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A new species of *Streblospio* (Polychaeta: Spionidae) from the northern Adriatic Sea (Mediterranean Sea)

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Abstract

The present study describes a new species of spionid polychaete, *Streblospio eridani* n. sp., from the Italian coasts of the Northern Adriatic Sea (Mediterranean Sea). This new spionid species was recorded from shallow lagoon and marine habitats: the soft bottoms of the Sacca di Goro (Po River Delta), on October 2017, and the Lido di Dante (Emilia Romagna), between 2016 and 2017. The re-assessment of spionid specimens from other lagoon systems (the Valli di Comacchio and the Valle di Gorino) led us to recognize the presence of this species in the northern Adriatic Sea since 2009. *Streblospio eridani* n. sp. had low occurrence and density at the marine site Lido di Dante and at the lagoon sites Valli di Comacchio and Valle di Gorino; whereas it reached the highest density of 21,213.3 ind. m^{-2} at the Sacca di Goro. Morphologically this new species is characterized by dorsal (occipital) papilla on the first chaetiger, sabre chaetae and hooded hooks first appearing on chaetigers 7, hooks with 4-5 pairs of small secondary teeth, pygidium with ventral lappets, and brooding branchiate structures between chaetigers 13 and 28. In the phylogenetic reconstruction, based on the mitochondrial cytochrome *c* oxidase subunit I (COI) gene, the Adriatic taxon results clearly indicate that this is distinct from the other species of the genus and formed a well-supported clade with other *Streblospio* sp. specimens from India. Nucleotide divergences calculated between the Adriatic specimens and the other *Streblospio* species are higher than the intraspecific range reported for the genus and support the description of a new species. Morphological characters important for differentiation of the new species herein described from congeneric species are discussed and an updated key for *Streblospio* species is provided.

Keywords: COI, Polychaete, Spionid, *Streblospio eridani* n. sp., northern Adriatic Sea

Introduction

Spionidae Grube, 1850 is among the most diverse of polychaete families: more than 500 species belonging to about 40 genera are known globally (Meiβner *et al.* 2014). Spionids are ubiquitous polychaetes, and commonly found, often in large numbers, in intertidal or subtidal soft sediments (Blake & Kudenov 1978; Meiβner & Götting 2015; Glasby *et al.* 2000; Rouse & Pleijel 2001) in most marine habitats. Among them, the genus *Streblospio* Webster, 1879 contains a low number of species, with few distinguishing morphological characters (Mahon *et al.* 2009).

To date, five species and one subspecies of the genus *Streblospio* are known: *Streblospio benedicti* Webster, 1879 originally described from northeast U.S.A. (northern Atlantic Ocean), *S. shrubsolii* (Buchanan, 1890) from southeast England (northern Atlantic Ocean), *S. benedicti japonica* Imajima, 1990 from Japan (northern Pacific Ocean), *S. gynobranchiata* Rice & Levin, 1998 from Florida and the Gulf of México (northern Atlantic Ocean), and the recently described *S. padventralis* Delgado-Blas, Díaz-Díaz & Viéitez, 2018 and *S. eunateae* Martínez & Adarraga, 2019, both from the coast of the Iberian Peninsula.

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Only two of the known species of the genus *Streblospio* are reported in the Mediterranean Sea: *Streblospio shrubsolii* (Lardicci *et al.* 1997; Munari 2013; Çinar *et al.* 2014; Mikac 2015; Bevilacqua *et al.* 2018; Mistri *et al.* 2018), and *S. gynobranchiata* (Çinar *et al.* 2005a, b, 2006, 2008, 2009, 2011a, 2012a, b, 2014; Çinar 2013; Çinar & Dagli 2013; Dagli & Çinar 2008; Dagli *et al.* 2011; Ergen *et al.* 2006; Kurt-Sahin & Çinar 2012; Zenetos *et al.* 2017), which is also recorded from the Black Sea (Radashevsky & Selifonova 2013, Boltachova *et al.* 2015; Selifonova & Bartsits 2018) and Caspian Sea (Taheri *et al.* 2009; Taheri & Foshtomi 2011; Ghasemi *et al.* 2014). In the present study we describe a new species of the genus *Streblospio*, *Streblospio eridani* n. sp. from coastal soft bottoms of the northern Adriatic Sea (a meeting point between Eastern and Western Mediterranean Sea), also giving ecological informations. An identification key to *Streblospio* species, which updates the one recently published by Delgado-Blas *et al.* (2018), is provided for the new species of *Streblospio* from the Adriatic Sea.

Description of sampling sites

The Sacca di Goro (Emilia Romagna, northern Adriatic Sea) is a wide (26 km^2) microtidal, and polyhaline, lagoon located in the southernmost Po Delta area (Fig. 1a, b). The lagoon has a maximum depth of 2.0 m. It receives nutrient-rich freshwater, primarily from the Po di Volano. The neighbouring Valle di Gorino (8 km^2) is a cul-de-sac of the Sacca di Goro (Fig. 1b), with a maximum depth of 1.5 m that receives freshwaters from the Po di Goro through a gate. The Sacca di Goro is spatially enclosed by a long natural sandbank and is characterized by limited water circulation. Being eutrophic, this ecosystem is very suitable for shellfish farming, and it is one of the largest clam-farming grounds in Europe.

The Valli di Comacchio (Fig. 1a, c), are a complex of brackish and hypereutrophized lagoons (width over 100 km^2) in the southern part of the Po River Delta (Emilia Romagna), whose depth ranges from 0.5 to 1.5 m and bottoms are typically muddy. The ecosystem is characterized by limited water renewal, being almost completely surrounded by earthen dikes. This lagoonal system is connected with the Adriatic Sea by 2 marine channels, and it also receives a small amount of continental waters. Occasional marine water inflow may come through the opening of Bellochio drain.

