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## Integrated data analysis allows the establishment of a new, cosmopolitan genus of marine Macrodasysida (Gastrotricha)

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Macrodasysida (phylum Gastrotricha) comprises 365 species distributed across 34 genera and 10 families. However, current classification is under revision due to the contradictory results of molecular and morphological cladistic analyses. Studies aimed at bridging the gaps took advantage of supplementary assessments of poorly known species and particularly from observations of new taxa showing original traits that could help to identify plesiomorphic character states in these anatomically diverse micrometazoa. We follow this path by describing three new interesting macrodasysidan species respectively from Italy, Brazil and Sweden. In many respects, the new species resemble most closely species of the genus *Macrodasys*; however, details of the external morphology, in combination with the different lay-out of the reproductive system and the tiny spermatozoa lacking a visible flagellum, suggest they belong to a new genus, possibly in the family Macrodasysidae. These hypotheses are supported by the phylogenetic relationships of 47 taxa inferred from analyses of the 18S rRNA gene, which found the new species clustering with *Thaidasys tongiorgii* in a subset of a larger clade containing *Macrodasys*. Accordingly, the establishment of the following taxa is proposed: *Kryptodasys* gen. nov., *K. marcocurinii* sp. nov., *K. carlosrochai* sp. nov. and *K. ulfjondeliusi* sp. nov.

Gastrotrichs are microscopic, benthic invertebrates found in both freshwater and marine ecosystems worldwide<sup>1</sup>. They constitute a phylum, recently united with the Platyhelminthes in a clade named Rouphozoa<sup>2,3</sup>. Gastrotricha includes, as of February 2019, 852 species, 511 of which are marine and 341 freshwater<sup>4</sup>. Gastrotricha is divided into two orders: Chaetonotida, common in both freshwater and marine ecosystems, and Macrodasysida, which is mostly marine<sup>5,6</sup>.

Macrodasysida includes about 370 interstitial species living in littoral and/or sublittoral sandy habitats. Classification and  $\alpha$ -biodiversity of the entire order are in a state of flux as shown by the recent ingroup taxa reassessments based on new phylogenetic data<sup>7–9</sup> and the persistent description of new species<sup>10–15</sup>. Taxonomic novelties have also emerged at supraspecific levels; in fact over the last five-six years, the number of genera and families has increased to 35 and 10, respectively, from the 31 genera and 8 families known before 2012<sup>8,9,16</sup>. The two genera described most recently, *Hummondasys* Todaro, Leasi & Hochberg, 2014 and *Thaidasys* Todaro, Dal Zotto & Leasi, 2015, were established on the base of peculiar specimens found in geographic areas never investigated before with regard to the gastrotrich fauna. Both genera are monotypic and so far are only known from their respective type locality: *Hummondasys* found at Negril in Jamaica, and *Thaidasys* collected at Phuket island in Thailand<sup>8,9</sup>. Curiously, among Chaetonotida, two genera were also recently described from areas poorly known, and similarly to the macrodasysidans, these genera are monotypic or nearly so: *Bifidochaetus* Kolicica & Kisielewski, 2015 and *Cephalonotus* Garraffoni, Arat ujo, Guidi, Lauren o & Balsamo, 2017<sup>17,18</sup>.

In a taxonomic framework, this scenario may lead to the wrong conclusion that nowadays relevant innovation regarding the Gastrotricha may originate only from remote areas and consist of rare and species-poor taxa having a restricted geographic distribution. In contrast with this vision, here we propose the establishment of a new

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genus based on the discovery of specimens belonging to a group of new species found in different regions of the world, including areas considered well investigated with regard to the gastrotrich fauna. The establishment of a new genus is based on the concordance between both the morphological characteristics of the studied specimens and the results of phylogenetic analyses based on the nucleotide sequence of the 18S rRNA gene. Furthermore, we show that species belonging to the new established genus were found in the past but they were erroneously affiliated to the genus *Macrodasys*.

## Results

### Taxonomic account

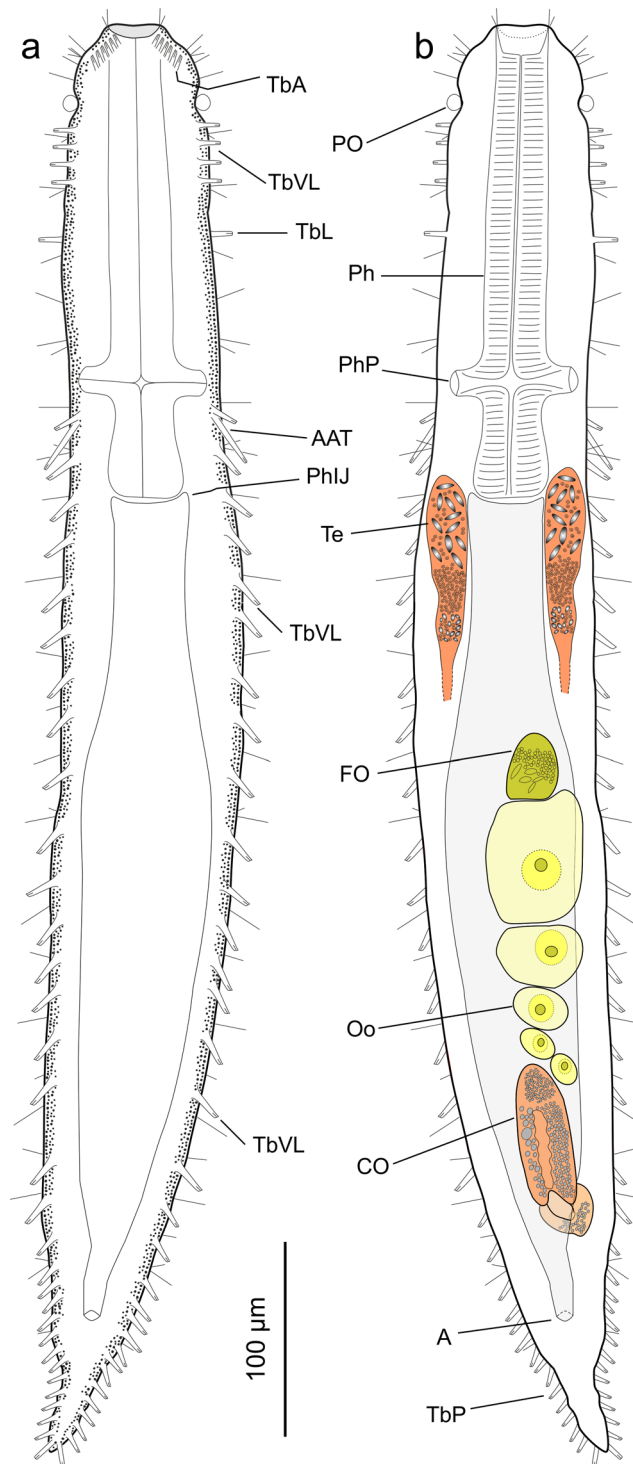
Phylum Gastrotricha Metschnikoff, 1865  
 Order Macrodasysida Remane, 1925 [Rao & Clausen, 1970]  
 Family Macrodasysidae Remane, 1924  
 Genus *Kryptodasys* gen. nov.  
 urn:lsid:zoobank.org:act:24D72D24-185C-8BCF-853C0E5023E

**Diagnosis.** Body vermiform, up to 1000  $\mu\text{m}$  in total length (TL), and up to 96  $\mu\text{m}$  in breadth, vaulted dorsally and flattened ventrally, with vacuolated cells along the body margins; epidermal glands generally few, small, scattered along the body. Cuticle naked, without spines and/or scales. Head ovoid or slightly bulbous, bearing pestle organs in a noticeable constriction. Trunk broadest in the mid-gut region, narrowing gently to the anus, then more quickly to the caudum; caudum unilobed, more often in the form of a short tail. Sensorial cilia distributed singly in dorsolateral and lateral columns along the body, sparingly around the head. Ventral locomotory ciliation rather sparse, arranged into two bands that run separately along most of the body but converging posterior to the anus into a single band. Anterior adhesive tubes (TbA), up to six per side, forming diagonal columns, which originate straight from the body surface and project forward; ventral adhesive tubes (TbV), absent; lateral adhesive tubes (TbL), absent or up to 2 along the pharyngeal region; ventrolateral adhesive tubes (TbVL), up to 26 per side, some along the pharyngeal region but most in the intestinal region; dorsal adhesive tubes (TbD), absent or up to 11 per side, most of which are present along the intestinal region; (TbDL), absent; posterior adhesive tubes (TbP), up to 10, along the the caudal margins. Paired accessory adhesive tubes, of two-three tubes per side, arising ventrolaterally from a common base anterior to the pharyngo-intestinal junction. Mouth terminal, of mid-size (up to 25  $\mu\text{m}$  in breadth), leading to a shallow buccal cavity (8–15  $\mu\text{m}$  in length); pharynx up to 293  $\mu\text{m}$  long and up to 31  $\mu\text{m}$  wide; pharyngeal pores well distant from the base, with dorsolateral openings. Pharyngo-intestinal junction (PhIJ) at U31–U43. Intestine straight, slightly wider at mid body; anal opening ventral at U89–U93. Hermaphrodite; testicles paired, elongate, beginning just anterior to the PhIJ; sperm ducts presumably open separately on the ventral surface; spermatozoa, stubby (5–10  $\mu\text{m}$  in length), rod-like to elliptical in shape, apparently devoid of a flagellum. Ovary solitary, rather posterior in the trunk region; oocytes growing from posterior to anterior with largest element dorsal to the mid intestine. Caudal organ, bulky, posterior to the ovary; glando-muscular in nature and approximately bullet-shaped; it bears a canal with a single opening at the posterior end; frontal organ, inconspicuous, anterior to the largest oocyte; non muscular in nature and approximately round-shaped; usually containing some spermatozoa and secretory material. Type-species, *Kryptodasys marcocurinii* sp. nov.; other species, *K. carlosrochai* sp. nov., *K. celticus* (Hummon, 2008), *K. hexadactylis* (Rao, 1970), *K. nobskaensis* (Hummon, 2008), *K. remanei* (Boaden, 1963) and *K. ulfondeliusi* sp. nov.

**Etymology.** *Kryptodasys* (*kryptós* Gr = hidden and *dasýs* Gr, hairy) the first word alludes to the uniqueness of this taxon, which has been overlooked for a long time while the second is traditionally used in the name of most gastrotrich genera and refers to their thick ciliation.

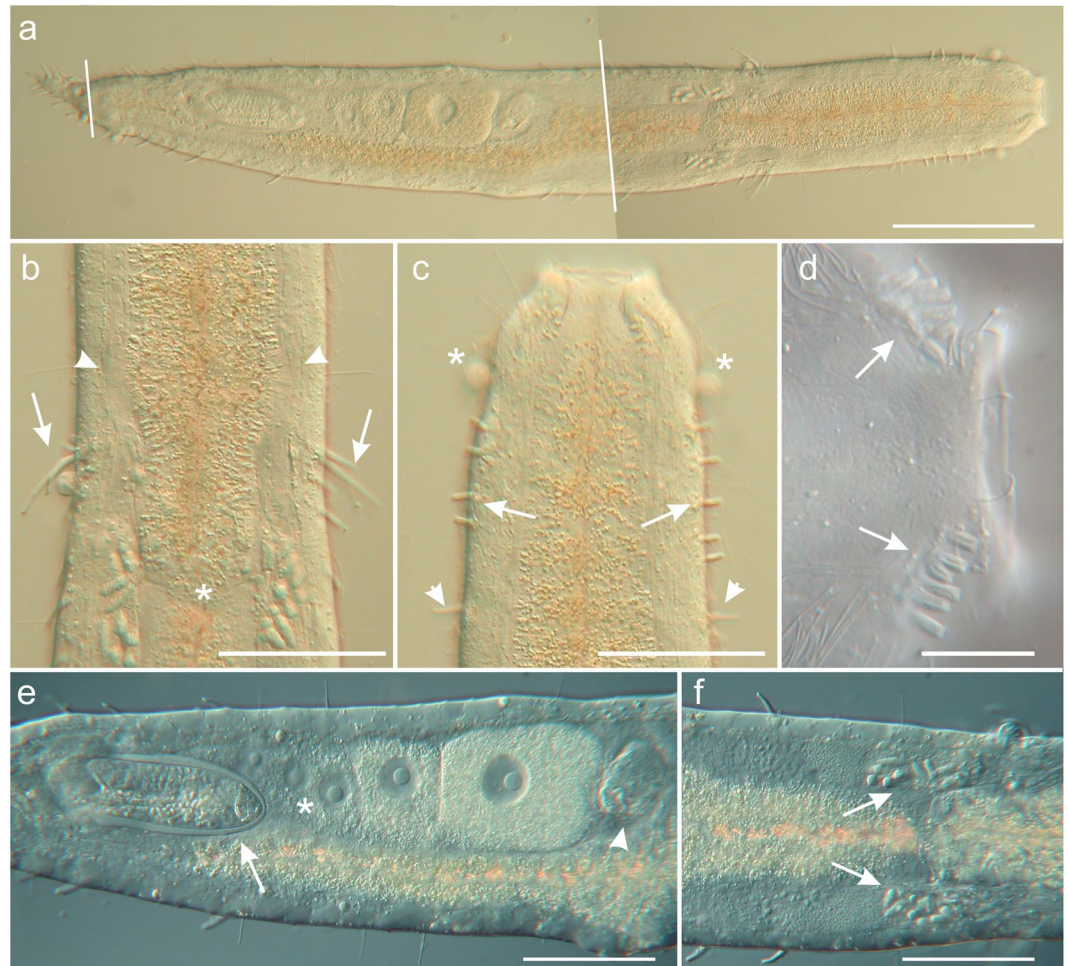
*Kryptodasys marcocurinii* sp. nov.  
 urn:lsid:zoobank.org:act:26B5FC81-CEEB-4741-9BA9-99DD162232B3  
 (Figs 1–4)

**Diagnosis.** Body elongate, 711–734  $\mu\text{m}$  in total length (TL), and up to 96  $\mu\text{m}$  in breadth; vaulted dorsally and flattened ventrally, with vacuolated cells along the body margins; epidermal glands generally few, small, scattered along the body. Cuticle naked, not forming spines and/or scales. Head slightly bulbous, bearing noticeable rounded pestle organs in a constriction. Trunk broadest in the mid-gut region, narrowing gently to the anus, then more quickly to the posterior end; caudum in the form of a short tail. Sensorial cilia organised singly in lateral and dorsolateral columns along the body, sparingly on the lateral sides of the head. Ventral locomotory ciliation rather sparse, arranged into two bands that run separately along most of the body but converging posterior to the anus into a single band. Anterior adhesive tubes (TbA), five to six per side, forming diagonal columns emerging directly from the body surface and projecting forward; ventral adhesive tubes (TbV) absent; ventrolateral adhesive tubes TbVL, up to 25 of which four to five are smaller and closer to each other in the anterior pharyngeal region (U07–U13), one in the posterior pharyngeal region (U31) and the other 19 equally spaced over the intestinal region from the pharyngo-intestinal junction (PhIJ) to the anus; lateral adhesive tubes (TbL), 1 per side in the pharyngeal region at U15; dorsolateral (TbDL) and dorsal adhesive tubes (TbD) absent; posterior adhesive tubes (TbP), 8–10, along the caudal margins. Paired accessory adhesive tubes (AAT), of two tubes per side, arising ventrolaterally from a common base anterior to the pharyngo-intestinal junction at U27. Mouth terminal, of mid-size (up to 26  $\mu\text{m}$  in width), leading to a shallow buccal cavity (15  $\mu\text{m}$  in length); pharynx up to 227  $\mu\text{m}$  long and up to 36  $\mu\text{m}$  wide; pharyngeal pores distant from the base with dorsolateral openings at U25. PhIJ at U33. Intestine straight, slightly wider at mid body; anal opening on the ventral side at U90. Hermaphrodite;



