that the anterior median scale of these four scales was the one closest to the body surface. The first median scale pointing to

Fig. 1. *Diplodasys rothei* n. sp. **A- F.** Appearence of the body-shape and the posterior end with caudal feet (cf) in \triangleright different specimens and different stages of movement. aat, anterior adhesive tubes; he, head; mo, mouth opening; ne, neck; tr, trunk. Arrows indicate constrictions of the body (dividing head, neck and trunk as well as occasional additional constrictions as in D). **G.** Posterior end with caudal feet. **H**₁-**H**₄. Four snapshots from a video sequence of one specimen in different phases of movement. **I.** Ventral view showing anterior (aat) and trunk adhesive tubes (tat). All images from light microscopy.



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Fig. 2. *Diplodasys rothei* n. sp. Schematic drawings of the holotype specimen. **A.** Combined internal and ventral view, ventral scale pattern is not displayed. Dotted areas indicate epidermal glands, grey areas represent the shape and arrangement of ventral locomotory cilia. Borders between anterior (TbA), ventrolateral (TbVL), and posterior (TbP) adhesive tubes are indicated by dashed lines. **B.** Dorsal view showing the arrangement of dorsal scales. Ornamentation is only drawn for some scales around U50. Borders between head spines (hs), neck spines (ns), and trunk spines (ts) are indicated by dashed lines. co, caudal organ; fs, frontal sac; ov, ovary (mature egg); pp, pharyngeal pores; tes, testes.

the posterior end (the 'middorsal scale') overlaps the anterior one and the two laterally adjacent scales are on top of the two median scales with all edges free (Fig. 2B, 4A,C). In one case it was observed that the 'middorsal scale' overlaps the lateral scale on one side (compare Fig. 3A and 3B). There may also be asymmetry in more caudal dorsal scales. In one specimen, the fourth median scale (counting from the 'middorsal scale' towards the posterior end) seems to be fused with one adjacent scale of a lateral row (see Fig. 2B).

The sculpture of the scales generally shows

an eye-shaped depression in the centre (approximately 3 by 5 µm) and numerous ornamentations around it in the form of small round depressions rather irregularly scattered (Figs. 2B, 3A, 4A,C,E,G). Within the central depressed area there is a cross-shaped (cruciform) elevated ornamentation (Fig. 4A,E), although some scales had more variable ornamentations, with less clear crosses or just simple transverse bars (see, e.g. Fig. 4A,E,G). Both ornamentations can occur on the same individual.



Fig. 3. *Diplodasys rothei* n. sp. **A-B.** Schematic drawings of the arrangement pattern of the "middorsal scale" (dark grey) and the three surrounding plates (light grey) in two different specimens. Note the slight difference of the dorsal scale ornamentation in two different specimens (compare Figs. 2B and 3A).

Ventral scales. The ventral side is covered with scales (Fig. 4B). These are smaller than the dorsal scales (approximately 5-6 µm wide). According to our observations the ventral scales are also imbricated. Ventral scales are usually visible by SEM, but very hard to observe by light microscopy. The orientation of the ventral scales gradually changes direction along the length of the animal: ventral scales at the head point with the free edge towards the opening of the mouth, but at a slightly lateral angle. At the "neck" the scales are pointing with the free edge towards the lateral sides of the animals and in the "trunk" region the scales are pointing posteriorly. On some of the scales, a slight ornamentation similar to that described on the dorsal scales is present. Cross-shaped structures as in the depressions of the dorsal scales were observed in one specimen. However, the observed structures were not as prominent as in the dorsal scales.

Spines. Several spined scales are present along the lateral sides of the animals. The spines are almost quadrangular in cross section, point laterally or postero-laterally and are slightly curved. Other spines, especially in the head and neck region, are straight (Figs. 1A–F, 2A, B, 4A, F, G). They are present in a lateral position and generally form the shapes described for most other *Diplodasys* species (commonly referred to as *Diplodasys* spines, see e.g. Kisielewski 1987, Todaro et al. 1992, Clausen 2004). In the head region there are 4, rarely 3 spines of variable size (3–7 µm) on each side

(Fig. 2A,B). In the neck region 5, rarely 4 spines are present (Fig. 2A,B). The central neck spine differs in its insertion. While four spines insert at a more ventral level and are equally spaced from each other, the central spine inserts on a slightly more dorsal level and may therefore be easily overlooked (Fig. 2A, 4A,B). In the trunk region, 22–28 spines (mean of 26 spines, n = 10 animals) are present.