Lido di Dante is a flat dissipative sandy beach 12 km south of the port of Ravenna (Emilia Romagna) (Fig. 1a, d), between the mouths of the Fiumi Uniti (0.9 km North) and Bevano rivers (2.6 km South). The seabed has a gentle slope of about 6 m/km, with a tidal amplitude ranging between 0.30 m and 0.85 m. Bottom sediments ranges from well-sorted fine to medium sand. During summer it is subject to periods of oxygen deficiency, and development of algae and mucilage.

Materials and methods

Following the review of the genus *Streblospio* provided by Rice & and Levin (1998), and the recent identification key to *Streblospio* species provided by Delgado-Blas *et al.* (2018), specimens of the genus *Streblospio* from the northern Adriatic Sea are herein described as a new species. The terminology used to describe morphological features is derived from Delgado-Blas *et al.* (2018) and the previous review of spionid polychaetes provided by Radashevsky (2012).

Specimens of the new species of *Streblospio* were collected during different monitoring surveys of benthic macroinvetebrates carried out along the coasts of the Emilia Romagna (north-eastern Italy), in the Northern Adriatic Sea (Fig. 1a): ten stations (P1-P5 and C1-C5) were sampled in the Po Delta lagoon Sacca di Goro (Fig. 1b) on October 2017, within the LIFE13 NAT/IT/000115 project; two stations, ID and OD (Fig. 1d), were sampled at the seaside resort Lido di Dante on August and September 2016, and 2017. The finding of a new species of *Streblospio* from these two sites, led us to examine specimens previously identified as *Streblospio* sp. (Munari and Mistri, unpublished data), all of which were collected at several sampling stations along the coast of the Emilia Romagna, i.e., Valle di Gorino (Fig. 1b) on June 2009 and Valli di Comacchio (Fig. 1c) on June 2013 and May 2014.

In order to investigate the community of benthic macroinvertebrates (i.e., organisms > 500 µm), at each stations (and at each sampling time), three replicate samples of sediments were taken with a Van Veen grab (area: 0.027 m^2; volume: 4 l); the contents of the grab were sieved through a 0.5 mm sieve. Material retained on the sieve was fixed in 5 % buffered formalin, and then transferred to alcohol. In the laboratory, macroinvertebrates were stained with Rose Bengal to facilitate sorting and identified to species level where possible. Specimens of *Streblospio eridani* n. sp. were measured using a compound microscope Nikon Eclipse (E200) equipped with a digital camera to achieve images and morphometric measures. Several chosen specimens were dehydrated and gold-coated for Scanning Electron Microscope (SEM) study following standard procedure reported by Munari (2014). Selected specimens of the new species are deposited at the Museum of Natural History of Ferrara (MNHF).