**Figure 1.** Line art illustrations of *Kryptodasys marcocurinii* sp. nov. **(a)** Habitus, ventral view. **(b)** Habitus, dorsal view, showing the internal organization with the male and female reproductive structures. Drawings are made mostly from the holotypic specimen. A = anus, AAT = accessory adhesive tubes, CO = caudal organ, FO = frontal organ, Oo = oocyte, Ph = pharynx, PhIJ = pharyngo-intestinal junction, PhP = pharyngeal pore, PO = pestle organ, TbA = anterior adhesive tube, TbL = lateral adhesive tube, TbP = posterior adhesive tube, TbVL = ventrolateral adhesive tube, Te = testicle.

testicles paired, elongate, beginning just anterior to the PhIJ; sperm ducts short, presumably open separately on the ventral surface; spermatozoa, stubby ( $9 \times 3 \mu\text{m}$ ), elliptical to pear-shaped, apparently devoid of a flagellum. Ovary solitary, rather posterior in the trunk region; oocytes growing from posterior to anterior with largest egg dorsal to the mid intestine at U59. Caudal organ, bulky, posterior to the ovary, centered at U78; glando-muscular



**Figure 2.** Differential interference contrast photomicrographs showing the morphology of *Kryptodasys marcocurinii* sp. nov. (a) Habitus, ventral view. (b) Close-up of the posterior pharynx region showing the accessory adhesive tubes (arrows), pharyngeal pores (arrowhead) and pharyngo-intestinal junction (asterisk). (c) Close-up of the anterior region, ventral view, showing the short ventrolateral adhesive tubes (arrows), lateral adhesive tubes (arrowhead) and pestle organs (asterisk). (d) Ventral view of the head, focussing on the anterior adhesive tubes (arrows). (e) Trunk region showing the ovary (asterisk), frontal organ (arrowhead) anterior to the largest egg, and caudal organ (arrows), posterior to the smallest oocyte. (f) Pharyngo-intestinal body region, showing the testicles with spermatozoa (arrows). Scale bars (a) = 100  $\mu\text{m}$ , (b,c,e,f) = 50  $\mu\text{m}$ , (d) = 20  $\mu\text{m}$ .

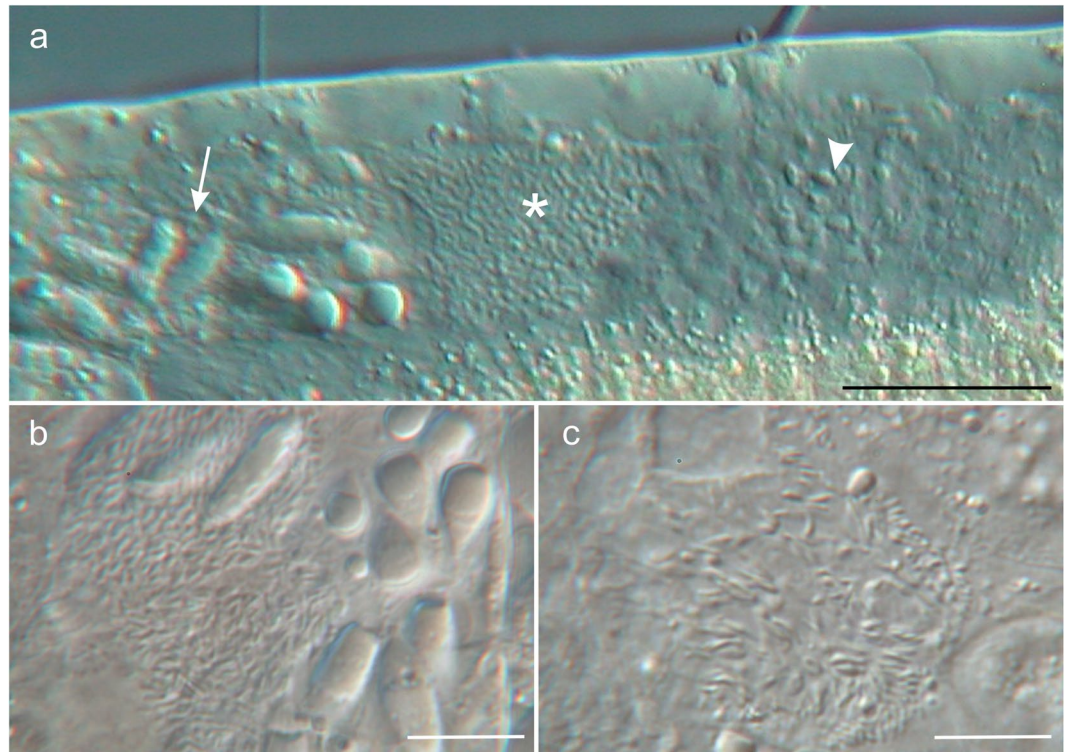
in nature and approximately bullet-shaped (71  $\mu\text{m}$  long  $\times$  26  $\mu\text{m}$  wide); it bears a canal with a single opening at the posterior end; frontal organ, sac-like, anterior to the largest oocyte, centered at U52; non muscular in nature and approximately ovoid in shape (36  $\times$  26  $\mu\text{m}$ ); usually containing some spermatozoa and secretory material.

**Etymology.** The species is named after Marco Curini-Galletti, a colleague, friend, and superb scuba diver, who in the numerous, joined sampling trips, scornful of danger, has on several occasions threatened his life to collect our samples, including the present ones.

**Type material.** Holotype: the 734  $\mu\text{m}$  long adult specimen shown in Figs 2–4 no longer extant (International Code of Zoological Nomenclature<sup>19</sup>, Articles 73.1.1 and 73.1.4; see also recommendation 73G–J of Declaration 45 - Addition of Recommendations to Article 73<sup>20</sup>), collected on 11/07/2005. *Additional examined material.* three adults, one subadult and one juvenile specimen, collected from the type locality and other locations; all were observed alive and went destroyed during the observation. Two additional adults were preserved in a 95% ethanol solution and subsequently used for DNA analysis (see below and Supplementary Table S1).

**Distribution and ecology.** Type locality - Sardinia: Grotta di Nereo (Nereo's cave, Lat. 40°33'70.5"N, Long. 08°09'62.9"E); occasional in frequency of occurrence and numerous in abundance in medium, moderately sorted sand collected at a depth of 30.7 m. Other locations: Grotta il Porticato, occasional in frequency and scarce in abundance at 20 m depth in coarse moderately sorted sand; Costa Paradiso, sparse in frequency and rare in abundance at 35 m depth in very coarse, well sorted sand. In all cases, values of temperature and salinity of the pore water at the time of samplings were 13 °C and 38 PSU, respectively. Values of the granulometric parameters are reported in Supplementary Table S2.





**Figure 3.** Differential interference contrast photomicrographs showing the male gonad of *Kryptodasys marcurinii* sp. nov. (a) Left testicle apparently compartmentalized, showing mature spermatozoa (arrows) in the anterior portion, fine granular material (asterisks) in the mid portion, and small cellular elements, possibly maturing sperm, mixed with granular material in posterior region (arrowhead). (b) Close-up of the anterior portion. (c) Close-up of the posterior portion. Scale bars (a) = 50  $\mu\text{m}$ , (b,c) = 10  $\mu\text{m}$ .

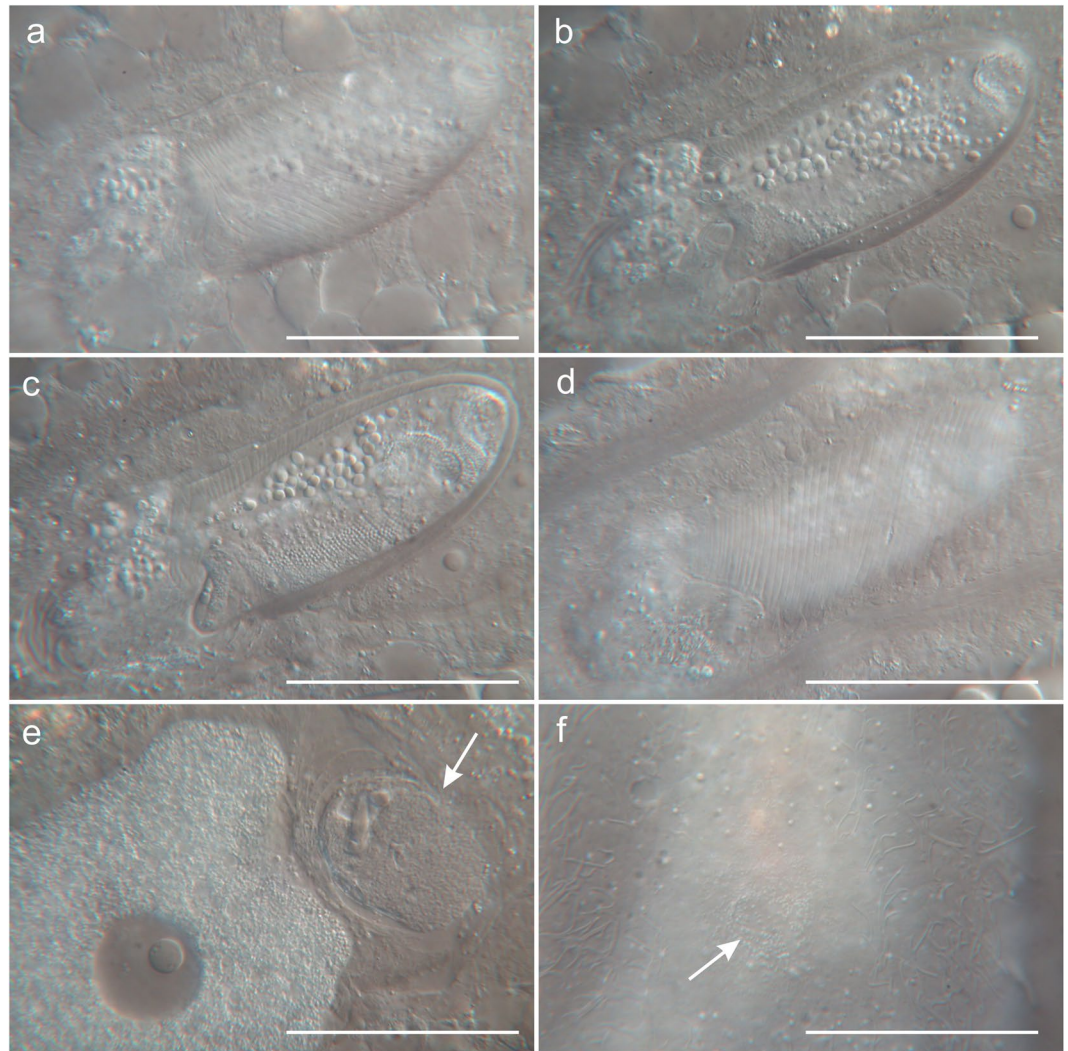
**Description.** Based mostly on the adult specimen with a total body length of 734  $\mu\text{m}$  shown in Fig. 2. Body vermiform and of medium width (Fig. 2a); vaulted dorsally and flattened ventrally, bearing vacuolated cells along the lateral and dorsolateral body margins; epidermal glands few, small, scattered along the body. Cuticle smooth, not forming spines and/or scales. Head slightly bulbous, bearing noticeable pestle organs in a constriction at U05 (Figs 1 and 2a). Body of similar width in the anterior third increasing slightly in breadth to mid trunk and thereafter narrowing gently to the anus, and then more quickly to the caudum; caudum in the form of a short tail (Figs 1 and 2a). Widths of head/mid pharynx/PhIJ/trunk/anus/base of tail, and locations along the length of the body are as follows: 64/79/85/96/43/25  $\mu\text{m}$  at U04/U16/U33/U55/U90/U93, respectively.

**Ciliation:** Sparse sensorial cilia (10–18  $\mu\text{m}$  long) insert on the dorsal and ventrolateral margin of the head, in addition to about 25–30 other elements (15–22  $\mu\text{m}$  long) organised singly in dorsolateral and lateral columns along the body. Ventral locomotor ciliature forms two longitudinal bands extending separately from under the head to the posterior trunk region but converging behind the anal opening into a single band; ciliary bands appear wider, denser and closer to each other along the pharyngeal region (Fig. 2b).

**Adhesive tubes:** TbA, six per side (6–8  $\mu\text{m}$  in length), forming diagonal columns, emerging directly from the body surface and projecting anteriorly (Figs 1a and 2d); TbV, absent; TbVL, 25 per side, four–five of which are smaller (10–12  $\mu\text{m}$  in length) and closer to each other in the anterior pharyngeal region from U07 to U13 (Figs 1a and 2b), one is in the posterior pharyngeal region at U31 and the remaining 19 (13–18  $\mu\text{m}$  in length) equally spaced along the intestinal region, from the PhIJ to the anus; TbL, one per side (13  $\mu\text{m}$  in length) in the pharyngeal region at U15 (Fig. 1); TbD and TbDL absent; TbP, ten per side (8–13  $\mu\text{m}$  in length), surrounding the caudum (Figs 1a and 2a). In addition, there are paired accessory adhesive tubes, two tubes per side, arising ventrolaterally from a common base anterior to the pharyngo–intestinal junction at U27; tubes are of different size, and posterolaterally directed; the shortest tube, 13  $\mu\text{m}$  in length, arises anteriorly while the longest is posterior and twice as long, 26  $\mu\text{m}$  (Figs 1 and 2a,b).

**Digestive tract:** Mouth is terminal, 26  $\mu\text{m}$  in width, with the mouth rim slightly protruding forward; buccal cavity rather shallow (15  $\mu\text{m}$  in length) and lined with a thin cuticle (Figs 1 and 2a); pharynx, 227  $\mu\text{m}$  in length, widens to 37  $\mu\text{m}$  toward the rear up; pharyngeal pores open dorsolaterally distant from the base at U25. Pharyngo-intestinal junction (PhIJ) at U33. Intestine is straight and increases in breadth from the PhIJ to mid-body where reaches 66  $\mu\text{m}$  in width and then gradually narrows toward the posterior body end; anal opening ventral at U90. In all of the examined specimens, the intestine contained yellowish/orange coloured material, probably biodebris, but not diatom frustules (Fig. 2e,f).