Ciliation. The locomotory cilia on the ventral side are grouped into two longitudinal bands medial to the adhesive tubes (Fig. 2A). By light microscopy, the columns may appear as a uniform band of cilia. However, with SEM, where cilia are sometimes artificially shrivelled, they appear to insert in bunches or clusters (Fig. 4B, see also Fig. 2A). In the head and neck region there are additional cilia between the longitudinal columns, forming a dense continuous ventral ciliation. The SEM examination revealed that these cilia also insert in clusters (Fig. 4B). An unpaired patch of locomotory cilia is present posterior to the ventral anus (Fig. 2A, 5D).

Cilia of different lengths, probably sensory cilia, are found around the mouth (Fig. 5E) and on the ventrolateral margin of the body. The longest of these are about 1.5 times longer than the lateral spines (approximately 15 μ m). These cilia were observed better in the light microscopic investigation of living animals, under SEM most cilia were obviously shrivelled and therefore indistinct.

Anterior adhesive tubes. Posterior to the rim of the mouth is a median group of 10–14 bilaterally arranged anterior adhesive tubes (TbA, Fig. 2A, 4D). The two most medial TbA are slightly thinner than the other tubes (Fig. 4D). Next to these, on each side, two to three tubes are grouped close together and a single tube follows after a narrow gap (Fig. 2A, 4D). One or two additional paired tubes are present posterior of the central group of tubes (Fig. 2A). The anterior adhesive tubes, 3–4 µm in length, are shorter than the ventrolateral tubes of the trunk.

Ventrolateral adhesive tubes. Paired longitudinal columns of ventrolateral adhesive tubes (TbVL) extend posterior of the second body constriction approximately at U32, i.e. in the trunk region, and extend almost to the posterior end at U87 (Fig. 2A). They are always positioned just lateral to the locomotory cilia and never visible from the dorsal side. One additional single adhesive tube is present on each side at the level of the transition between head and neck at approximately U22 (Fig. 2A). The neck region itself has no adhesive tubes.

There are 18–21 TbVL per side (n=2), in juveniles this number may be lower (14 TbVL per side have been counted in one subadult specimen). The TbVL insert as bundles of 1–3 tubes; the bundles are more or less equally spaced. The tubes per bundle differ in length (6.5–8.5 μ m). One ventrolateral adhesive tube per side is closely associated with the third spine from the posterior end. It is directly ventral of the spine and much longer than the other TbVL (up to 13 μ m, see Figs. 1C, 2A, 5C).

Caudal pedicles and posterior adhesive tubes. The trunk narrows towards the posterior end and has a pair of short caudal pedicles (approximately 5–8 µm long) that bear the TbP (Figs. 2A, 5D,E). Three TbP are present on each pedicle. Two terminal adhesive tubes insert ventrally and protrude from underneath the dorsal scales (Fig. 5A,B,D). One adhesive tube is present on the medial side of each pedicle. A small cluster of 2–4 tubes is present anterior and ventral of each pedicle (Fig. 5D). These posterior adhesive tubes are separated from the trunk tubes by a comparably large gap. In one specimen investigated by SEM, small spines rise from the centre of the dorsal scales covering the caudal pedicles (Fig. 5A). The ventral surface of the caudal pedicles is rather smooth.

Gut system. Diplodasys rothei n. sp. has a gut system that is subdivided into an anterior muscular pharynx (U10 to U38), approximately 70 µm long, and a posterior intestine that begins at the region of the pharyngeo-intestinal junction at U38 and spans approximately 115 µm (measurements from the holotype specimen) up to U98 where it ends in an inconspicuous ventral anus (Fig. 2A). Anterior of U20, the pharynx and its lumen dramatically widen to form the large, funnel-shaped buccal cavity that is known as the 'oral hood' in many species of the Thaumastodermatidae. Posteriorly, between U30 and U38, the pharynx has a distinct swelling. In this region (approximately at U33) the paired ventrolateral pharyngeal pores, which are difficult to observe by light microscopy, are also present. The width of the pharynx of the holotype is 40, 25, 18, 20, 20, 17, and 15 µm at U10, 15, 20, 25, 30, 33, and 38, respectively. The intestine is straight and does not vary a lot in width (mean width approximately 15 µm). However, the intestine tapers gradually from U85 on (13, 10, and 5 µm at U85, 90, and 95, respectively).

Epidermal glands. There are large (mean diameter of 10 to 15 µm) epidermal glands arranged in two lateral to dorsolateral columns along the gut tube. The number of glands per column varies a lot, there are, for instance, only three or four glands per side in the holotype specimen (see Fig. 2A) while other animals possess up to eight glands in a row (see Fig. 1F). The shape of each gland is roundish but with a distinct neck visible when following different focal plains under high magnification. The content of each gland is granular and appears colourless in living specimens while a somehow brownish appearance is possible in fixed animals.