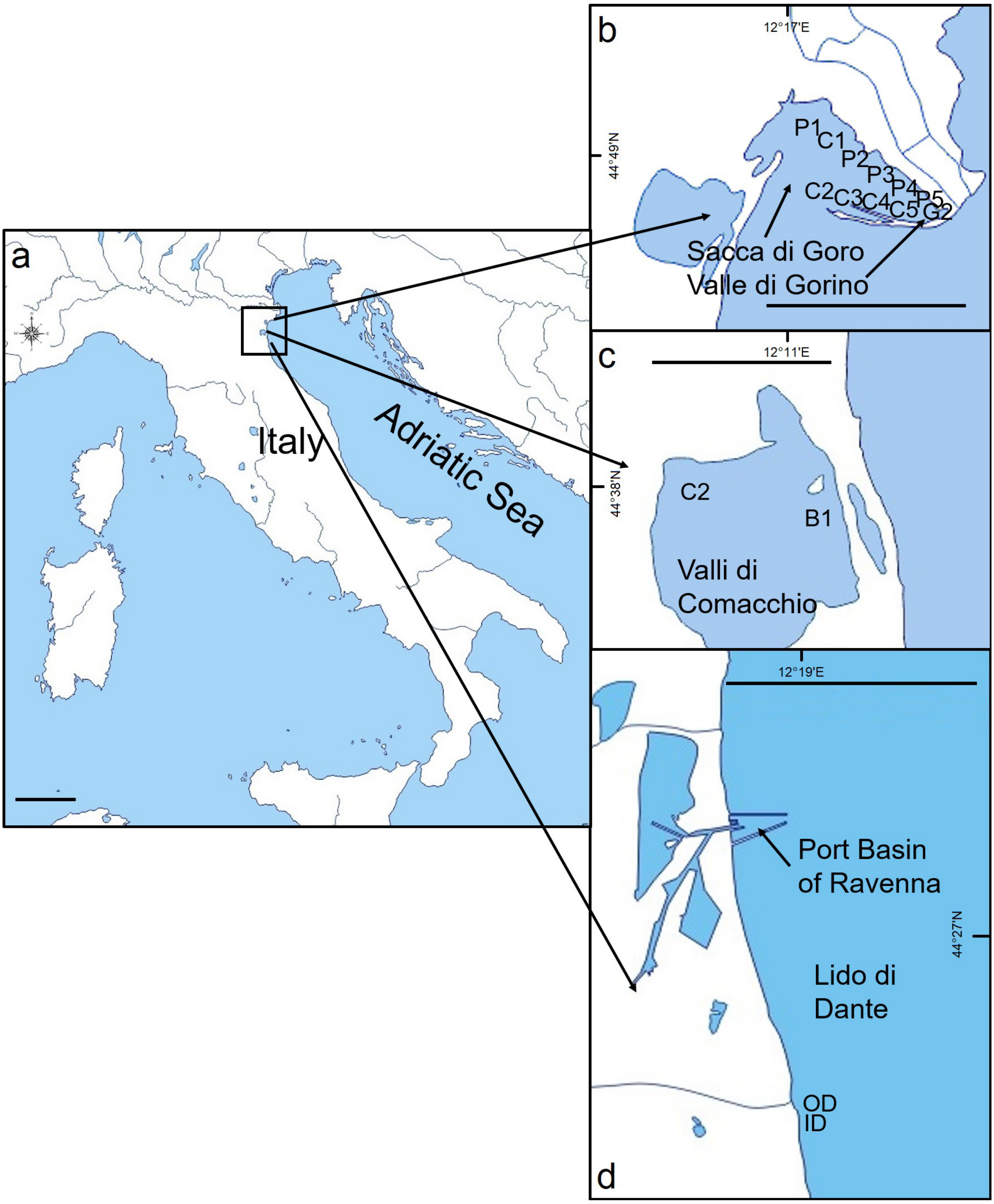

FIGURE 1. a map of the study area and location of sampling stations along the Italian coasts of the northern Adriatic Sea. **b** sampling stations in the Sacca di Goro and the Valle di Gorino. **c** sampling stations in the Valli di Comacchio. **d** sampling stations in the Lido di Dante. Scale bars: a =100 km; b-d = 10 km.

A saturated solution of Methyl Green in ethanol was used on some specimens for temporary staining in order to highlight distinct staining color pattern.

During the sampling campaigns water parameters were measured in situ with a probe.

Total genomic DNA of 14 specimens from station P2 was extracted using the DNeasy Blood & Tissue kit (Qiagen) according to the manufacturer's protocol. The mitochondrial cytochrome *c* oxidase subunit I gene (COI) was amplified using the primer pair LCO1490 and HC02198 (Folmer *et al.* 1994), following the PCR conditions listed in Schulze *et al.* (2000). The obtained PCR products (643 bp long) were cleaned using the HT ExoSAP-IT (Applied Biosystems™) and sequencing was carried out at the BMR Genomics Sequencing Service (University of Padova, Italy) with the same primers employed in the amplification reaction. As the COI sequences obtained for the 14 samples of *Streblospio* sp. were identical, only one was deposited in the International Nucleotide Sequence Database Collaboration (INSDC) repositories with the following GenBank accession number: LR597481. The identity of the obtained sequence was checked by using the BLAST program available at the USA National Center for Biotechnology Information (NCBI) web server (http://www.ncbi.nlm.nih.gov).

To infer the phylogenetic position of the collected specimens, a dataset of 40 COI sequences of samples belonging to *S. benedicti* Webster, 1879, *S. gynobranchiata* Rice & Levin, 1998, *S. shrubsolii* (Buchanan, 1890) and *Streblospio* sp. was constructed. *Prionospio steenstrupi* Malmgren, 1867 (AF138955) was chosen as outgroup according to Mahon *et al.* (2009). Sequences for comparison were downloaded from the USA National Center for Biotechnology Information (NCBI) web server (http://www.ncbi.nlm.nih.gov). A multiple sequence alignment (499 bp) was obtained using MUSCLE (www.ebi.ac.uk/Tools/msa/muscle/).

Phylogenetic analyses were performed with MEGA v. 5.1 program (Tamura *et al.* 2011) using Neighbour Joining (NJ), Maximum Parsimony (MP), and Maximum Likelihood (ML) methods. For ML, the model that best fit the data according to the ModelTest software implemented in MEGA v. 5.1, under the BIC criterion (Schwarz 1978), was HKY + G. Non-parametric bootstrap re-sampling (Felsenstein 1985) was performed to test the robustness of the tree topology (1000 replicates).

Results

We identified a total of 7250 individuals, 7227 out of which from the Sacca di Goro, 13 from the Lido di Dante, 6 from the Valli di Comacchio, 4 from the Valle di Gorino. In all the sampled stations of the 4 sites the specimens belonging to the new species were counted in order to obtain also knowledges on the spatial distribution of the individuals. In particular, were collected: 1) from the Sacca di Goro (in October 2017) 1367 specimens at station C1, 454 specimens at station C2, 305 specimens at station C3, 837 specimens at station C4, 1720 specimens at station C5, 1117 specimens at station P1, 942 specimens at station P2, 281 specimens at station P3, 124 specimens at station P4, 80 specimens at station P5; 2) from the Lido di Dante 3 specimens at station ID and 1 specimen at station OD in August 2016, 7 specimens at station ID in September 2016, 1 specimen at station ID in August 2017, 1 specimen at station ID in September 2017; 3) from the Valle di Gorino 4 specimens at station G2 in June 2009; 4) from the Valli di Comacchio 1 specimen at station C2 in June 2013, and 5 specimens at station B1 in May 2014.

Not all of them were used as type materials because they did not add further information to those obtained from specimens selected as types. Best preserved and complete specimens (representative of the variability among individuals) were measured, thoroughly analyzed, and deposited at the Museum of Natural History of Ferrara (MNHF), as holotype and paratypes.

The main environmental data (e.g., sediment characteristics, depth) of the sampling stations at each sampling site are provided in Table 1. Sampling stations in the Sacca di Goro and Valle di Gorino were characterised by muddy (silty clay) sediments, those in the Valli di Comacchio by clay and clayey silt, whereas those in the Lido di Dante by sandy sediments (Table 1).