**Reproductive tract:** Hermaphroditic; testicles paired and elongate; they begin just anterior to the PhIJ and span posteriorly for about 95  $\mu\text{m}$ , from U31 to U46 (Figs 1b and 2a,f). Testicles appear to be anatomically and

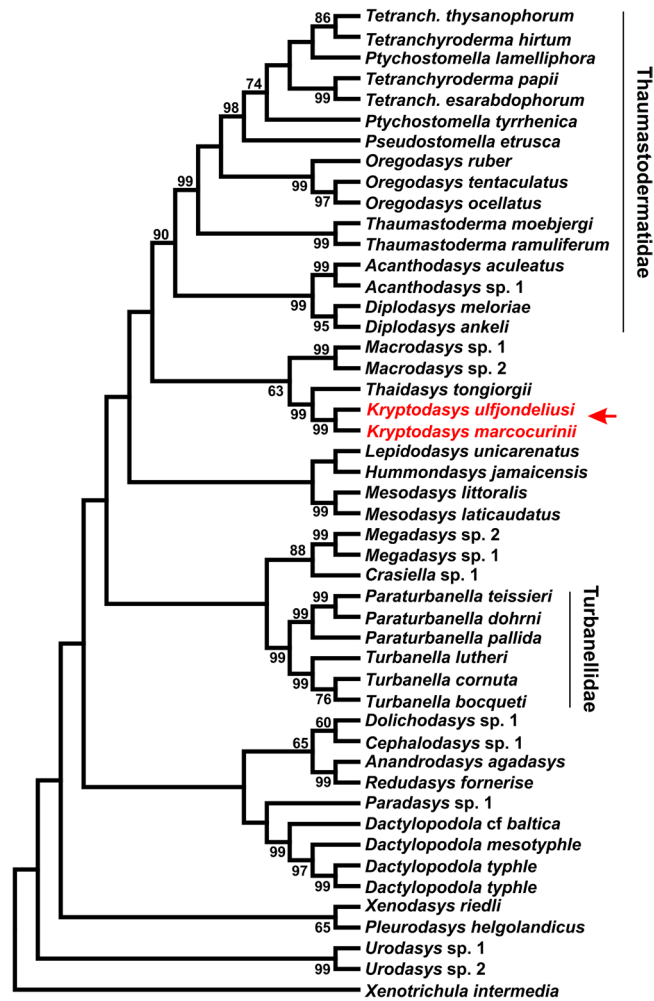


**Figure 4.** Differential interference contrast photomicrographs showing the accessory reproductive organs of *Kryptodasys marcocurinii* sp. nov. (a–d) Caudal organ at different focal planes. (e) Frontal organ, containing some spermatozoa and granular material. (f) External pore of the frontal organ on the ventral body side (arrow). Scale bars (a–d) = 50  $\mu\text{m}$ , (e,f) = 30  $\mu\text{m}$ .

functionally compartmentalized as the anterior portion contains large and better structured cellular elements (likely spermatozoa), the mid portion shows fine granular material, while the posterior portion holds granular material mixed with small cellular elements (likely maturing sperm) (Fig. 3). Each testicle apparently seems to continue posteriorly with a short sperm duct, which presumably opens on the ventral surface. Ultrastructural studies are needed to ascertain the exact organization and function of testicles and ducts. Spermatozoa are stubby (9  $\mu\text{m}$  in length and 3  $\mu\text{m}$  in width), elliptical to pear-shaped, apparently lacking a flagellum (Figs 1b, 2f and 3a,b). Ovary single, rather posterior in the trunk region; oocytes growing from posterior to anterior with largest oocyte dorsal to the mid intestine at U59. (Figs 1b and 2a,e). Caudal organ noticeable, posterior to the ovary, centered at U78 (Figs 1b and 2a,e); glando-muscular in nature and approximately bullet-shaped, 85 long and 26  $\mu\text{m}$  wide (Fig. 4a–d); it bears a canal with a single opening at its posterior end; the entire organ opens on the ventral surface, anterior to the anus at U83. Frontal organ, sac-like, anterior to the largest oocyte, centered at U52; non muscular in nature and roughly ovoidal in shape (26  $\mu\text{m}$  long and 34  $\mu\text{m}$  wide); usually containing some spermatozoa and secretory material (Figs 1b, 2a,e and 4e). An inconspicuous opening, surrounded by secretory material, was observed on the ventral surface in correspondence of the frontal organ; it is interpreted as its external pore (Fig. 4f); the internal pore of the frontal organ was not seen.

**Variability and remarks.** The three additional measured adult specimens ranged from 711 to 720  $\mu\text{m}$  in total length. All of them had fully developed male and female gonads, and accessory reproductive organs, similar to the holotype. The adhesive apparatus was also similar to that of the holotype, however, the 720  $\mu\text{m}$  long adult had five TbA and only four TbVL in the anterior region of the pharynx. The observed subadult, 680  $\mu\text{m}$  in total length, showed on each side four TbA, six TbP and 18 TbVL, four of which were in the anterior region and one in





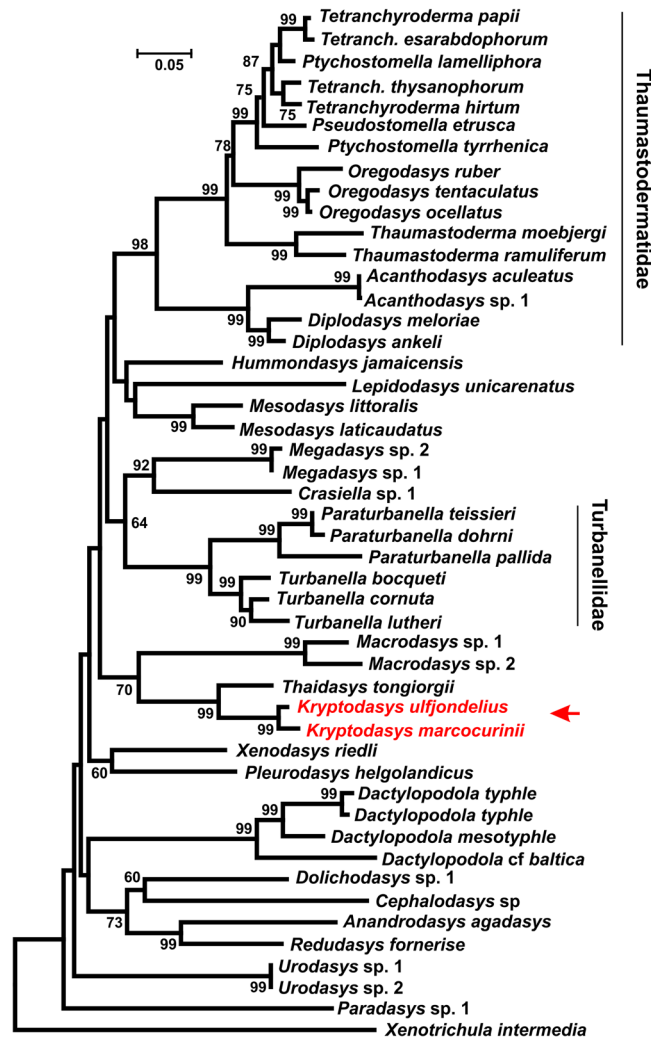
**Figure 5.** Phylogenetic relationships of 47 Gastrotricha Macrodasysida including two species of the new genus *Kryptodasys* inferred from MP analysis of 18S rRNA gene. The chaetonotidan *Xenotrichula intermedia* (Fam. Xenotrichulidae) is the outgroup. The most parsimonious tree (length = 4095; consistency index = 0.356007, composite index = 0.232281) is presented. Numerical values at nodes represent bootstrap support.

the posterior region of the pharynx; the lateral adhesive tubes present in the pharyngeal region of the adult were also present in the subadult; likewise present were the paired accessory adhesive tubes. By contrast, the subadult specimens was lacking gonads and frontal organ while the caudal organ was starting to become visible. The juvenile, 434  $\mu\text{m}$  in total length, showed on each side three TbA, three TbP and six TbVL, one of which was present in the anterior pharyngeal region. The TbL and the accessory adhesive tubes were present also. Ontogenetic variations with regard to metric and meristic traits (e.g., number of adhesive tubes) are a normal occurrence during gastrotrich growth. However, our data also indicates variability among adults, although it is minimal; variation was observed in the total length and the number of TbA and TbVL present in anterior region of the pharynx. On the other hand, other traits appear to be present throughout all of the life stages (at least the documented ones), as is the case of the single TbL in the pharyngeal region and the paired accessory adhesive tubes, both present from the juvenile age to adulthood. This new species will be differentiated later in light of the other two new species descriptions (see Supplementary Material, Appendix S1).

**Phylogenetic analysis.** The ultimate alignment counted 1906 positions, 850 of which appear constant and 723 informative under the parsimony criterion. MP, ML and Bayesian analysis yielded highly congruent topologies, with most of the shared clades bearing high statistic support at nodes: i.e., Bayesian posterior probability and bootstrap values  $>98\%$  and  $>60$ , respectively (Figs 5–7).

Some of the well supported groups are (1) the heavily sampled families Turbanellidae and Thaumastodermatidae and their traditional subgroupings (i.e., subfamilies and genera); (2) the sister-group relationship between *Anandrodasys agadasys* (Hochberg, 2003) and *Redudasys fornerise* Kisielewski, 1987; and (3) the recently recognised alliance between *Megadasys* Schmidt, 1974 and *Crasiella* Clausen, 1968.

On the other hand, Cephalodasyidae and Macrodasysidae under no circumstances appear as monophyletic due to the association between representatives of different families and/or the scattering along the phylogenetic trees



**Figure 6.** Phylogenetic relationships of 47 Gastrotricha Macrodasysida including two species of the new genus *Kryptodasys* inferred from ML analysis of 18S rRNA gene. The chaetonotidan *Xenotrichula intermedia* (Fam. Xenotrichulidae) is the outgroup. The tree with the highest log likelihood (−20472.3991) is presented. Numerical values at nodes represent bootstrap support.

of their respective species. Multisampled genera appear also as monophyletic in the obtained topologies (except *Ptychostomella* Remane, 1926 and *Tetranchyroderma* Remane, 1926).

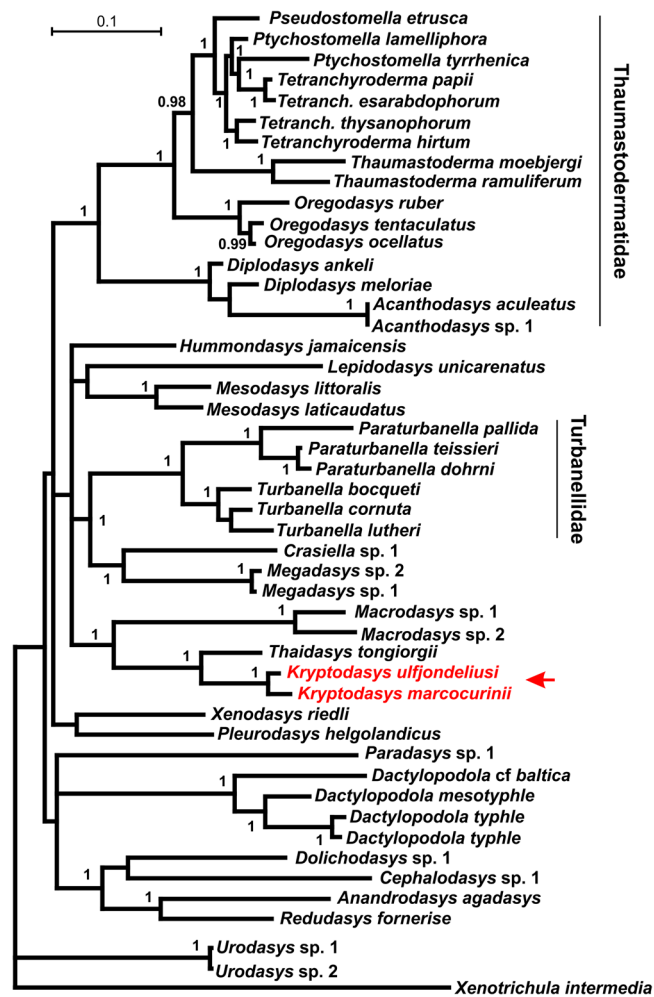
Concerning the two new taxa included in the genus *Kryptodasys* gen. nov., all of the obtained trees show them forming a monophyletic clade that is sister to *Thaidasys tongiorgii* (Fam. Macrodasysidae). In turn, the clade *Thaidasys* + *Kryptodasys* gen. nov. appears in a sister-group relationship with *Macrodasys* (Fam. Macrodasysidae); all these clades received high statistical support at nodes (Figs 5–7).

## Discussion

**Remarks on diagnostic features and morphology.** Body size and shape, general organization of the adhesive apparatus, and the arrangement and composition of the reproductive apparatus of the three new taxa are comparable to species of the genus *Macrodasys*. However, the new species appear to be distinct from the overwhelming majority of *Macrodasys* species due to peculiarities of their reproductive apparatus and the morphology of their spermatozoa. Specifically, the new species have the frontal organ (=seminal receptacle, female in function) located anterior to the largest egg (Figs 2e and 4e; Supplementary Figs S3c, S6b and S7c), while species of *Macrodasys* have the frontal organ located posterior to the largest egg. Anatomically speaking, this means that while the frontal organ and caudal organ (=copulatory organ, male in function) of the new taxa are separated by the ovary and its cellular products (maturing oocytes), in *Macrodasys* the two organs are positioned next to each other in the same body compartment. Regarding the male gametes, the new species possess rather short, smooth spermatozoa lacking a regular flagellum (Figs 2f and 3a,b; Supplementary Figs S3a,c, S6b and S7b), in contrast with the species of *Macrodasys*, which possess filiform sperm, with a cork-screw-like anterior portion and a long flagellum showing the usual  $9 \times 2 + 2$  axonemal organization<sup>21</sup>.

Studies have shown that the highly differentiated shape of the accessory reproductive organs, the organization of the reproductive apparatus, and the structure and ultrastructure of the spermatozoa bear phylogenetic signals





**Figure 7.** Phylogenetic relationships of 47 Gastrotricha Macrodasysida including two species of the new genus *Kryptodasys* inferred from Bayesian analysis of 18S rRNA gene. The chaetonotidan *Xenotrichula intermedia* (Fam. Xenotrichulidae) is the outgroup. Numerical values at nodes represent posterior probabilities.

that may be utilized, usefully, for taxonomic and classification purposes<sup>7,22,23</sup>. Thus, in our opinion, the morphological differences highlighted above are indicative of a clear distinctness (autoapomorphies) of the new taxa from *Macrodasys* and suggest the establishment of a new genus to accommodate them. The phylogenetic analyses based on a molecular marker (18S rRNA gene) provides support for such a hypothesis (see phylogenetic results above and phylogenetic remarks below). Therefore, the establishment of the new taxon, *Kryptodasys* gen. nov. is proposed.

The three new described species (see also Supplementary Material, Appendix S1), can easily be distinguished from each other based on several autoapomorphic characteristics of the adult specimens. Some of the main distinctive traits are reported hereafter (see also Supplementary Table S3). *K. marcocurinii* sp. nov. is of larger size, compared to *K. carlosrochai* sp. nov. and to *K. ulfjondeliusi* sp. nov.; for instance, the Sardinian species has a TL up to 734 µm while the other two species are 515 µm and 595 µm in total length, respectively. The Sardinian species also has many more adhesive tubes compared to the Brazilian and the Swedish species; for example, in the former species, the TbA are up to 6 per side vs 3 and 4 tubes present, respectively, in the other two species; there are up to 25 TbVL in the Sardinian species vs 10 and 18 tubes present in the Brazilian and Swedish species, respectively. The arrangement of the TbVL is also different in the three species. More specifically, *K. marcocurinii* shows 5–6 tubes in the pharyngeal region, 4–5 of which are of smaller size and closer to each other in the anterior region; in *K. carlosrochai* there is a single tube in the pharyngeal region located halfway along the pharynx and its size is not different from the other TbL; in *K. ulfjondeliusi* there are three tubes in the pharyngeal region of ‘normal’ size that are equally spaced along the posterior half of the pharynx. Moreover, *K. carlosrochai* is unique also in that its accessory adhesive tubes are made up of 3 tubes each while in the other two species these organs are made up of 2 tubes only. *K. ulfjondeliusi* is further distinguishable as it possesses TbD, which are absent in the other two species.