Fig. 4. *Diplodasys rothei* n. sp. **A.** Dorsal view of anterior part, the square indicates the scales changing direction. \triangleright **B.** Ventral view of neck region with neck spines, clusters of cilia and few adhesive tubes. **C.** Focus on the scales changing direction (anterior is to the left). **D.** Ventral view of head with mouth opening (mo) and anterior adhesive tubes (TbA). **E.** Dorsal view of head with sensory cilia (sci). **F.** Detail from dorsal scales and spines in the trunk region. **G.** Dorsal scales and lateral spines in anterior part of the body showing a different orientation of spines. C from light microscopy, all other images from SEM.



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Reproductive system. *Diplodasys rothei* n. sp. is a simultaneous hermaphrodite. Several specimens of *D. rothei* n. sp. observed under light microscope had a mature egg in the posterior quarter of the trunk, positioned approximately between U70 and U85 and dorsal to the intestine. The mature egg is ovoid in shape and measures 15–30 µm (width and length), sometimes even larger (e.g. 35–40 µm, see Fig. 1E). It is densely filled with vesicles (around 0.5 µm in diameter); a central big nucleus with distinct nucleolus is also present (diameter of nucleus is 10 µm). An ovary with developing eggs and oocytes was hardly visible. It is probably situated posterior to the mature egg.

A pair of testes lies at both sides of the intestine and spans a distance between U40 and U85, approximately 120 μ m in the holotype specimen. Each testis is a simple, tube-shaped organ with slight local swellings along its length (diameter varies between 5 and 7 μ m). From U75, each testis tapers considerably into a caudally projecting vas deferens (1–3 μ m in diameter). Between U80 and U85 each vas deferens bends medially and probably opens into the caudal organ. Filiform spermatozoa with probable spiralled heads can be observed along the whole gonad.

Diplodasys rothei n. sp. has a set of two accessory reproductive organs, presumably functioning as a sperm-storing and as a sperm-transferring device. Both organs are difficult to observe even under high magnifications and using contrasting methods such as the DIC. However, the presence of spermatozoa within their lumina proves their existence and general shape. A certain distance anterior to the mature egg, between U60 and U70, there is a spherical cluster of coiled spermatozoa (15 µm in diameter), positioned dorsal to the intestine. We interpret this structure as the 'frontal sac', a sperm storing receptacle already known for the genus *Diplodasys*. In direct posterior adjacency to the mature egg there is another cluster of coiled spermatozoa (between U80 and U90). Its shape is trilobate like a shamrock with two lobes projecting frontally and one caudally. This accessory reproductive organ is situated slightly more ventral than the mature egg. Both vasa deferentia seem to enter the putative caudal organ at a level between the paired anterior lobes and the unpaired posterior one. Its dimension is approximately 15 by 20 µm (width and length).

Discussion

So far, there are nine described species in the genus Diplodasys (see Hummon & Todaro 2010). Regarding the general structure and arrangement of the cuticular armament, species of Diplodasys can be divided into a group of species that have dorsal scales that are all directed posteriorly (Diplodasys minor, D. meloriae, and D. caudatus) and a group that have anteriorly directed scales in the anterior half and posteriorly directed scales in the posterior half of the trunk (Diplodasys ankeli, D. pacificus, D. swedmarki, and D. sanctimariae). Diplodasys remanei falls somewhat apart from the other species since it is the only slim and wormshaped form without the characteristic depressions that subdivide the body into head, neck, and trunk section. For Diplodasys platydasyoides, no information on the shape and orientation of the dorsal scales exists. However, it is the only Diplodasys species that has lateral tetrancres instead of the characteristic Diplodasys-spines (an evolutionary relationship between the spines in Diplodasys and Acanthodasys, and the "ancres" of other taxa of the Thaumastodermatidae exists, see Rieger & Rieger 1977). The here described Diplodasys rothei n. sp. definitely belongs to the group of species with a changing direction of covering scales, i.e. it is much likely related to D. ankeli, D. pacificus D. swedmarki, and D. sancti*mariae*. Within this group, the latter two species differ from the new one because of the structure and shape of the dorsal scales. D. sanctimariae has a pattern of regularly arranged "pores" all over its scales (including the central depression, see Hummon & Todaro 2009), while D. swedmarki possesses rectangular scales in the anterior half and so-called "additional plates" in the posterior half (Kisielewski 1987).