Systematics

Family SPIONIDAE Grube, 1850

Genus *Streblospio* Webster, 1879

Species *Streblospio eridani* n. sp.
(Figs 2-6)

Type material. Holotype: Sacca di Goro (2nd October 2017), complete specimen (MNHF I.AL.19.0002-1), collected at Station C3 (44°47.717'N, 12°20.620'E) by C. Munari in muddy sediment between 0.5 and 1.7 m depth (Table 1).

Paratypes: Sacca di Goro (October 2017), 9 specimens (MNHF I.AL.19.0002-2/10), collected at P1 (44°49.758'N, 12°18.105'E); 5 specimens (MNHF I.AL.19.0002-11/15) collected at station P2 (44°48.676'N, 12°20.748'E); 3 specimens (MNHF I.AL.19.0002-16/18) collected at station P5 (44°47.793'N, 12°22.177'E); 10 specimens (MNHF I.AL.19.0002-19/28) collected at station C1 (44°49.061'N, 12°19.395'E); 4 specimens (MNHF I.AL.19.0002-29/32) collected at station C5 (44°47.435'N, 12°22.328'E); 2 specimens (MNHF I.AL.19.0002-33-34) collected at station C2 (44°47.783'N, 12°19.422'E). Valle di Gorino (June 2009), 4 specimens, (MNHF I.AL.19.0002-35/38) collected at station G2 (44°47.481'N, 12°21.262'E). Valli di Comacchio, June 2013, one specimen (MNHF I.AL.19.0002-39) collected at station C2 (44°37.944'N, 12°7.375'E); May 2014, 2 specimens (MNHF I.AL.19.0002-40/41) collected at station B1 (44°36.912'N, 12°12.731'E). Lido di Dante (September 2017), 3 specimens (MNHF I.AL.19.0002-42/44) collected at station ID (44°23.168'N, 12°19.294'E).

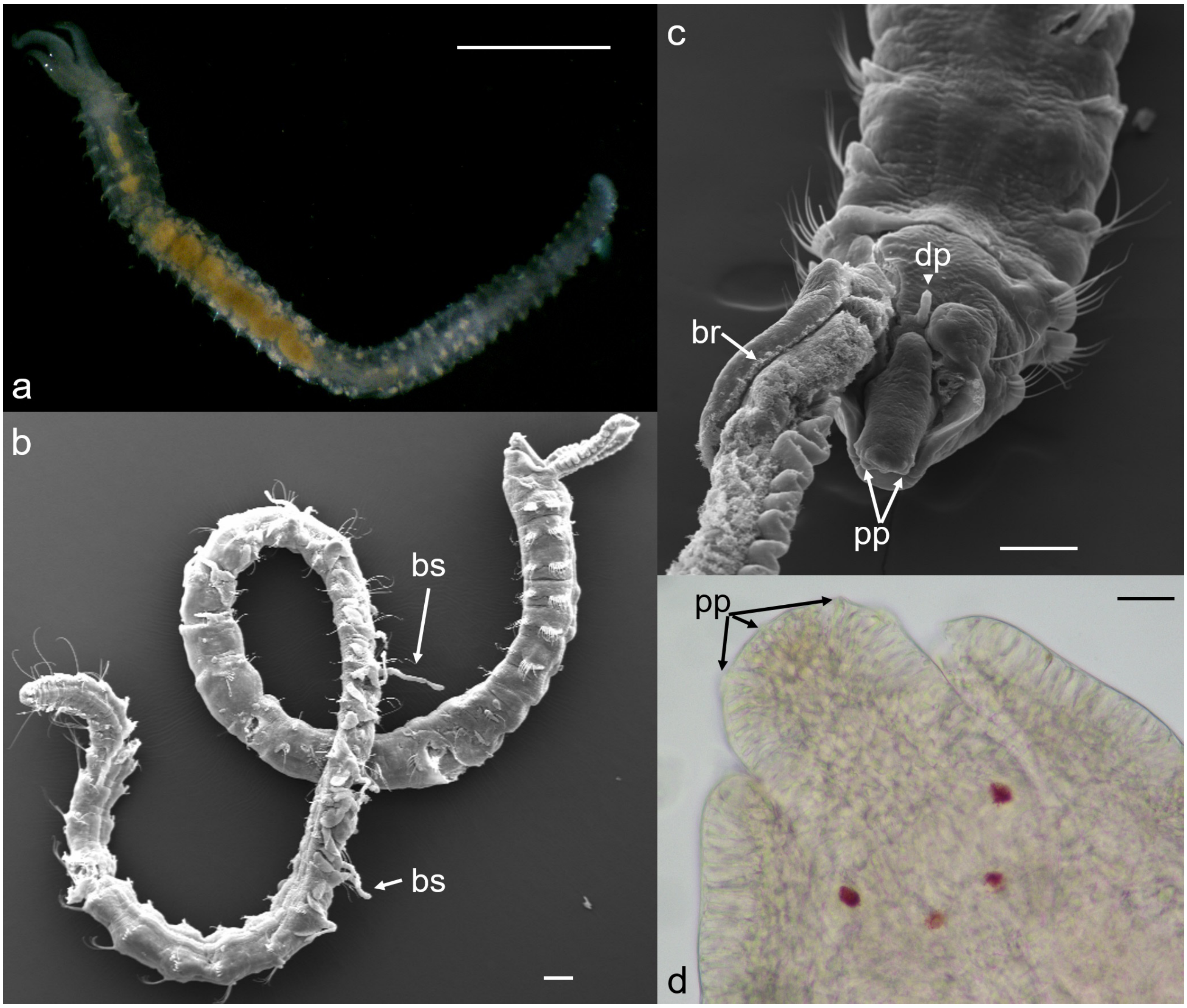

FIGURE 2. *Streblospio eridani* n. sp. **a** holotype (MNHF I.AL.19.0002-1), complete, dorsal view. **b** specimen lacking palps, with finger-like brooding structures (as the holotype), lateral view. **c** anterior region with prostomium and peristomium, fronto-dorsal view. **d** prostomium with prostomial papillae on the fronto-lateral surface (paratype MNHF I.AL.19.0002-33), and 2 pairs of eyes, dorsal view. Abbreviations: br, branchia; bs, brooding structures; dp, dorsal papilla like a small occipital antenna; pp, prostomial peaks (or sensory knobs). Scale bars: a = 1mm; b–c = 100 µm; d = 10 µm.