**A revision of *Macrodasys* species.** Currently, the genus *Macrodasys* includes 37 species, making it the second largest macrodasysidan genus after *Tetranchyoderma* (Thaumastodermatidae). *Macrodasys* was established

by Adolf Remane based on the distinctive morphological characteristics of a novel ‘...aberrante Gastrotrichen...’ found in the bay of Kiel (Germany), named *Macrodasys buddenbrocki*<sup>24</sup>. *M. buddenbrocki* Remane, 1924 was described and illustrated as having paired testicles filled with filiform spermatozoa, and two accessory reproductive organs, the most anterior of which is located posterior to the largest egg cells (see Pg. 24, Fig. 2 in Remane<sup>24</sup>). This organ, described as being connected to the sperm ducts, was thought by Remane to function as a *vesicula seminalis*, thus being male in function. It is now known that in *Macrodasys* sperm ducts empty separately on the ventrolateral sides via independent pores; consequently, Remane’s *vesicula seminalis* is currently interpreted as a seminal receptacle, thus female in function, and it is called a frontal organ<sup>25</sup>. The second accessory sexual organ of *M. buddenbrocki* is located more posterior in the trunk region. This additional organ, considered by Remane to be a *bursa copulatrix* (female in function), is currently interpreted as a copulatory organ and is called a caudal organ<sup>25</sup>.

Most of the species presently affiliated to the genus *Macrodasys* have been described as having such traits, including the four additional species described by Remane himself in the years following the discovery of *M. buddenbrocki*<sup>26–28</sup>. However, some *Macrodasys* species, described later on by other authors, do not appear to comply fully with the organization of the reproductive system typical of the genus. These species include: *M. remanei* Boaden, 1963 from north Wales (U.K.), *M. hexadactylis* Rao, 1970 from the coast of Andhra (India) and *M. nobskaensis* Hummon, 2008 from Massachusetts (USA). One of the biggest differences is that in these three species, a structure interpretable as the frontal organ is positioned in front to the largest egg cells as opposed to posterior to the egg cells characteristic of other species of *Macrodasys*<sup>29–31</sup>. In our opinion, this evident discrepancy distinguishes these three species from the ‘genuine’ species of the genus *Macrodasys*; the general morphology of these species is also more similar to our new species. Consequently, we propose to transfer these three species to the genus *Kryptodasys*. Other morphological traits support the re-classification of these three species according to our proposal. For instance, *M. remanei* and *M. hexadactylis* both bear paired accessory adhesive tubes arising ventrolaterally, well anterior to the pharyngo-intestinal junction (see Fig. 8 in Boaden<sup>29</sup> and Fig. 1 in Rao<sup>30</sup>); this is an anatomical characteristic that is also present in all three *Kryptodasys* species described from Sardinia, Brazil and Sweden but not in other species of *Macrodasys*. In addition, *M. hexadactylis* possesses short spermatozoa, lacking a typical flagellum (see Fig. 5 in Rao<sup>30</sup>), a condition shared with the three new *Kryptodasys* species but which is in contrast with the filiform spermatozoa of the ‘genuine’ *Macrodasys* taxa. Future research could shed light on the presence of the paired accessory adhesive tubes in *M. nobskaensis* and on the morphology of the spermatozoa in *M. remanei* and *M. nobskaensis*, information that is currently unavailable.

Beside the three species indicated above, another species, currently classified in the genus *Macrodasys*, should be transferred to the genus *Kryptodasys* i.e., *M. celticus* Hummon, 2008.

In describing this species, Hummon<sup>31</sup> remarks: “Unusual is the female reproductive system of *Macrodasys celticus* n. sp., eggs developing front to rear, combined with a caudal organ that is located just behind the largest egg, along with an absence of any structure where the frontal organ would be found”. Because the combination of such characteristics have no equal among gastrotrichs, if real, they would require the erection of (at least) a new genus to allocate these specimens. However, the situation might be much simpler. In fact, if the two structures thought by the author to be developing oocytes positioned anterior to the largest egg are re-interpreted as the frontal organ, then the organization of the reproductive apparatus of this species is similar to that of species of the genus *Kryptodasys*. The shape and position of the caudal organ along with the arrangement of the TbA in diagonal columns make the resemblance of *M. celticus* and *Kryptodasys* species even stronger and strengthen our hypothesis. Again, future investigations should verify in *Kryptodasys celticus* (= *Macrodasys celticus*) the presence of the accessory adhesive tubes and shape of the spermatozoa, information currently lacking.

An emended diagnosis of the genus *Macrodasys* along with a list of the species that fit such a diagnosis, and a taxonomic key to species of the genus *Kryptodasys* are reported in the Supplementary Material, Appendix S1.

**Phylogenetic remarks.** In the last two decades, phylogenetic studies using molecular genetic data have proved to be very helpful in clarifying relationships among and within a large number of taxonomic groups. Regarding Gastrotricha, previous phylogenetic studies based on gene sequences (18S rRNA gene alone or in combination with the 28S rRNA and Cox1 genes) have offered support for some of the traditional grouping established on anatomical characteristics (morphological traits) e.g., the families Thaumastodermatidae and Turbanellidae, most genera etc., but have also highlighted unpredicted associations e.g., between species that seem to have very little in common with regard to the general anatomy or between taxa that were affiliated to distinct families<sup>16,32,33</sup>. Some of the new phylogenetic alliances revealed by these early molecular studies have then been confirmed by subsequent investigations, and considered to be very likely by re-assessing in an evolutionary framework the morphological characteristics of the species involved<sup>7,16</sup>. These outcomes have permitted the re-classification of some taxa and consequently reduced some of the major divergences existing between e.g., the traditional classification based on morphological characteristics and the new phylogenetic scenarios put forward by the molecular analyses. In this novel framework, other studies based on molecular data have provided clues about the origin and phylogenetic alliances in cases where the morphological information had proved to be non-resolutive<sup>9,34</sup> while others have strengthened early hypotheses based on morphological clues<sup>35,36</sup>. These experiences leave little doubts about the high significance of the molecular studies in assessing the evolutionary relationships within Gastrotricha. Results of the present study provide robust evidence (i.e., high congruence of the obtained trees, and high statistical support at the crucial nodes) regarding the status and the phylogenetic position of the new species and genus within Macrodasysidae. In our opinion, this hypothesis should be considered very likely, mostly because in a wider framework, the topology of the current phylogenetic trees confirms the in-group evolutionary scenario of the Macrodasysida illustrated by other recent studies<sup>8,9,16,37</sup>. Moreover, while at a morphological level there are not traits that could suggest different, potential alliances (e.g., armored cuticle, spermiducts confluent in a single male pore etc.), the new species share several similarities with the other

members of Macrodasysidae especially with *Macrodasys* (see above). While the position of the new genus within Macrodasysidae appears robust, we point out that in the current analysis (and in others as well), another taxon associated with the Macrodasysidae by traditional taxonomy, *Urodasys*, does not band with the other macrodasysids. This conflicting condition should be explicitly addressed in future research e.g., by improving the taxonomic sampling of *Urodasys* species.

**Conclusive remarks.** A growing body of evidence suggests the evolutionary alliances hypothesized by analyses based on molecular markers (e.g., 18S rRNA gene) to be very likely and consequently extremely valuable in the ongoing process of the natural classification of the Gastrotricha.

This is particularly true in the case of robust out-puts e.g., the resulting trees bear high support at nodes and/or the topologies obtained from different analysis are virtually the same. Our current study seems to be strictly in line with all the above. Consequently, the uniqueness of the new species described here, and their place within the Macrodasysidae, as indicated by the phylogenetic trees, appears to be very likely. Furthermore, the phylogenetic position of the new species within the family, most closed to the genus *Thaidasys* (molecular information), and the layout of their reproductive system distinct from that of *Thaidasys* and *Macrodasys* (morphological information) both support the hypothesis that the formal classification of the new species requires the erection of a new genus, which is named named *Kryptodasys*. A review of the relevant literature, found that species belonging to the new established genus *Kryptodasys* were already recorded in the past, but erroneously affiliated to the affine genus *Macrodasys*. A signal that such event may plague other portions of Gastrotricha taxonomic spectrum and, at the same time, an invitation to further challenge the traditional classification with data based on molecular markers, to reach a sole, concordant phylogeny. Finally, data show that *Kryptodasys*, similarly to most other genera of Gastrotricha, is cosmopolitan in distribution (*sensu* Sterrer<sup>38</sup>), suggesting that this very likely applies also to the very few monotypic genera currently known only from restricted areas, often from a single location, a call to extend the research.

## Methods

**Sampling.** Specimens belonging to the new genus were found during unrelated investigations in different geographic areas. The very first specimens belonging to the genus were found by MAT in 2001 during investigation at the Elba Island (Italy). Subsequently, animals with similar characteristics were found over the years in: Sardinia (Italy), São Paulo State (Brazil), Alboran Sea (Spain), Florida (USA), Gullmarsfjord (Western Sweden) and Lanzarote (Canary Islands, Spain). In general, specimens were found in low numbers, often less than five specimens; as a consequence, data granting an adequate description are available, to date, only for the animals collected in Sardinia, Brazil and Sweden. These specimens are described herein as three new species. In all cases, animals were found in sublittoral sand collected using 500 ml plastic jars<sup>39</sup>. The Sardinian gastrotrichs were found from the north western coast of the island, in 2005 near Capo Caccia in sediment taken at a depth of 19–30 m, and subsequently in 2010 at Costa Paradiso in sand collected at a depth of 35 m. Sampling was performed by scuba diving at both locations<sup>40</sup>. The Brazilian specimens were found in 2002 in sand from the island of Ilhabela collected at a depth of 2–4 m, while the Swedish worms were found in 2008 at Östersidan at a depth of 0.5–2.5 m. Both the Brazilian and Swedish samples were collected by skin diving<sup>41–43</sup>. Additional details are provided in the type material section of each species.

Use and handling of the animals subject of the present study (Gastrotricha) is not regulated/prohibited; furthermore, collection from the wild does not require special authorization if carried out in public areas (beaches) as it was in the present case.

**Sample processing and morphological analysis.** In all cases, the collected samples were transferred to the local field laboratory and processed within one week from collection. Gastrotrichs were extracted daily from aliquots of sediment by the narcotization-decantation technique, using a sea-water isosmotic (6–7%) magnesium chloride solution. The fauna-containing supernatant was then poured directly into a 3–5 cm-diameter Petri dish and scanned for specimens under a Wild M3/M8 dissecting microscope. Individual gastrotrichs were picked out with a micropipette, wholemounted on microscope slides and studied alive under Nomarski optics<sup>44</sup>; specimens were photographed and measured during observation. The following equipment was used: Sardinia, Leitz Dialux 20 microscope equipped with a Nikon 995 digital camera; Brazil, Zeiss Axioscop 2 Plus microscope fitted with a Nikon 995 digital camera; Sweden, Nikon Eclipse 80i microscope equipped with a Nikon Digital Sight DSFi1 digital camera. After identification, some specimens were stored in 95% ethanol for future DNA analysis (see below).

**Species description and illustration.** The description of the new species (see also Supplementary Material, Appendix S1) follows the convention of Hummon *et al.*<sup>45</sup>; the position of some morphological characters along the body are given in percentage units (U) of total body length. CorelDRAW® Graphics Suite v. X5 was used to prepare the line art illustrations and to assemble the figures.

**Granulometric analysis and ecology.** Study of the substratum was performed following Giere *et al.*<sup>46</sup>. In short, sand from each site was rinsed with tap water and dried in oven at 60 °C for 48 hr. Then, 150 g of dry sediment was analysed by passing through a stack of six sieves with the following phi mesh size: –1, 0, 1, 2, 3, 4, 5. Sieves were shaken for 15 min on a mechanical sieve shaker. The sediment fraction retained on each sieve was weighed to 0.01 g and the resulting data entered into a computer program founded on the calculation of Seward-Thompson & Hails<sup>47</sup> to obtain the following parameters: mean grain size, sorting coefficient, skewness and kurtosis<sup>48</sup>. Sediment size and sorting classes are based on Wentworth tables<sup>49</sup>. The ecological characteristics



of the new species such as frequency and abundance are determined according the rationale originally proposed by Hummon *et al.*<sup>50</sup> and subsequently implemented in several other studies e.g., Todaro *et al.*<sup>8,14,15</sup>.

**Molecular analysis.** The position of the new species along the Macrodasysida phylogenetic tree was inferred from the analysis of the 18S rRNA gene of 45 terminals belonging to the near complete taxonomic spectrum of the order: 45 species of 25 genera in ten families. A basal species of Chaetonotida, *Xenotrichula intermedia* Remane, 1924 (Xenotrichulidae), was selected to be the out-group. Most of the used sequences were obtained from GenBank (Supplementary Table S1). Of the three new species described herein, sequences were obtained only from *Kryptodasys marcocurinii* sp. nov. and *K. ulffjondeliusi* sp. nov. In both cases, sequences were derived from single whole specimens; DNA extraction and gene amplification were carried out according to Todaro *et al.*<sup>51</sup>. The PCR products were cleaned with the QIAquick PCR Purification Kit (QIAGEN) and shipped for sequencing to Macrogen, Korea ([www.macrogen.co.kr](http://www.macrogen.co.kr)). Sequences were assembled using Staden v1.6.0<sup>52</sup> and the alignment performed with MUSCLE as implemented in MEGA 6<sup>53</sup>. The final data matrix, which consisted of 48 terminals and 1895 nucleotide characters, was later opportunely formatted and analysed cladistically using three distinct methods: (1) Maximum Parsimony (MP), (2) Maximum Likelihood (ML), and (3) Bayesian inference (BI). MP and ML were conducted using MEGA 6 while BI was run using MrBayes 3.1.2<sup>54</sup>. For ML and BI methods, the General Time Reversible model setting with estimated proportion of invariable sites and gamma distributed rate variation across sites, favoured by both the lnL and the AICc criteria in MEGA 6 and MrModeltest v2.3<sup>55</sup>, was used. In the MP and ML analyses, statistical support for the tree branches was obtained using 1000 bootstrap replicates. For the BI analysis, two independent trails with four chains each were run for six million generations; after a burn-in of 15000 generations, chains were sampled every 100<sup>th</sup> generation. A consensus tree (50%) was obtained using TreeView<sup>56</sup>.

**Nomenclatural acts.** This article meets the requirements of the amended International Code of Zoological Nomenclature<sup>19</sup> as the article itself, and the nomenclature acts it contains have been registered in ZooBank under urn:lsid:zoobank.org:pub:6630A1EA-3197-4780-9DE9-8560C44F6F4A

## Data Availability

All appropriate data created by the authors (i.e., drawings, photographs etc.) are available within the article and/or its supplementary material. Molecular data supporting the findings are openly available from GenBank under the accession numbers reported in Supplementary Table S1.

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## Author Contributions

M.A.T. Planned the study. M.A.T., M.D. and K.T. carried out the field and/or laboratory parts. M.A.T., M.D. and R.H. analysed the data. M.A.T. provided materials/reagents/analysis tools. M.A.T. wrote the first draft; all authors reviewed the manuscript.