With regards to the structure of the cuticle, the Bahamian specimens are most similar to *D. ankeli* and *D. pacificus*, especially concerning the microstructure and orientation of the dorsal scales and characteristics of the lateral spines. The dorsal scales of *D. rothei* n. sp. have a similar microstucture and cruciform pattern as described for *D. pacificus* by Schmidt (1974) and reported for *D. ankeli* (see Figs. 10F and 11 of Rieger & Rieger 1977) and may therefore be a common pattern of all three species. However, in *D. ankeli*, the cruciform pattern is not reported in all available descriptions. The pattern of overlap of the four



Fig. 5. *Diplodasys rothei* n. sp., posterior end. **A.** Caudal feet (cf) from dorsal, the two feet are close together and hard to distinguish. Caudal feet carry small spines (not labelled) and posterior adhesive tubes (pat). sp, last lateral spine. **B.** Caudal feet (cf) viewed from dorsal, no small spines are present in this specimen. Encircled are the terminal pair (1) and the single adhesive tube on the inner side of the caufal foot (2). **C.** One long ventrolateral adhesive tube is associated with the third spine from the posterior (arrow). **D.** Ventral view of the caudal feet and region anterior of them. Encircled are three groups of adhesive tubes as labelled in B and additionally the group anterior of the caudal feet (3). **E.** Overview of the ventral side showing distribution of anterior (TbA), ventrolateral (TbVL) and posterior (TbP) adhesive tubes. ppo, pharyngeal pores. All images from SEM.

dorsal scales at the point where they change their direction is almost similar in the Bahamas specimens and *D. pacificus* (lateral scales above median scales), but differs from the pattern in *D. ankeli*, where the two median scales are above the lateral scales. Anterior median scales with an undulating anterior margin such as in the new species were also figured by Schmidt (1974, Fig. 17B) for *D. pacificus* and may be present in *D. ankeli* too (see Lee & Chang 2006, Fig. 1A).

Concerning the number of lateral spines, the Bahamian specimens are broadly similar to most species in the genus, possessing less than 35 spines per side (D. minor has 46 spines per side). However, the distribution of the spines differs among the species. In *D. rothei* n. sp., there are four pairs of head spines, which is similar to the condition in D. minor, D. pacificus, D. meloriae and D. sanc*timariae*. The number of neck spines per side (5) in the new species is most similar to *D. pacificus*. However, spine size differs among the species. In most described species, the lateral spines are longer than 10 µm (up to 13 µm in D. ankeli and 16 µm in D. sanctimariae). In the new species, the lateral spines have a maximum length of 10 µm, which is again comparable with the situation in D. pacificus that has a spine-length between 7 and 9.5 µm (Schmidt 1974).

A clear difference between *D. rothei* n. sp., D. ankeli, and D. pacificus is the number and distribution of anterior adhesive tubes (TbA). The TbA of *D. pacificus* form lateral transverse rows of seven tubes on both sides of the animal, they are separated by a clear median gap in between the rows. The new species has two medial rows of TbA – the anterior row consists of 3 pairs of TbA and the posterior row consists of 2 pairs of TbA; a solitary pair of TbA is also present just lateral to the first row of TbA. In this respect, the new species resembles D. ankeli, at least concerning the presence of two post-oral rows of TbA. However, the number of TbA differs considerably in the two species: D. ankeli has a high number of TbA (a total of 26-32 in two rows according to Kisielewski 1987, Todaro et al. 1992, Clausen 2004, Lee & Chang 2006). Furthermore, D. ankeli seems to be a slightly bigger species than D. rothei n. sp. Length indications for *D. ankeli* are for instance 256-320 µm (Todaro et al. 1992), 349 µm (Lee & Chang 2006), 360 µm (Wilke 1954), or up to 470 µm (Clausen 2004), while D. rothei n. sp. measures 209-306 µm.

Concluding, the Bahamian specimens appear as a unique form that is somewhat intermediate between the European populations of D. ankeli and the Pacific populations of D. pacificus, but with distinct differences to both species, most importantly the number and arrangement of TbA. Therefore, we regard the Bahamian specimens as a new species. The intermediate geographic position of the Bahamas between Europe (D. ankeli) and the Eastern Pacific (D. pacificus) may give further support for hypothesising a new morphological species. To date, D. pacificus has only been reported from the Galapagos (Schmidt 1974), while D. ankeli has been reported from several locations in Europe (e.g. Wilke 1954, Kisielewski 1987, Todaro et al. 1992, Balsamo et al. 1994, Clausen 2000) and Korea (Lee & Chang 2006). However, there is a further record of D. ankeli from the central Caribbean but without any distinct information on the locality or morphometric data of the observed specimens (Hummon 2010). This could imply that *D. ankeli* and *D. rothei* n. sp. are closely related sibling species that co-occur, at least, in the TNWA.