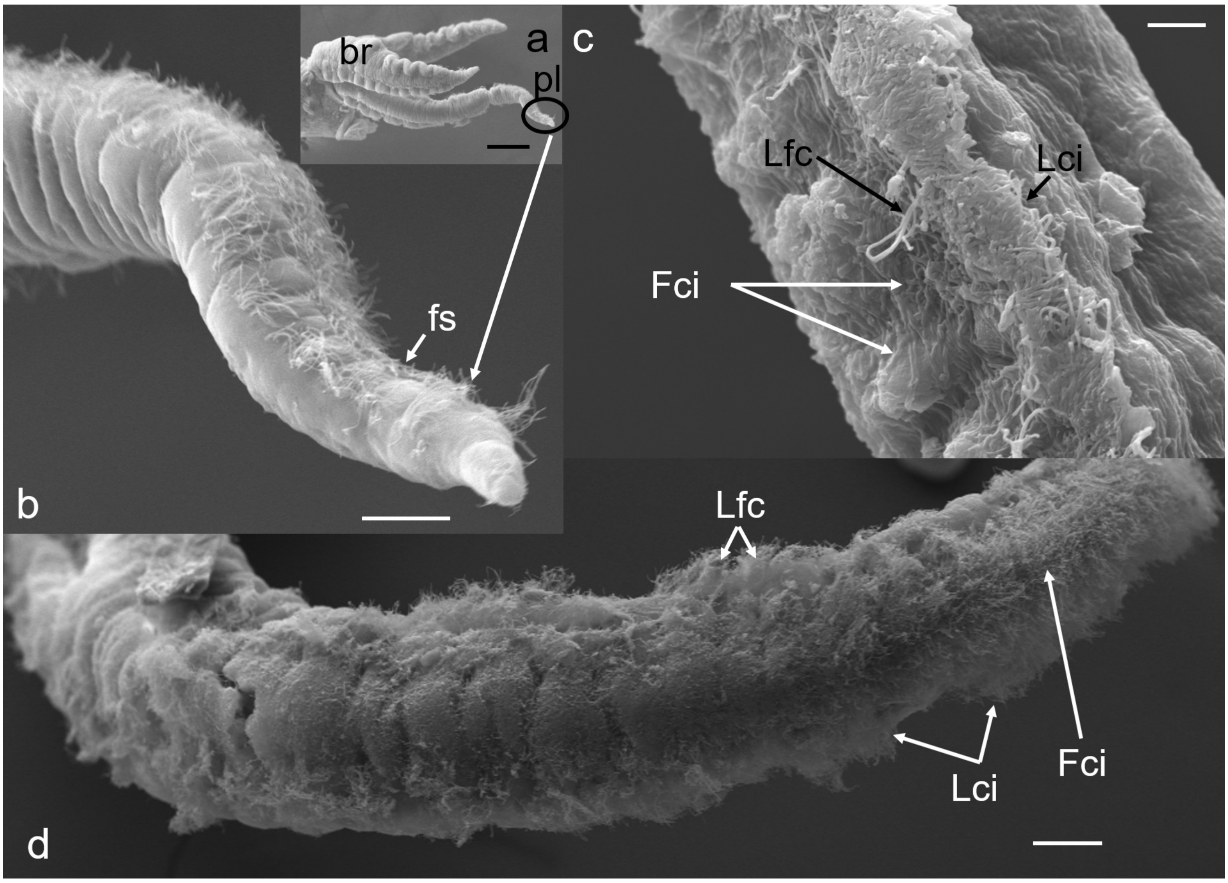

FIGURE 3. *Streblospio eridani* n. sp. ciliated palps. **a** anterior region, with branchiae and palps, lateral view. **b** distal end of palp, frontal surface, lateral view. **c** detail of palp with cilia on frontal surface, latero-frontal view. **d** detail of frontal surface of ciliated palp, frontal view. Abbreviations: br, branchiae; pl, palps; fs, frontal surface; Lci, lateral cilia; Fci, frontal cilia, Lfc, latero-frontal cirri. Scale bars: a = 100 µm; b = 20 µm; c = 3 µm; d = 30 µm.