## Additional Information

**Supplementary information** accompanies this paper at <https://doi.org/10.1038/s41598-019-43977-y>.

**Competing Interests:** The authors declare no competing interests.

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## SUPPLEMENTARY MATERIAL

### Appendix S1

The following nomenclatural acts have been registered in Zoobank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) under:

urn:lsid:zoobank.org:pub:6630A1EA-3197-4780-9DE9-8560C44F6F4A

### **Integrated data analysis allows the establishment of a new, cosmopolitan genus of marine Macrodasyida (Gastrotricha)**

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## **Taxonomic account**

Phylum Gastrotricha Metschnikoff, 1865

Order Macrodasyida Remane, 1925 [Rao & Clausen, 1970]

Family Macrodasyidae Remane, 1924

Genus *Kryptodasys* gen. nov.

urn:lsid:zoobank.org:act:24D72D24-185C-4D2A-8BCF-853C0E5023E

*Kryptodasys carlosrochai* sp. nov.

urn:lsid:zoobank.org:act:B5F35A4D-1743-435B-B22E-27893FBBD8D7

(Figs S1–S4)

(= nov. gen. nov. spec. Todaro & Rocha, 2004)

**Diagnosis.** Body elongate, 485–515  $\mu\text{m}$  in total length (TL), and up to 72  $\mu\text{m}$  in width; flattened ventrally and vaulted dorsally, with vacuolated cells along the body margins; epidermal glands generally few, small, scattered along the body. Cuticular covering smooth, devoid of scales and/or spines. Head slightly trapezoidal, bearing noticeable rounded pestle organs in a constriction. Trunk broadest in the mid-gut region, narrowing gently to the anus, then more quickly to the caudum; caudum in the form of a short tail. Sensory hairs arranged singly in lateral and dorsolateral columns along the body, sparsely on the lateral sides of the head. Ventral locomotor ciliature in the form of two bands of sparse and short cilia extending separately from under the head to the posterior trunk region but converging behind the anus into a single band. Anterior adhesive tubes (TbA), three per side, forming diagonal columns inserting directly on the body surface and projecting forward; ventral adhesive tubes (TbV), absent; ventrolateral adhesive tubes (TbVL), up to 10, of which one is in the anterior pharyngeal region (U18) and eight along the intestinal region; third and fourth tubes of the intestinal region separated by a space almost twice as much as the distance between the others. Lateral-, dorsal- and dorsolateral adhesive tubes absent; posterior adhesive tubes (TbP), up to four surrounding the caudum. Paired accessory adhesive tubes, of three tubes per side, arising ventrolaterally from a common base anterior to the pharyngo-intestinal junction at U33. Mouth terminal, rather small (up to 13  $\mu\text{m}$  in diameter), leading to a short buccal cavity (6  $\mu\text{m}$  in length); pharynx up to 175  $\mu\text{m}$  in length and up to 36  $\mu\text{m}$  in width; pharyngeal pores distant from the base with dorsolateral openings at U28. Pharyngo-intestinal junction (PhIJ) at U36. Intestine straight, widest at mid-body (U53); anus ventral at U89. Hermaphroditic; testicles paired, elongate, beginning just anterior to the PhIJ; sperm ducts short, presumably open separately on the ventral

surface; Spermatozoa, squat (4  $\mu\text{m}$  long and 1.5  $\mu\text{m}$  wide), in the form of little nails, apparently lacking a flagellum. Caudal organ, posterior to the ovary, centered at U77 (Figs S1B, S2D); glando-muscular in nature and approximately bullet-shaped, 54  $\mu\text{m}$  long and 14  $\mu\text{m}$  wide; it bears a canal with a single opening at the posterior end. Frontal organ, sac-like, anterior to the largest oocyte, centered at U51; non muscular in nature and roughly round in shape (20  $\mu\text{m}$  long and 20  $\mu\text{m}$  wide); it shows some internal compartmentalization, with the posterior portion containing a number of spermatozoa while the anterior portion contains secretory material.

**Etymology.** The species is named after Carlos Rocha, colleague and friend, who organized a series of investigations on marine meiofauna of the State of São Paulo (Brazil), during one of which the species was originally found.

**Type specimen.** Holotype: the 509  $\mu\text{m}$  long adult specimen shown in Figures S2, S3 no longer extant (International Code of Zoological Nomenclature, Articles 73.1.1 and 73.1.4; see also recommendation 73G–J of Declaration 45 - Addition of Recommendations to Article 73, ICZN 2017), collected on 28/04/2002. *Additional examined material.* Two adult specimens from the same sandy sample; all specimens were observed alive and are no longer extant.

**Distribution and ecology.** Type locality - Brazil, São Paulo, Praia de Castelhanos, on the east coast of the island of Ilhabela (Lat. 23°51'27.8" S; Long. 45°17'20.5" W); occasional in frequency of occurrence, and scarce in abundance at a depth of 2.0–4.0 m, in fine, poorly sorted sand. values of salinity and temperature of the interstitial water at the time of sampling were 34.8 ‰ and 23° C, respectively. Values of the granulometric parameters are reported in Table S1.

**Description.** Based mostly on the adult specimen with a total body length of 509  $\mu\text{m}$  (Figs S2, S3). Body elongate and of medium width; flattened ventrally and vaulted dorsally, with vacuolated cells along the lateral and dorsolateral body margins (Figs S1, S2A, S4A); epidermal glands few, small, scattered along the body. Cuticular covering smooth, devoid of scales and/or spines. Head slightly bulbous, bearing rounded pestle organs in a constriction (36  $\mu\text{m}$ ) at U04 (Figs S1, S2A, C, D). Body showing two additional constrictions, in the pharyngeal region at U14 (37  $\mu\text{m}$ , and just anterior to PhIJ at U35 (40  $\mu\text{m}$ ), then increasing slightly in breadth to mid trunk and there after narrowing gently to the anus, and subsequently more quickly to the caudum; caudum in the form of a short tail (Figs S1, S2A, S4A). Widths of head/mid pharynx/PhIJ/trunk/anus/base of tail, and locations along the length of the body are as follows: 40/43/49/52/25/13  $\mu\text{m}$  at U04/U20/U35/U60/U89/U92, respectively.

**Ciliation.** Sparse sensorial cilia (8–15  $\mu\text{m}$  in length) insert on the dorsal and ventrolateral margin of the head, in addition about 20–25 sensory hairs (12–20  $\mu\text{m}$  in length) arranged singly in lateral and



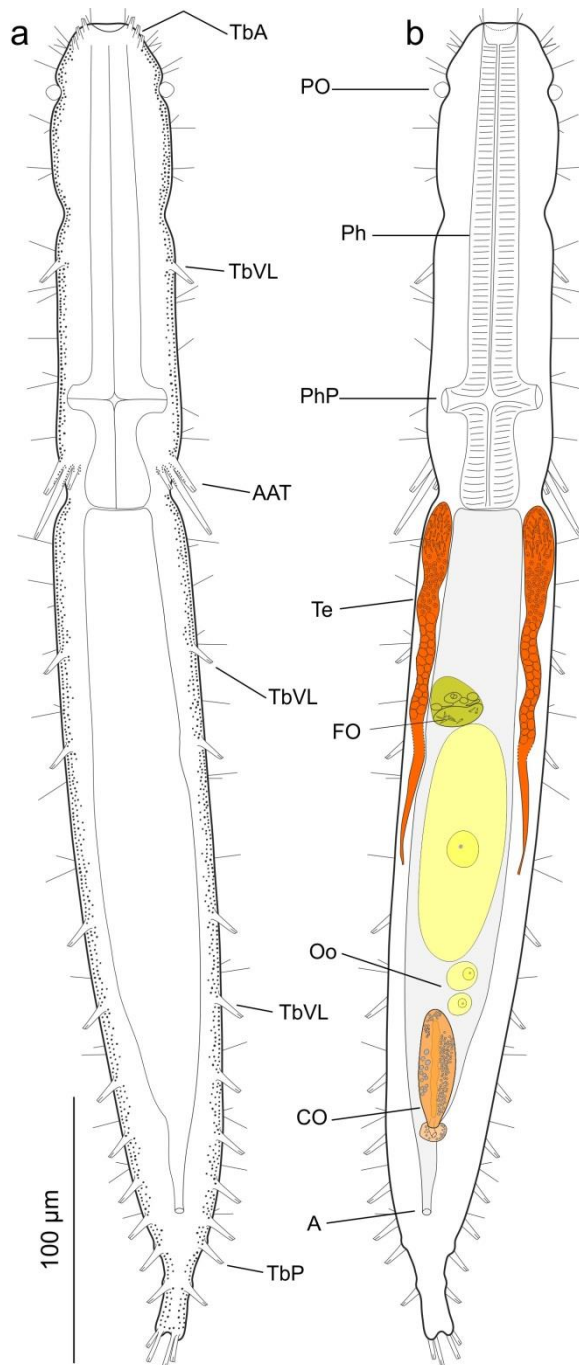
dorsolateral columns along the body. Ventral locomotor ciliature forms two longitudinal bands extending separately from under the head to the posterior trunk region but converging behind the anus into a single band; cilia in the bands are rather sparse and short (8–10  $\mu\text{m}$  in length; Fig. 6D). *Adhesive tubes.* TbA, three per side (4–6  $\mu\text{m}$  in length), forming diagonal columns, inserting directly on the body surface and projecting forward (Figs S1A, S3D); TbV, absent; TbVL, nine per side (8–13  $\mu\text{m}$  in length), one of which in the anterior pharyngeal region at U18, and eight along the intestinal region from U47 to U89. The third and the fourth tube of the intestinal region are separated by a distance that is almost the double the distance between the others (Fig. S1). TbL, TbDL and TbD absent; TbP, four per side (6–12  $\mu\text{m}$  in length), two of which at the distal end of short tail (Figs S1, S2A). In addition, there are paired accessory adhesive tubes, of three tubes per side, arising, in a row, ventrolaterally from a common base, anterior to the pharyngo-intestinal junction at U33 (Figs S1, S2A, S4). Tubes are of different size, and posterolaterally directed. The longest tube, up to 32  $\mu\text{m}$  in length, emerges in between the other two; the shortest tube, up to 9  $\mu\text{m}$  in length, is the most medial (i.e., closest to the body midline) while the other, up to 14  $\mu\text{m}$  in length, is the most lateral (Figs S1A, S2A, B).

*Digestive tract.* Mouth is terminal, 13  $\mu\text{m}$  in diameter; buccal cavity rather shallow (6  $\mu\text{m}$  in length) and lined with a thin cuticle (Figs S1B, S2A); pharynx, 173  $\mu\text{m}$  in length, widens toward the rear up to 21  $\mu\text{m}$ ; pharyngeal pores open dorsolaterally far off from the base, at U28. Pharyngo-intestinal junction (PhIJ) at U36. Intestine increases slightly in breadth from the PhIJ to mid-body where it reaches 32  $\mu\text{m}$  in width and then gradually narrows toward the posterior body end; anus ventral at U89. In all of the examined specimens, the intestine contained yellowish/orange coloured material, probably biodetritus, but not diatoms frustules (Figs S2A, S3).

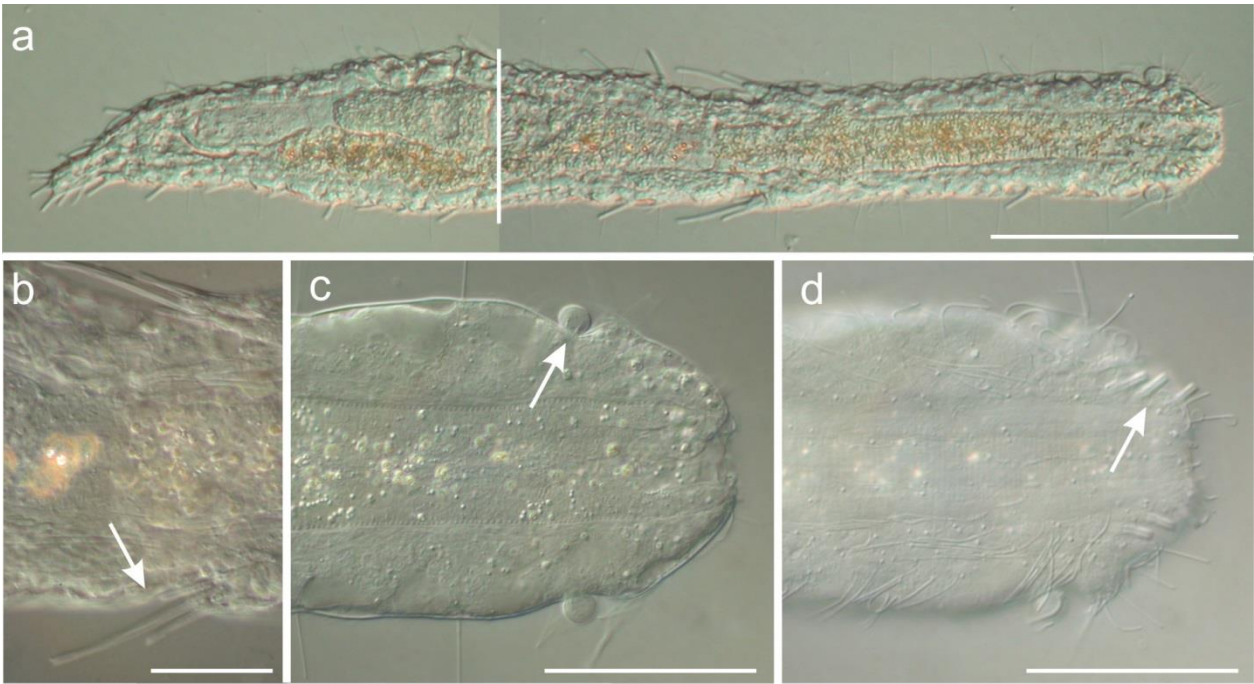
*Reproductive tract.* Hermaphroditic; testicles paired and elongate; they begin just anterior to the PhJI and span posteriorly for about 97  $\mu\text{m}$ , from U35 to U50 (Figs S1B, S2C). Testicles appear to be anatomically and functionally compartmentalized as the anterior portion contains larger and better structured cellular elements (likely spermatozoa), the mid portion shows smaller roundish bodies (likely maturing sperm) while the posterior longer portion holds large cells (primary germinal cells, Figs S1B, S3A). Each testicle apparently extends posteriorly with a short sperm duct, which presumably opens on the ventral surface. Ultrastructural studies are needed to ascertain the exact organization and function of testicles and ducts. Spermatozoa appear stubby (4  $\mu\text{m}$  long and 1.5  $\mu\text{m}$  wide), in the form of little nails, apparently lacking a flagellum (Figs S1B, S3A, C, D). Ovary single, in the second third of the trunk; oocytes maturing in a caudo-cephalic direction with largest oocyte dorsal to the mid intestine centered at U61 (Figs S1B, S3B, C). Caudal organ noticeable, posterior to the ovary, centered at U77 (Figs S1B, S2A, S3B); glando-muscular in nature

and approximately bullet-shaped, 54  $\mu\text{m}$  long and 14  $\mu\text{m}$  wide; it bears a canal with a single opening at its posterior end; the entire organ opens on the ventral surface, anterior to the anus at U82. Frontal organ, sac-like, anterior to the largest oocyte, centered at U51; non muscular in nature and roughly round in shape (20  $\mu\text{m}$  long and 20  $\mu\text{m}$  wide); it shows some internal compartmentalization, with the posterior portion containing a number of immotile spermatozoa while the anterior portion contains secretory material and vacuolated elements (Figs S1B, S3C, D). Neither internal nor external opening were observed.