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References

- Balsamo, M., E. Fregni & P. Tongiorgi (1994). Marine and freshwater Gastrotricha from the island of Montechristo (Tuscan Archipelago, Italy), with the description of new species. Boll. Zool. 61: 217–227.
- Clausen, C. (2000). Gastrotricha Macrodasyida from the Tromsø region, northern Norway. Sarsia 85: 357-384.
- (2004). Gastrotricha from the Faroe Bank. Sarsia 89: 423–458.
- Hochberg, R. (2008). Gastrotricha of Bocas del Toro, Panama: a preliminary report. Meiofauna Marina 16: 101–107.

- Hummon, W. D. (1974). Gastrotricha from Beafort, North Carolina, U.S.A. Cahiers de Biologie Marine 15: 431–446.
- (2010). Marine Gastrotricha of the Caribbean Sea: a review and new descriptions. Bull. Mar. Sci. 86: 661–708.
- Hummon, W. D. & M. A. Todaro (2009). Italian marine Gastrotricha: VI. Seven new species of Macrodasyida. Zootaxa 2278: 47–68.
- Kisielewski, J. (1987). New records of marine Gastrotricha from the French coasts of Manche and Atlantic. I. Macrodasyida, with descriptions of seven new species. Bull. Mus. Nat. Hist. Nat., 4e Ser. 9: 837–877.
- Lee, J. M. & C. Y. Chang (2006). Marine Gastrotrichs of the genus *Diplodasys* (Macrodasyida: Thaumastodermatidae) from Korea. Kor. J. Syst. Zool. 22: 109–115.
- Miloslavich, P., J. M. Díaz, E. Klein, J. J. Alvarado, C. Díaz, J. Gobin, E. Escobar-Briones, J.-J. Cruz-Motta, E. Weil, J. Cortés, A. C. Bastidas, R. Robertson, F. Zapata, A. Martín, J. Castillo, A. Kazandjian & M. Ortiz (2010). Marine Biodiversity in the Caribbean: Regional Estimates and Distribution Patterns. PLoS ONE 5 (8): e11916. doi:10.1371/journal. pone.0011916
- Rao, G. C. & P. N. Ganapati (1968). Some New Interstitial Gastrotrichs from the Beach sands of Waltair cost. Proc. Ind. Acad. Sci. 67 B: 35–53.

- Rao, G. C. & C. Clausen (1970). Planodasys marginalis gen. et sp. nov. and Planodasyidae fam. nov. (Gastrotricha Macrodasoidea). Sarsia 42: 73–82.
- Remane, A. (1927). Neue Gastrotricha Macrodasyoidea. Zool. Jahrb. Abt. Syst. 54: 203–242.
- (1936). Gastrotricha und Kinorhyncha. In: Dr. H.
 G. Bronns, Klassen und Ordnungen des Tierreichs, Vol. 4: Vermes: Askhelminthes, Trochhelminthes: 1–385.
- Renaud-Debyser, J. (1963). Recherches ecolologiques sur la faune interstitielle des sables Bassin d'Arcachon, Ile de Bimini, Bahamas. Vie Milieu 15 Suppl.: 1–157.
- Rieger, G. E. & R. M. Rieger (1977). Comparative fine structure study of the gastrotrich cuticle and aspects of cuticle evolution within the Aschelminthes. Z. Zool. Syst. Evolut.-forsch. 15: 81–124.
- Schmidt, P. (1974). Interstitielle Fauna von Galapagos. IV. Gastrotricha. Mikrofauna des Meeresbodens 26: 1–76.
- Todaro, A., M. Balsamo & P. Tongiorgi (1992). Marine gastrotrichs from the Tuscan Archipelago (Tyrrhenian Sea): I. Macrodasyida, with description of three new species. Ital. J. Zool. 59: 471-485.
- Todaro, M. A., T. Kånneby, M. Dal Zotto & U. Jondelius (2011). Phylogeny of Thaumastodermatidae (Gastrotricha: Macrodasyida) Inferred from Nuclear and Mitochondrial Sequence Data. PLoS ONE 6 (3): e17892. doi:10.1371/journal.pone.0017892
- Wilke, U. (1954). Mediterrane Gastrotrichen. Zool. Jahrb. Syst. Ökol. Geogr. Tiere 82: 497–550.