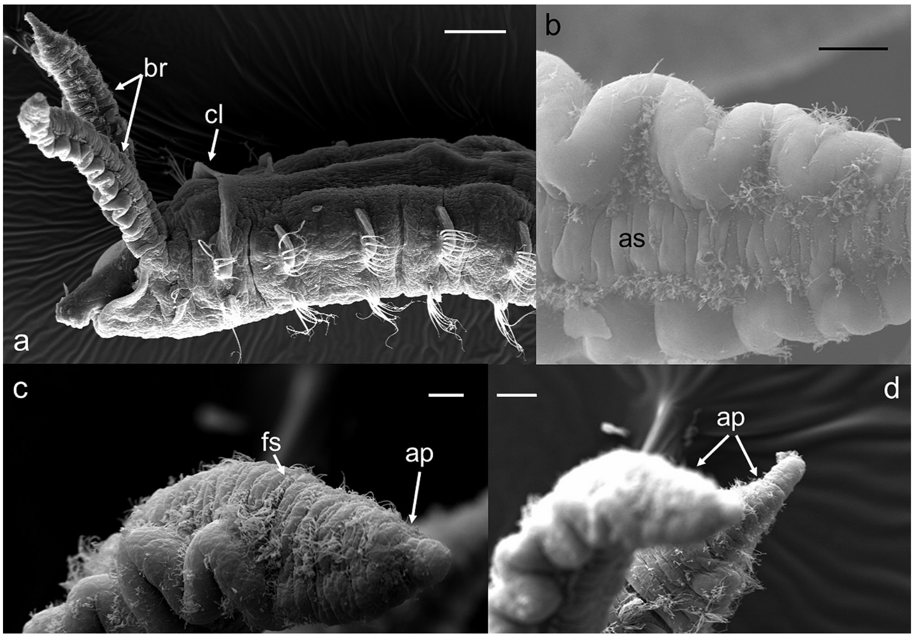

FIGURE 4. *Streblospio eridani* n. sp., ciliated branchiae. **a** anterior region with ciliated branchiae on chaetiger 1, dorso-lateral view. **b** detail of abfrontal surface, lateral view. **c** detail of ciliated branchiae, distal end, with distal digitiform appendage, dorsal view. **d** detail of ciliated branchiae and distal digitiform appendage, lateral view. Abbreviations: br, branchiae; cl, collar; ap, digitiform appendage; fs, frontal surface; as, abfrontal surface. Scale bars: a = 100 µm; b = 20 µm; c = 10 µm; d = 20 µm.

Description. Holotype complete specimen (Fig. 2a), 5.10 mm long, 0.39 mm wide (including parapodia) at chaetiger 6, with 40 chaetigers; female, with oocytes, and brooding structures between chaetigers 15 and 26. Paratypes complete (Fig. 2b), in number of 26, ranging from 36 to 50 chaetigers, 4.74–14.93 mm long, and 0.22–0.45 mm wide (including parapodia), complete although some paratypes lost palps or branchiae during handling and examination. Paratypes incomplete, in number of 17, 0.22–0.44 mm wide, with 8–32 chaetigers.

Prostomium elongate and anteriorly rounded (Fig. 2c), partially enveloped by peristomial extensions; presence of prostomial peaks (sensory knobs similar to papillae) along the fronto-lateral edge of the prostomium, on the dorsal and ventral surface (Fig. 2c), and easily visible even with a light microscope (Fig. 2d). Two pairs of brownish, small eyes in a trapezoidal arrangement (Fig. 2d); a few specimens with 5 eyes. Eyes no longer visible in some specimens preserved in alcohol. One pair of palps situated on midlateral side of prostomium (Fig. 3a), ciliated on frontal surfaces (Fig. 3b), extending to chaetigers 6 in the holotype, and to chaetigers 5–10 in paratypes. Palps have three groups of cilia (i.e., lateral cilia, frontal cilia and latero-frontal cirri; Fig. 3c, d). Peristomium with short dorsolateral wings (Fig. 2b), surrounding base of palps (Fig. 2c), and fused with the first chaetiger.

One pair of thick, ciliated branchiae inserted just posterior to palps (on chaetiger 1), extending back to chaetigers 5 in the holotype, and to chaetigers 5–11 in paratypes, with distal digitiform appendage (Fig. 4a, d); slightly thicker than palps. Branchiae with central axis and two laterally flattened surfaces (Fig. 4a, b, c); frontal surface densely ciliated on inner part (oriented towards midline of body) and sparsely ciliated on its outer part; abfrontal surface sparsely ciliated with two main tracts of cilia along long axis of branchia (Fig. 4b). A few specimens having branchiae with transverse bands of dark pigment disappearing after fixation over time. Length of palps and branchiae depends on state of contraction or expansion. A small conical dorsal papilla (like a small occipital antenna) on chaetiger 1 between branchiae (Fig. 2c), difficult to see without removing them.

Parapodia on chaetiger 1 with neuropodial postchaetal lamellae only, neuropodial lamellae protruding, small flap-like, ear-shaped (Fig. 2c, 4a, 6a), and slightly inclined; notopodial lamellae lacking, and only few capillaries present. Notopodium of chaetiger 2 with broad postchaetal lamellae continuous with dorsal hood extending across dorsum connecting left and right notopodia (Figs 2c, 4a, 6a), and forming a high dorsal entire membranous collar (Figs 4a, 6a); anterior margin of hood widens upward and forms a pouch open anteriorly (Figs 4a, 6a); notopodial postchaetal lamellae of chaetiger 2 rounded on ventral edge. Neuropodium of chaetiger 2 with broad flap-like postchaetal lamellae, rectangular shaped (Figs 2c, 6a). Neuropodial lamellae rounded from chaetiger 3. Notopodial and neuropodial postchaetal lamellae small and flap-like on anterior chaetigers, and absent on posterior chaetigers: notopodial postchaetal lamellae progressively reducing from chaetiger 8 until they disappear beyond chaetigers 11-18; neuropodial lamellae progressively reducing from chaetiger 7 until disappearing between chaetigers 10 and 12. Neuropodial and notopodial prechaetal lamellae absent.