**Variability and remarks.** The two additional measured adult specimens ranged from 485 to 515  $\mu\text{m}$  in total length, with a pharynx up to 175  $\mu\text{m}$  in length. Both of them had fully developed male and female gonads, and accessory reproductive organs, similar to the holotype. The adhesive apparatus in general was also similar to that of the holotype, but the 515  $\mu\text{m}$  long adult had an additional TbVL per side in the intestinal region (10 TbVL in total). One of the specimens had the accessory adhesive tubes made up of three tubes each, like the holotype (Fig. S4), the other possessed paired accessory adhesive tubes made up of two tubes instead of three tubes. We think that the normal condition for the species is to have the accessory tubes composed of three tubes and the state exhibited by one of the adults to be an aberration. Factors causing the aberration are unknown. This new species will be differentiated below after the description of the third new species.

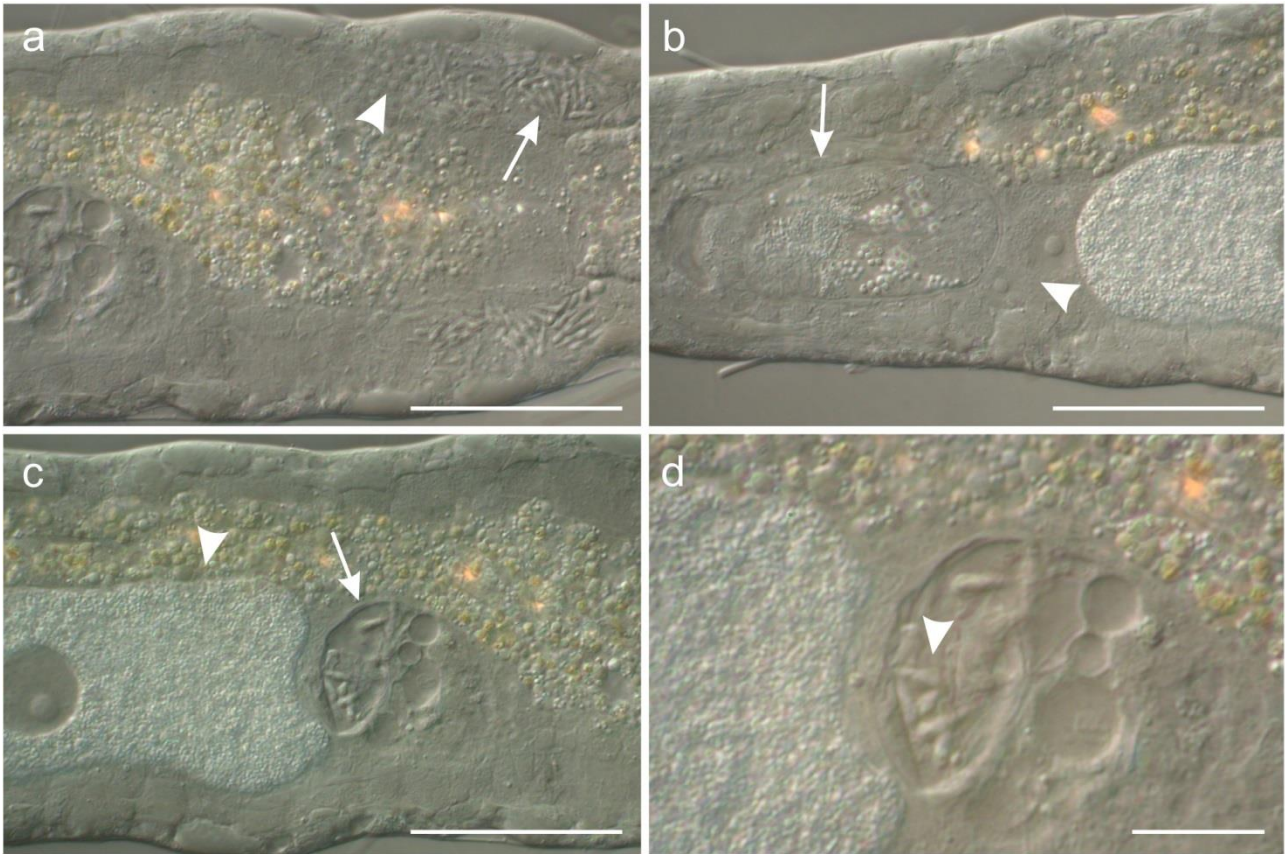


**Figure S1.** Line art illustrations of *Kryptodasys carlosrochai* sp. nov. **(a)**, habitus as seen from the ventral side. **(b)** habitus as seen from the dorsal side, showing the internal anatomy with the male and female reproductive structures. Drawings are made mostly from the holotypic specimen. Abbreviations: A = anus, AAT = accessory adhesive organ, CO = caudal organ, FO = frontal organ, Oo = oocyte, Ph = pharynx, PhIJ = pharyngo-intestinal junction, PhP = pharyngeal pore, PO = pestle organ, TbA = anterior adhesive tube, TbP = posterior adhesive tube, TbVL = ventrolateral adhesive tube, Te = testicle.

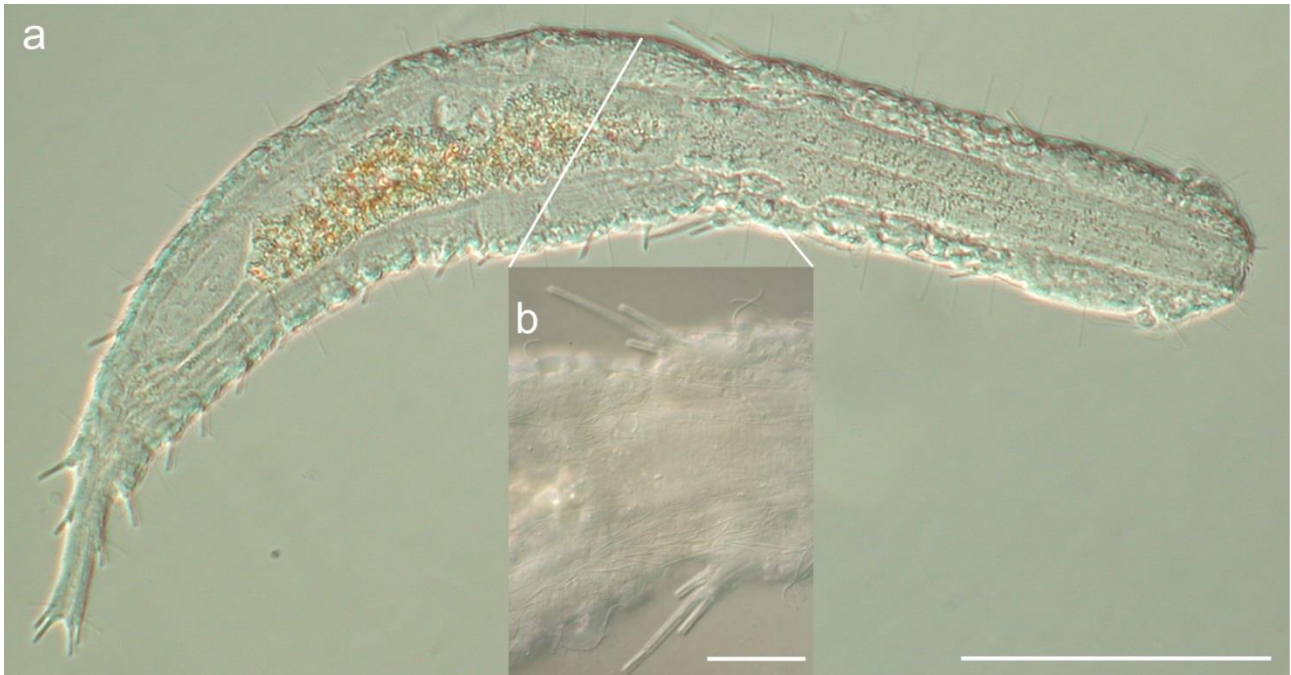


**Figure S2.** Differential interference contrast photomicrographs, showing the morphology of *Kryptodasys carlosrochai* sp. nov. **(a)** habitus as seen from the ventral side. **(b)** close-up of the posterior pharyngeal region, showing the accessory adhesive organs (arrow). **(c)** close-up of the anterior region, showing the pestle organs (arrow). **(d)** close-up of the anterior region, ventral view, showing the anterior adhesive tubes (arrows). Scale bars **(a)** = 100  $\mu\text{m}$ , **(b)** = 20  $\mu\text{m}$ , **(c, d)** = 30  $\mu\text{m}$ .





**Figure S3.** Differential interference contrast photomicrographs, showing gonads and accessory reproductive organs of *Kryptodasys carlosrochai* sp. nov. **(a)**, testicles apparently compartmentalized, showing mature spermatozoa (arrow) in the anterior portion followed by small cellular elements, possibly maturing sperm, mixed with granular material (arrowhead). **(b)** posterior trunk region, showing the ovary (arrowhead) followed by the caudal organ (arrow). **(c)** mid trunk region, showing the frontal organ (arrow), anterior to the largest egg (arrowhead). **(d)** close-up of the frontal organ showing some sperm inside. Scale bars **(a–c)** = 30  $\mu\text{m}$ , **(d)** = 10  $\mu\text{m}$ .



**Figure S4.** Differential interference contrast photomicrographs of an additional studied adult specimen of *Kryptodasys carlosrochai* sp. nov. (a) habitus as seen from the ventral side. (b) close-up of the posterior pharyngeal region, showing the accessory adhesive organs. Scale bars (a) = 100  $\mu\text{m}$ , (b) = 20  $\mu\text{m}$ .

*Kryptodasys ulfjondeliusi* sp. nov.

urn:lsid:zoobank.org:act:BF9614B6-9D50-4292-B106-A0E1624008D8

(Figs S5–S7)

**Diagnosis.** Body elongate, 545–595  $\mu\text{m}$  in total length (TL), and up to 71  $\mu\text{m}$  in width; flattened ventrally and vaulted dorsally, with vacuolated cells along the body margins; epidermal glands generally few, small, scattered along the body. Cuticular covering smooth, devoid of scales and/or spines. Head ovoid, bearing rather small, rounded pestle organs in a constriction. Trunk broadest in the mid-gut region, narrowing gently to the anus, then more quickly to the unilobed caudum. Sensory hairs arranged singly in lateral and dorsolateral columns along the body, more densely on dorsal and lateral sides of the head. Ventral locomotor ciliature in the form of two bands of cilia extending separately from under the head to the posterior trunk region but converging behind the anus into a single band. Anterior adhesive tubes (TbA) up to four per side, forming diagonal columns inserting directly on the body surface and projecting forward; ventral adhesive tubes (TbV) absent; ventrolateral adhesive tubes (TbVL), up to 18, three of which in the pharyngeal region and

15 along the intestinal region; TbL, two per side in the pharyngeal region; TbDL absent; TbD, five per side, one in the pharyngeal region and four along the intestinal region. TbP, five per side, two of which at the distal end. In addition, there are paired accessory adhesive tubes, of two tubes per side, arising ventrolaterally from a common base, anterior to the pharyngo-intestinal junction; tubes are rather difficult to see and of different size. Mouth terminal, 19  $\mu\text{m}$  in diameter; buccal cavity mug shaped, 24  $\mu\text{m}$  in length; pharynx up to 233  $\mu\text{m}$  in length and 27  $\mu\text{m}$  in width; pharyngeal pores open dorsolaterally far-off from the base at U32. PhIJ at U43. Intestine straight, wider in the first half; anus ventral at U92. Hermaphroditic; testicles paired, elongate, beginning just anterior to the PhIJ; sperm ducts rather long, that presumably open separately at U67. Spermatozoa, small, apparently lacking a flagellum and shaped like stout spindles. Ovary single, in the posterior third of the body; oocytes maturing in a caudo-cephalic direction, with the largest oocyte centered at about U71. Caudal organ, posterior to the ovary; glando-muscular in nature and approximately bullet-shaped, its opening on the ventral surface, anterior to the anus at U86. Frontal organ, sac-like, anterior to the largest oocyte, centered at U51; non muscular in nature and roughly rounded or wider than longer in shape; usually it contains 1-2 immotile spermatozoa surrounded by secretory material.

**Etymology.** The species is named after Ulf Jondelius, colleague and friend, who organized a series of investigations on Gastrotricha of Sweden, during one of which the species was originally found.

**Type specimen.** Holotype: the 593  $\mu\text{m}$  long adult specimen shown in Figure S6 no longer extant (International Code of Zoological Nomenclature, Articles 73.1.1 and 73.1.4; see also recommendation 73G–J of Declaration 45 - Addition of Recommendations to Article 73, ICZN 2017), collected on 24/07/2009. *Additional examined material.* Two adults and one juvenile from the same sandy sample; all specimens were observed alive and are no longer extant. Three additional identified specimens were fixed in 95% ethanol solution, kept in the collection of the first author and later used for DNA analysis (see below).

**Distribution and ecology.** Type locality - Sweden, Gullmarsfjorden, Östersidan, Klubban beach; on the west coast just outside of the Klubban Biological Station (Uppsala University) (Lat. 58° 15'06'' N; Long. 11° 27'55'' E); occasional in frequency of occurrence, and scarce in abundance at a depth of 2.0–4.0 m, in medium, poorly sorted sand with some detritus. Values of salinity and temperature of the interstitial water at the time of sampling were about 26‰ and 20° C, respectively. Values of the granulometric parameters are reported in Table S1.

**Description.** Based mostly on the adult specimen with a total body length of 593  $\mu\text{m}$  shown in Figure 10. Body elongate and of medium width; flattened ventrally and vaulted dorsally, with

vacuolated cells along the lateral and dorsolateral body margins (Fig. S5, S6A); epidermal glands few, small, scattered along the body. Cuticular covering smooth, devoid of scales and/or spines. Head distinct, ovoid in shape, bearing rounded, rather small, pestle organs in a constriction at U04 (Figs S5, S6A–C, S7A). Body of similar width in the anterior portion, increasing slightly in breadth from just anterior of the PhIJ to mid trunk and then narrowing gently to the unilobed caudum (Figs S5, S6A, C). Widths of head/mid pharynx/PhIJ/trunk/anus, and locations along the length of the body are as follows: 63/59/63/71/40  $\mu\text{m}$  at U07/U25/U43/U57/U92, respectively.

The holotype shows an evident relief in the posterior trunk region at U74; the relief is on the left side when seen from the dorsal side and represent the residual scar of an oviposition, which in these animals occurs by rupture of the body wall.

*Ciliation.* Sparse sensorial cilia (10–16  $\mu\text{m}$  in length) insert on the dorsal and ventrolateral margin of the head, in addition about 35–50 sensory hairs (18–30  $\mu\text{m}$  in length) arranged singly in lateral and dorsolateral columns along the body. Ventral locomotor ciliature forms two longitudinal bands running separately from under the head to the posterior trunk region but converging behind the anus into a single band; bands are denser and poorly spaced under the head and anterior pharyngeal region (Fig. S6C).

*Adhesive tubes.* TbA, four per side (5–6  $\mu\text{m}$  in length), forming diagonal columns, inserting directly on the body surface and project forward (Figs S5A, S7C); TbV, absent; TbVL, 18 per side (9–13  $\mu\text{m}$  in length), three of which in the pharyngeal region (two anterior and one posterior to the pharyngeal pore), and 15 along the intestinal region, more or less evenly spaced from the pharyngo-intestinal junction to the anus. TbL, two per side (9  $\mu\text{m}$  in length), in the pharyngeal region anterior to the pharyngeal pores; TbDL absent; TbD, 5 per side (4–6  $\mu\text{m}$  in length), one in the pharyngeal region, anterior to the pharyngeal pores and four along the anterior  $\frac{3}{4}$  of the intestinal region. TbP, five per side (4–10  $\mu\text{m}$  in length), two of which at the distal end (Figs S5, S6A). In addition, there are paired accessory adhesive tubes, of two tubes per side, arising ventrolaterally from a common base, anterior to the pharyngo-intestinal junction at U34. The tubes, which are rather difficult to see, are of different size, and posterolaterally directed. The shortest tube, 10  $\mu\text{m}$  in length, arises anteriorly while the longest is posterior and double of that length, 26  $\mu\text{m}$  (Figs S5A, S6C).

*Digestive tract.* Mouth is terminal, 19  $\mu\text{m}$  in diameter; buccal cavity is mug shaped, 24  $\mu\text{m}$  in length and lined with a thin cuticle (Figs S5B, S6A, S7A); pharynx, 233  $\mu\text{m}$  in length, widens toward the rear up to 27  $\mu\text{m}$ ; pharyngeal pores open dorsolaterally far-off from the base, at U32. Pharyngo-intestinal junction (PhIJ) at U43. Intestine is wider in the first half (35  $\mu\text{m}$  in width) then gradually narrows toward the posterior body end; anus ventral at U92. In all of the examined adult specimens

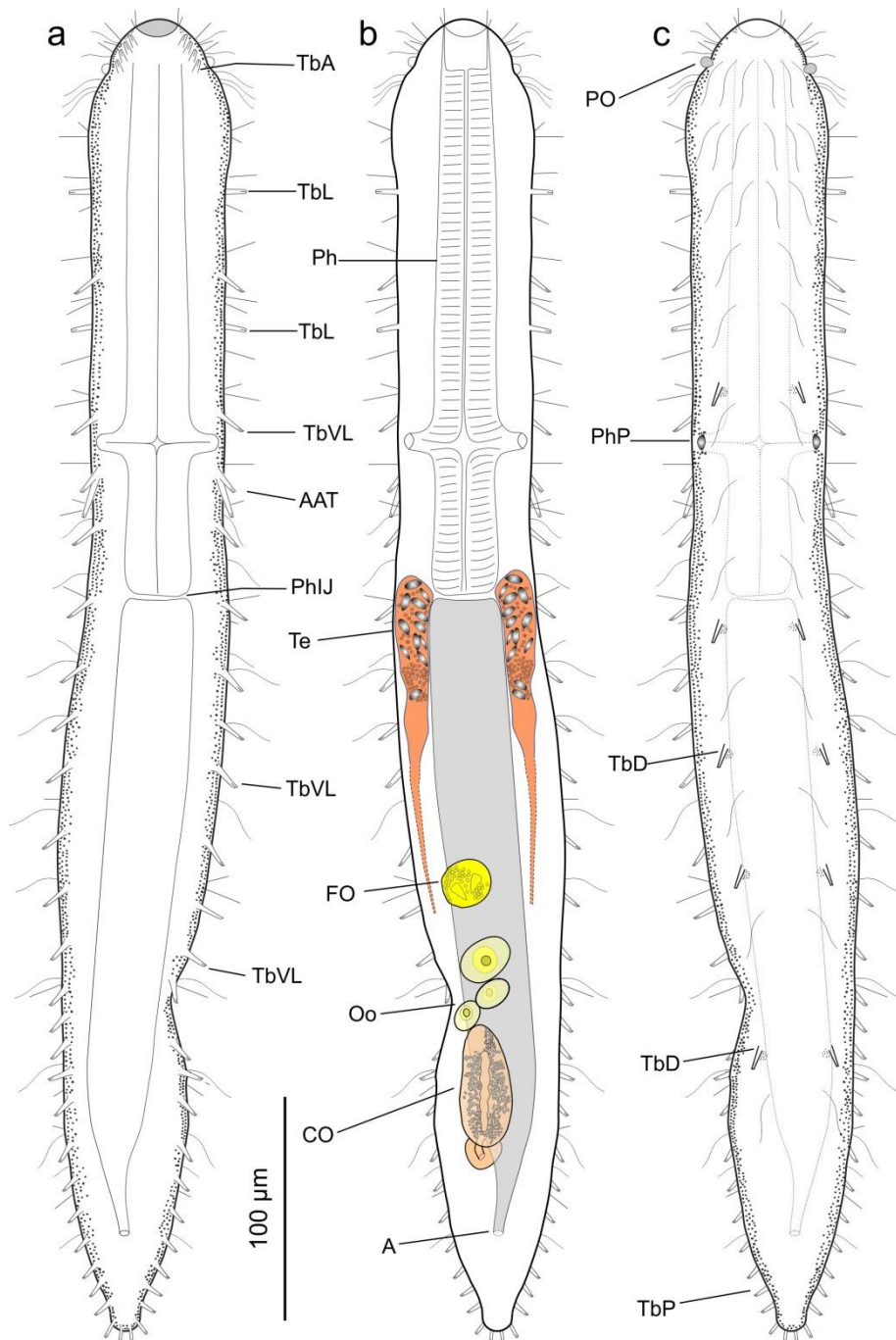


the intestine contained granular, refringent material, but not diatom frustules (Fig. S6B); in the juvenile specimens, the gut contained a single round microalgal cell (Fig. S7F).

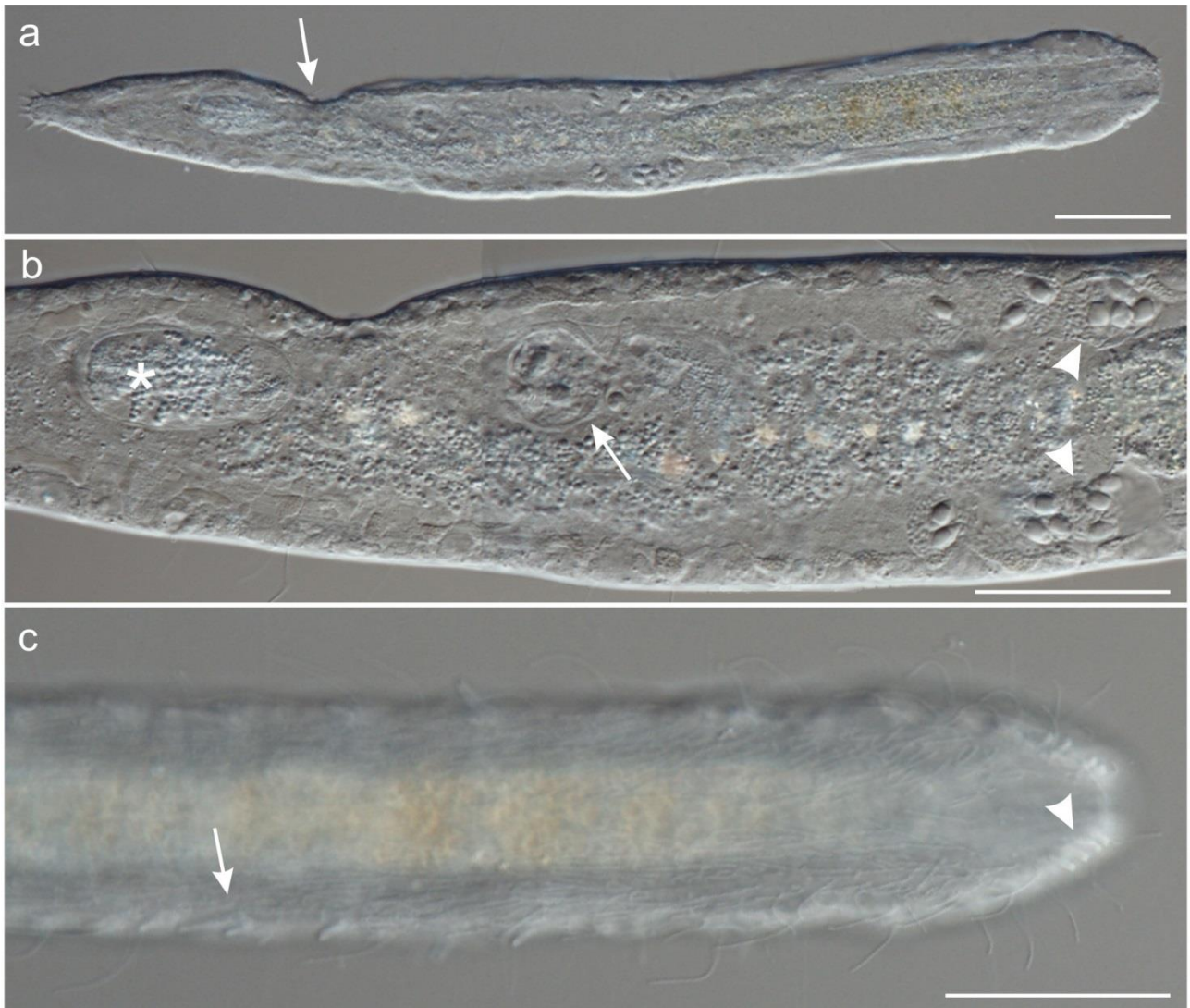
*Reproductive tract.* Hermaphroditic; testicles paired and elongate; they begin just anterior to the PhIJ and span posteriorly for about 84  $\mu\text{m}$ , from U42 to U56 (Figs S5B, S6A). Each gonad contains mainly larger and better structured cellular elements (likely spermatozoa) in its anterior third; in the following third it shows some putative spermatozoa mixed with granular material, while in the posterior third it appears to be rather empty; it continues in an elongate sperm duct that seems to open on the ventral surface at about U67. Spermatozoa appear stubby (10–11  $\mu\text{m}$  long and 4–6  $\mu\text{m}$  wide), in the form of stout spindles, apparently lacking a flagellum (Figs S5B, S6B, S7B). Ovary single, in the second third of the trunk; oocytes maturing in a caudo-cephalic direction; in the two additional studied adults the largest oocyte was located dorsal to the second half of the intestine, centered at about U71 (Fig. S7A). Caudal organ, posterior to the ovary, centered at U80 (Figs S5B, S6B); glando-muscular in nature and approximately bullet-shaped, 53  $\mu\text{m}$  long and 22  $\mu\text{m}$  wide; it bears a canal with a single opening at its posterior end; the entire organ opens on the ventral surface, anterior to the anus at U86. Frontal organ, sac-like, anterior to the largest oocyte, centered at U51; non muscular in nature and roughly round in shape (20  $\mu\text{m}$  in diameter); it contains 1-2 immotile spermatozoa surrounded by secretory material (Figs S5B, S6B, S7B, C). Neither internal nor external openings were observed.

**Variability and remarks.** The three additional measured adult specimens ranged from 545 to 598  $\mu\text{m}$  in total length, with the pharynx up to 233  $\mu\text{m}$  in length. All of them had fully developed male and female gonads, and the accessory reproductive organs were, in general, similar to those showed by the holotype (Fig. S7A–D). However, in these animals, the frontal organ was not rounded, like the one shown by the holotype, but appeared to be compressed to some extent along its length (i.e., caudo-cephalic axis), probably due to the pressure exerted on it by the largest, growing oocytes (Fig. S7A, C). The adhesive apparatus in general was also similar to that of the holotype, but the 545  $\mu\text{m}$  long specimen had only three TbA per side and 16 TbVL. Curiously, the same animal showed some asymmetries concerning the adhesive apparatus: the TbP numbered regularly five on one side but four on the other, while the accessory adhesive tubes on the left side included a single tube only instead of two tubes as it is usual for the species.

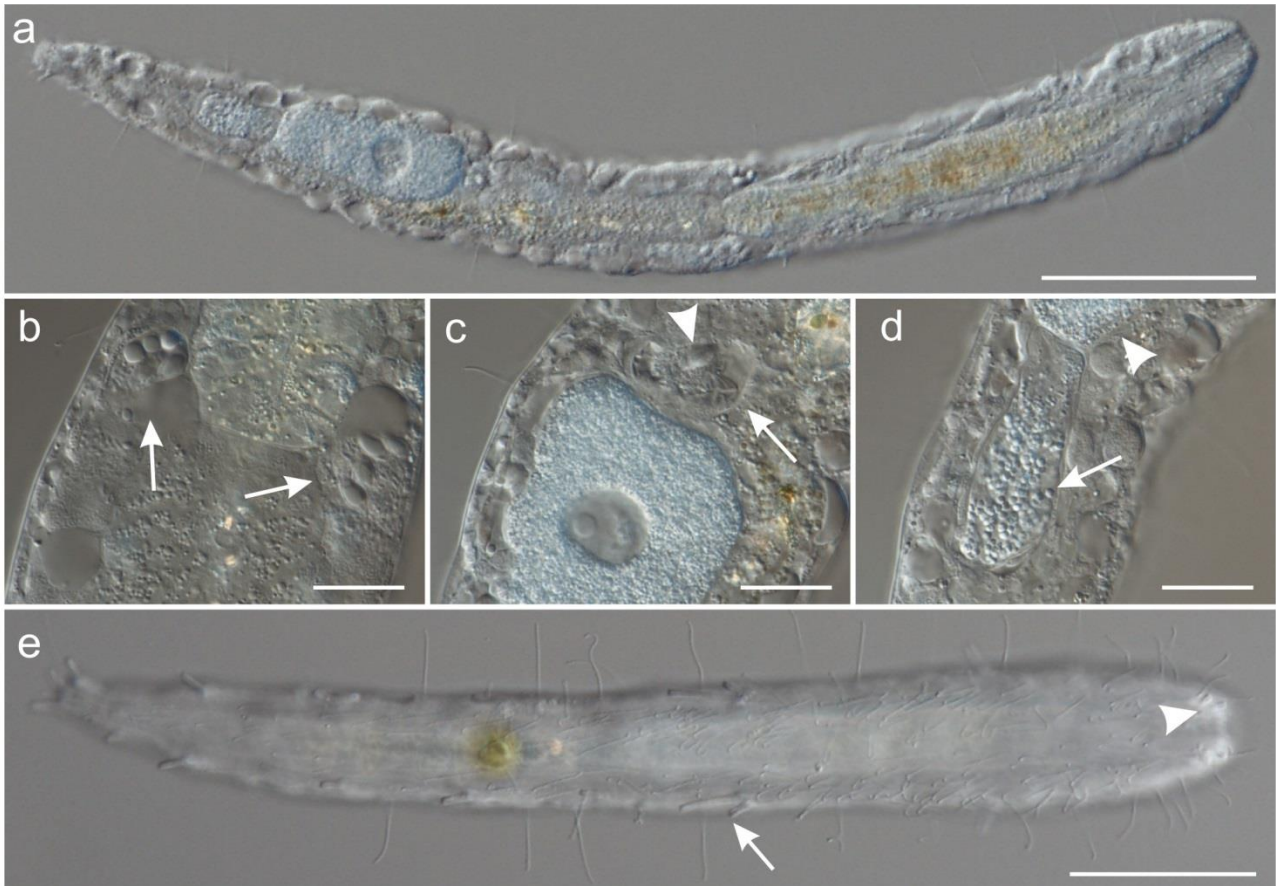
The measured juvenile specimen was 328  $\mu\text{m}$  in total length (Fig. S7F) with a 143  $\mu\text{m}$  long pharynx (PhIJ at U38). It showed, on each side, two TbA, three TbP and four TbVL, all of which along the intestinal region, starting from the PhIJ. The specimen in addition showed a single TbL at about U21 and the accessory adhesive tubes, of two tubes each, at U42 (Fig. S7F). Unfortunately, no information was acquired with regard to the presence and/or number of dorsal tubes (TbD).