All notopodial chaetae capillary; neuropodial chaetae include capillaries, and 1-2 sabre chaetae and multidentate hooded hooks; capillaries on anterior chaetigers are unilimbate and slightly hirsute (Fig. 5a, b), posteriorly slender, alimbate and less hirsute. Chaetiger 1 with 1–4 capillary notochaetae (Figs 2c, 4a, 6a) (2 capillaries in holotype), and 3-6 neuropodial capillary chaetae (4 capillaries in holotype) (Figs 2c, 4a, 6a). Notopodium of chaetiger 2 with 5-11 capillary chaetae (8 capillaries in holotype); neuropodium of chaetiger 2 with 4-9 capillary chaetae (7 capillaries in holotype). Chaetigers 3 to 6 with 6-10 notopodial (7-9 capillaries in holotype) and 7-11 neuropodial (8-9 capillaries in holotype) capillary chaetae. Notopodium of chaetiger 7 with only 6-9 capillary chaetae (8 capillaries in holotype). Neuropodial hooded hooks and sabre chaetae first appearing on chaetiger 7, and continuing almost to end of body; each hook with 4-5 pairs of small teeth in addition to main unpaired tooth (Fig. 6b); without secondary hoods. Neuropodium of chaetiger 7 with 2-4 hooded hooks (3 hooks in the holotype) accompanied by 2-4 slender capillary chaetae in a single row (2 capillaries in the holotype), and 1-2 sabre chaetae (1 sabre chaeta in the holotype). Sabre chaeta stout, curved, alimbated, and slightly granulated. On the middle body region, chaetigers 20-24, notopodia with only 4-6 capillary chaetae; neuropodia with 3-5 hooded hooks, 2-3 companion capillary chaetae and 1-2 sabre chaetae (Fig. 6c). On posterior region of the body, notopodia with 2-5 thin long alimbate capillary chaetae (3 in holotype); neuropodia with 6-10 hooded hooks (8 hooks in holotype), 1-3 alimbate capillaries and one sabre chaeta (Fig. 6d). Last three chaetigers with notopodia having only 2 long and slender capillaries, and neuropodia without companion capillaries. The second-last chaetiger with neuropodia having 4-6 hooded hooks (4 in holotype) and up to one sabre chaeta (Fig. 6d); the last chaetiger with neuropodia having 2-4 hooded hooks (2 in holotype) and without sabre chaetae. Pygidium with one pair of ventral lappets, without anal cirri (Fig. 6d). Finger-like extensions of lateral body wall in females (200 specimens examined, 23 out of these deposited as paratypes (all female) present from chaetigers 13–18 to chaetigers 27-28 (15-26 in holotype); extensions oriented dorsally and posteriorly, and resembling branchiae (Figs 2b, 7a); the base of these brood structures similar to pouches arising between noto- and neuropodium. Oogenesis intraovarian, it begins in chaetigers anterior (ahead) to those of the first branchiate brooding structures. Oocytes first occur laterally (i.e., in a lateral row), within paired ovaries attached to genital blood vessels, from chaetigers 7-8. In the subsequent chaetigers oocytes are present at the base and within the branchiate brooding structures (Fig. 7b, c); oocytes of different sizes, slightly oval-shape, 25.82 - 135.56 µm (n = 230) in diameter. The brooding structures contain about two to three eggs. Posterior to branchiate region on females are three or four dorsal appendages in rows between left and right notopodia (Fig. 7d); these extensions present on posterior chaetigers to near end of body.

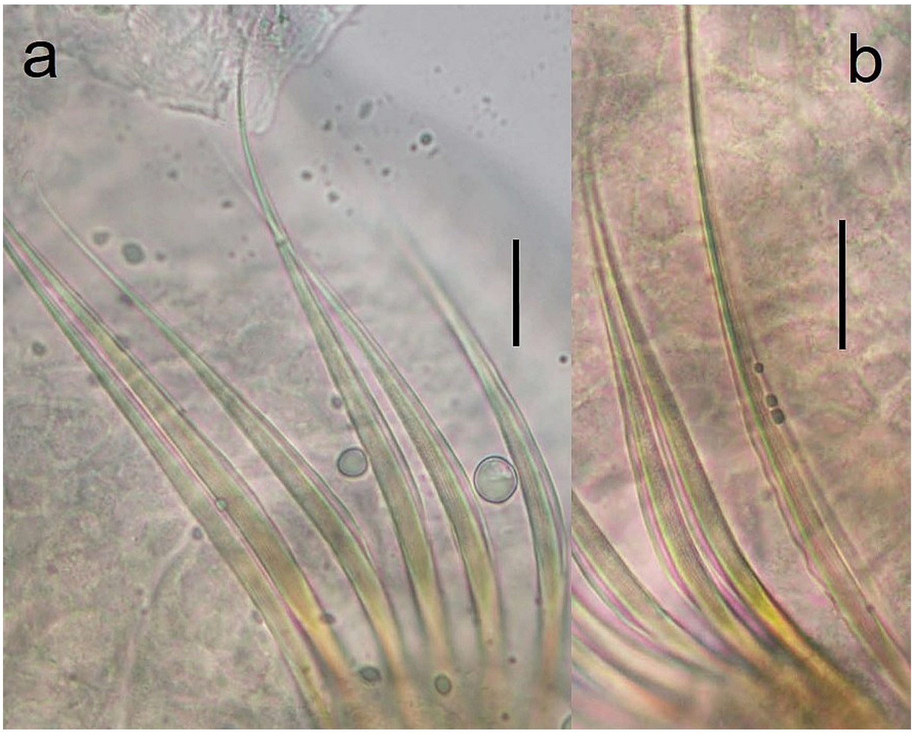

FIGURE 5. *Streblospio eridani* n. sp. **a** unilimbate anterior notocaetae; **b** unilimbate anterior neurochaetae. Scale bars: a-b= 10 µm.

Methyl green staining pattern. Specimens of the new species herein described exhibit methyl green staining reaction. Prostomium and the anterior half (first 8-9 chaetigers) of the body are stained intensely, with darker dorsolateral and ventral bands of stain; the staining has a discoloration on the dorsal pouch (Fig. 7e).