**Figure S5.** Line art illustrations of *Kryptodasys ulfjondeliusi* sp. nov. **(a)** habitus as seen from the ventral side. **(b)** habitus, showing the internal anatomy with the male and female reproductive structures. **(c)** habitus as seen from the dorsal side. Drawings are made mostly from the holotypic specimen. A = anus, AAT = accessory adhesive tubes, CO = caudal organ, FO = frontal organ, Oo = oocyte, Ph = pharynx, PhIJ = pharyngo-intestinal junction, PhP = pharyngeal pore, PO = pestle organ, TbA = anterior adhesive tube, TbD = dorsal adhesive tube, TbL = lateral adhesive tube, TbP = posterior adhesive tube, TbVL = ventrolateral adhesive tube, Te = testicle.



**Figure S6.** Differential interference contrast photomicrographs, showing the morphology of *Kryptodasys ulfjondeliusi* sp. nov. **(a)** habitus as seen from the dorsal side; the scar seen on the left side (arrow) testifies a recent oviposition which has happened by rupture of the body wall. **(b)** internal anatomy of the trunk region, showing the testicles with stubby spermatozoa (arrowheads), the frontal organ with some sperm inside (arrow) and the caudal organ (asterisk). **(c)** anterior region, ventral view, showing the accessory adhesive tubes (arrow) and the anterior adhesive tubes (arrowhead); Scale bars **(a)** = 100  $\mu\text{m}$ , **(b, c)** = 50  $\mu\text{m}$ .



**Figure S7.** Differential interference contrast photomicrographs of additional studied specimens of *Kryptodasys ulfjondeliusi* sp. nov. (**a–d**) adult specimen. (**a**) habitus as seen from the dorsal side. (**b**) testicles with stubby spermatozoa (arrows). (**c**) frontal organ (arrow) anterior the largest egg; some sperm are visible inside the frontal organ (arrowhead). (**d**) caudal organ (arrow) posterior to the ovary (arrowhead). (**e**) subadult specimen, showing the accessory adhesive tubes (arrow) and the anterior adhesive tubes (arrowhead). Scale bars (**a**) = 100  $\mu\text{m}$ , (**b–d**) = 20  $\mu\text{m}$ , (**e**) = 50  $\mu\text{m}$ .



## Taxonomic key to species of the genus *Kryptodasys*

- 1 TbDL present ..... 2  
– TbDL absent ..... 4
- 2 A single pair of TbD in the pharyngeal region ..... *K. ulffondeliusi* sp. nov.  
– Three pairs of TbD in the pharyngeal region ..... 3
- 3 TbD equally spaced along the pharyngeal region ..... *K. celticus* (Hummon, 2008)  
– Two pairs of TbD clustering anterior to the pharyngeal pores and a single pair near the PhIJ .....  
..... *K. nobskaensis* (Hummon, 2008)
- 4 Accessory adhesive tubes consist of 3 tubes ..... 5  
– Accessory adhesive tubes consist of 2 tubes ..... 6
- 5 LT up to 515  $\mu\text{m}$ ; TbVL up to 10 per side ..... *K. carlosrochai* sp. nov.  
– LT up to 1000  $\mu\text{m}$ , TbVL up to 30 per side ..... *K. remanei* (Boaden, 1963)
- 6 Five to six pairs of smaller TbVL in the anterior pharyngeal region ..... *K. marcocurinii* sp. nov.  
– TbVL all of similar size ..... *K. hexadactylis* (Rao, 1970)

## *Macrodasys* diagnosis (emended)

Body elongate, up to 1033  $\mu\text{m}$  in total length (LT), and up to 97  $\mu\text{m}$  in width, flattened ventrally and vaulted dorsally, with vacuolated cells along the body margins; epidermal glands generally few, small, scattered along the body. Cuticular covering smooth, devoid of scales and/or spines. Head slightly ovoid bearing pestle organs, and occasionally ocellar granules. Trunk broadest in the mid-gut region, narrowing gently to the anus, then more quickly to the caudum; caudum unilobed, more often in the form of a short tail. Sensory hairs arranged singly in lateral and dorsolateral columns along the body, sparsely on the lateral sides of the head. Ventral locomotor ciliature covering the entire field or in the form of two bands extending separately from under the head to the posterior trunk region; cilia appear denser in the anterior region. TbA, up to 13 per side, forming an arc or occasionally in two rows, which insert directly on the body surface; TbV, absent; TbL, occasionally present; TbVL, up to 40 per side, some in the pharyngeal region but most along the intestinal region; TbD, rarely present; TbDL, occasionally present in the posterior trunk region; TbP, up to

20, surrounding the caudum. Accessory adhesive tubes, absent. Mouth terminal, of mid-size (up to 27  $\mu\text{m}$  in diameter), leading to a short buccal cavity (8–15  $\mu\text{m}$  in length); pharynx up to 257  $\mu\text{m}$  long and up to 40  $\mu\text{m}$  wide; pharyngeal pores far off from the pharyngeal base, with dorsolateral openings. PhIJ at U30–U45. Intestine increases in width from the PhIJ to mid-body and gradually narrows toward the posterior body end; anus ventral at U85–U94. Hermaphroditic; testicles paired, elongate, usually beginning at the PhIJ; sperm ducts open separately on the ventral surface; spermatozoa, filiform with a cork-screw shaped anterior portion and a long, smooth flagellum. Ovary single, in the second third of the trunk; oocytes maturing in a caudo-cephalic direction with largest oocyte dorsal to the mid intestine. Caudal organ, noticeable, posterior to the ovary; glando-muscular in nature; it bears a canal with a single opening at the posterior end; usually the caudal organ is clearly subdivided in a narrow anterior portion and a wider posterior portion. Frontal organ, just posterior to the largest oocyte; in general compact, occasionally elongate; divided in anterior seminal receptacle and posterior spermatheca. Seminal receptacle with weakly muscolarised wall, showing a small, anterior pore (internal pore), occasionally slightly circularised (nozzle) and a second pore (external pore) that opens on the ventral surface. Type-species *Macrodasys buddenbrocki* Remane, 1924; other species: *M. achradoctalis* Evans, 1994; *M. acrosorus* Hummon & Todaro, 2009; *M. affinis* Remane, 1936; *M. africanus* Remane, 1950; *M. africanus ponticus* Valkanov, 1957; *M. ancocytalis* Evans, 1994; *M. andamanensis* Rao, 1993; *M. balticus* Roszczak, 1939; *M. blysoctalis* Evans, 1994; *M. caudatus* Remane, 1927; *M. cephalatus* Remane, 1927; *M. cunctatus* Wieser, 1957; *M. deltoctalis* Evans, 1994; *M. digronus* Hummon & Todaro, 2009; *M. dolichocytalis* Evans, 1994; *M. fornerise* Todaro & Rocha, 2004; *M. gerlachi* Papi, 1957; *M. glylius* Hummon, 2010; *M. imbricatus* Hummon, 2011; *M. indicus* Kutty & Nair, 1969; *M. lakshadweepense* Hummon, 2008; *M. macrurus* Hummon, 2011; *M. meristocytalis* Evans, 1994; *M. neapolitanus* Papi, 1957; *M. nigrocellus* Hummon, 2011; *M. ommatus* Todaro & Leasi, 2013; *M. pacificus* Schmidt, 1974; *M. plurosorus* Hummon, 2008; *M. scleracrus* Hummon, 2011; *M.*

*stenocytalis* Evans, 1994; *M. syringodes* Hummon, 2010; *M. thuscus* Luporini, Magagnini & Tongiorgi, 1973; *M. waltirensis* Rao & Ganapati, 1968.

## SUPPLEMENTARY MATERIAL

### Tables

**Table S1.** Gastrotrich taxa involved in the molecular analyses. GenBank accession number, geographic origin and reference.

Taxon	Accession	Origin	Reference
<b>Cephalodasyidae</b>			
<i>Cephalodasys</i> sp.1	AY963691	White Sea, Russia	Petrov et al., 2007
<i>Dolichodasys</i> sp. 1	AM231778	San Isidoro, Italy	Todaro et al., 2006
<i>Mesodasys laticaudatus</i>	JF357657	Albinia, Italy	Todaro et al., 2011
<i>Mesodasys littoralis</i>	JF357658	Bou Ficha, Tunisia	Todaro et al., 2011
<i>Paradasys</i> sp. 1	AM231781	Ionian sea, Italy	Todaro et al., 2006
<i>Pleurodasys helgolandicus</i>	JN203486	Ibiza, Spain	Todaro et al., 2012
<b>Dactylopodolidae</b>			
<i>Dactylopodola</i> cf. <i>baltica</i>	JF357650	Ras Alard, Kuwait	Todaro et al., 2011
<i>Dactylopodola mesotyphle</i>	JF357651	Punta Ala, Italy	Todaro et al., 2011
<i>Dactylopodola typhle</i>	JF357652	Bou Ficha, Tunisia	Todaro et al., 2011
<i>Dactylopodola typhle</i>	JF357653	Torre Civette, Italy	Todaro et al., 2011
<b>Hummondasyidae</b>			
<i>Hummondasys jamaicensis</i>	KM083602	Negril, Jamaica	Todaro et al., 2014
<b>Lepidodasyidae</b>			
<i>Lepidodasys unicarenatus</i>	JF357665	Pianosa, Italy	Todaro et al., 2011
<b>Macrodasysidae</b>			
<i>Kryptodasys macrocurinii</i>	MK880150	Sardinia, Italy	Present study
<i>Kryptodasys ulfjondeliusi</i>	MK880151	Gullmarsfiord, Sweden	Present study
<i>Macrodasys</i> sp. 1	JF357654	Torre Civette, Italy	Todaro et al., 2011
<i>Macrodasys</i> sp. 2	JF357670	Bohuslän, Sweden	Todaro et al., 2011
<i>Thaidasys tongiorgii</i>	KR072683	Phuket Island, Thailand	Todaro et al., 2015
<i>Urodasys</i> sp. 1	DQ079912	Florida, USA	Sørensen et al., 2006
<i>Urodasys</i> sp. 2	AY218102	NA	Giribet et al., 2004
<b>Planodasyidae</b>			
<i>Crasiella</i> sp.1	JN203488	Ilhabela, Brazil	Todaro et al., 2012
<i>Megadasys</i> sp. 1	JF357656	Porto Cesareo, Italy	Todaro et al., 2011
<i>Megadasys</i> sp. 2	JF357655	Grotta del Ciolo, Italy	Todaro et al., 2011
<b>Redudasyidae</b>			
<i>Anandrodasys agadasys</i>	JN203487	St. John Island, USA	Todaro et al., 2012
<i>Redudasys fornerise</i>	JN203489	Represa do Broa, Brazil	Todaro et al., 2012
<b>Thumastodermatidae</b>			
<i>Acanthodasys</i> sp. 1	JF357638	Capraia, Italy	Todaro et al., 2011
<i>Acanthodasys aculeatus</i>	JF357639	Capraia, Italy	Todaro et al., 2011
<i>Diplodasys ankei</i>	JF357624	Meloria, Italy	Todaro et al., 2011
<i>Diplodasys meloriae</i>	JF357640	Meloria, Italy	Todaro et al., 2011
<i>Oregodasys ocellatus</i>	JF357642	Meloria, Italy	Todaro et al., 2011



<i>Oregodasys ruber</i>	JF357625	Meloria, Italy	Todaro et al., 2011
<i>Oregodasys tentaculatus</i>	JF357626	Meloria, Italy	Todaro et al., 2011
<i>Pseudostomella etrusca</i>	JF357633	Albinia, Italy	Todaro et al., 2011
<i>Ptychostomella lamelliphora</i> (=sp1)	JF357643	Ilhabela, Brazil	Todaro et al., 2011
<i>Ptychostomella tyrrhenica</i>	JF357634	Albinia, Italy	Todaro et al., 2011
<i>Tetranchyroderma esarabdophorum</i>	JF357627	Mahdia, Tunisia	Todaro et al., 2011
<i>Tetranchyroderma hirtum</i>	JF357628	Capraia, Italy	Todaro et al., 2011
<i>Tetranchyroderma papii</i>	JF357637	Sardegna, Italy	Todaro et al., 2011
<i>Tetranchyroderma thysanophorum</i>	JF357630	Albinia, Italy	Todaro et al., 2011
<i>Thaumastoderma moebjergi</i>	JF357671	Bohuslän, Sweden	Todaro et al., 2011
<i>Thaumastoderma ramuliferum</i>	JF357631	Meloria, Italy	Todaro et al., 2011
<b>Turbanellidae</b>			
<i>Paraturbanella dohrni</i>	JF357659	Punta Ala, Italy	Todaro et al., 2011
<i>Paraturbanella pallida</i>	JF357660	Capraia, Italy	Todaro et al., 2011
<i>Paraturbanella teissieri</i>	JF357661	Punta Ala, Italy	Todaro et al., 2011
<i>Turbanella bocqueti</i>	JF357662	Tramore, Ireland	Todaro et al., 2011
<i>Turbanella cornuta</i>	JF357663	Chioggia, Italy	Todaro et al., 2011
<i>Turbanella lutheri</i>	JF357669	Torö, Sweden	Todaro et al., 2011
<b>Xenodasyidae</b>			
<i>Xenodasys riedli</i>	JN203490	St. John Island, USA	Todaro et al., 2012
<b>Xenotrichulidae*</b>			
<i>Xenotrichula intermedia</i>	JF357664	Mahdia, Tunisia	Todaro et al., 2011

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\* Order Chaetonotida; NA, Data not available.

**Table S2.** Granulometric parameters of the sediment at the investigated locations where the three new *Kryptodasys* species were found.

Location	Mean grain size (phi)	Sorting	Skewness	Kurtosis
<b>Sardinia, Italy</b>				
Grotta di Nereo	1.28	0.88	1.25	2.76
Grotta il Porticato	0.51	0.84	0.80	2.93
Costa Paradiso	-0.02	0.48	1.22	7.19
<b>Ilhabela, Brazil</b>				
Praia de Castelhanos	2.02	1.10	-1.04	2.70
<b>Gullmarsfjorden, Sweden</b>				
Klubban beach	1.84	1.22	-0.52	3.52

**Table S3.** Number of specimens found and studied/measured, along with the main traits of the adults of the three new *Kryptodasys* species described in the present article; measurements in  $\mu\text{m}$ .

	<i>K. marcocurinii</i>	<i>K. carlosrochai</i>	<i>K. ulfjondeliusi</i>
Specimens found	8	3	7
Specimens studied/measured	4 adults, 1 subadult, 1 juvenile	3 adults	3 adults, 1 juvenile
<b>Traits of the adults</b>			
Total length	711-734	485-515	545-598
Pharynx length	227	173-175	230-233
PhIJ at U	33	36	43
TbA per side	5-6	3	3-4
TbVL per side	24-25	Up to 10	16
TbVL in the pharyngeal region	5-6	1	3
TbL per side	1	absent	2
TbD per side	absent	absent	5
TbP total	8-10	4	9-10
N. tubes in the accessory adhesive organs	2	3	2

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