Type locality. Sacca di Goro, Po Delta lagoon, Emilia Romagna, Italy, Adriatic Sea.

Habitat and ecology. Adults of *Streblospio eridani* n. sp. were collected between 0.5 and 3.5 m depth in sand, silty clay, clayey silt and clay sediments (Table 1). In the eutrophic Sacca di Goro, this species became numerically dominant in the community in October 2017, and it occurred in silty clay sediments to a depth of 1.7 m, at sampling stations characterized by wide salinity range (4.5-33.7 PSU). In that period, its density ranged from 986.7 ind. m^{-2} (Station P2) to 21,213.3 ind. m^{-2} (Station C5), and it accounted for 52-97.8 % of the total abundance of the benthic macroinvertebrates (Table 1). *Streblospio eridani* n. sp. was associated with *Chironomus salinarius* Kieffer, 1915, indicator of organic enrichment and stagnant waters; other abundant species were tolerant or opportunistic, such as spionid polychaetes, *Capitella* Blainville, 1828 species complex, *Hydroides dyanthus* (Verrill, 1873) and the bivalve *Arcuatula senhousia* (Benson, 1842).

Re-examination of old material from the Valli di Comacchio (dating back to 2013, in clayey sediments, 1.5 m depth) and the Valle di Gorino (dating back to 2009), revealed that some specimens (6 and 4, respectively), originally identified as *Streblospio* sp., in fact belong to *S. eridani* n. sp., and that some of them (i.e., two specimens from the Valle di Gorino, and one from the Valli di Comacchio) had oocytes. In the Valle di Gorino and Valli di Comacchio the new species occurred in low density (Table 1). The community of the Valli di Comacchio in which the new species occurred was extremely similar to that of the Sacca di Goro, and characterized by opportunistic (e.g., spionid polychaetes, oligochaetes and chironomids) and tolerant species (i.e., the bivalve *Cerastoderma galucum* (Bruguière, 1789)). The community of the Valle di Gorino was characterized by stress tolerant species such as the amphipod *Corophium orientale* Schellenberg, 1928, the gastropod *Ecrobia ventrosa* (Montagu, 1803), and the polychaete *Hediste diversicolor* (O.F. Müller, 1776). In the marine site, Lido di Dante, *S. eridani* n. sp. occurred in sandy sediments in low density, representing only from 0.1% (station ID August and September 2017) to 0.5% (station ID September 2016) of the total abundance of the benthic macroinvertebrate community. Its density ranged from 12.3 ind. m^{-2} (stations: OD August 2016, ID August 2017, ID September 2017) to 86 ind m^{-2} (station ID September 2016). These sampling stations were characterized during the study periods by salinities of 33.2-37.3 PSU. Also at the Lido di Dante, *Streblospio eridani* n. sp. occurred in association with other opportunistic spionids belonging to the genera *Minuspio* Foster, 1971, *Prionospio* Malmgren, 1867 and *Polydora* Bosc, 1802.

TABLE 1. Main environmental characteristics of the sampling stations (coordinates, depth, sediment granulometric composition, salinity), with densities and percentage (%) of *Streblospio eridani* n. sp. on the total abundance of macrobenthic invertebrates.

Area	Station	Latitude	Longitude	Depth (m)	Sediment	Salinity	Densities ^{1}(ind. m-^{2})±SE	%
Sacca di Goro	C1	44°49.061'N	12°19.395'E	1.7	Silty clay	Meso/ Polyhaline	16859.7±3225.5	97.8
	C2	44°47.783'N	12°19.422'E	1.5	Silty clay		5599.3±921.8	63.2
	C3	44°47.717'N	12°20.620'E	1.5	Silty clay		3761.7±960.3	52.0
	C4	44°47.599'N	12°21.616'E	1.2	Silty clay		10323.0±1608.6	64.5
	C5	44°47.435'N	12°22.328'E	0.7	Silty clay		21213.3±1118.2	95.9
	P1	44°49.758'N	12°18.105'E	1.7	Silty clay		13776.3±2212.2	64.8
	P2	44°48.676'N	12°20.748'E	1.3	Silty clay		11618.0±1724.5	68.6
	P3	44°48.353'N	12°21.223'E	1	Silty clay		3465.7±1235.4	95.3
	P4	44°48.079'N	12°21.615E	1	Silty clay		1529.3+277.5	93.2
	P5	44°47.793'N	12°22.177'E	0.5	Silty clay		986.7±259.4	90.9
Valle di Gorino	G2	44°47.481'N	12°21.262'E	1.5	Silty clay	Meso/ Polyhaline	49.3±28.5	0.3
Valli di Comacchio	C2	44°37.944'N	12° 7.375'E	1	Clay	Euhaline/ Hyperhaline	12.3±7.1	0.1
	B1	44°36.912'N	12°12.731'E	1	Clayey silt		61.7±25.7	2.3
Lido di Dante	ID	44°23.168'N	12°19.294'E	2.5	Sand	Euhaline	12.3±7.1- 86.3±14.2	0.1-0.5
	OD	44°23.433'N	12°19.262'E	3.5	Sand		12.3±7.1	0.4

ind. m^{-2} ± SE = average individuals m^{-2} ± standard error

Remarks. Recently, Delgado-Blas *et al.* (2018) suggested raising the status of *Streblospio benedicti japonica* Imajima, 1990 to full species level as *S. japonica* Imajima, 1990 on the basis of several morphological characters (i.e., number of segments, segment on which sabre chaetae occur, number of accessory teeth on neuropodial hooks, and presence of ventral lappets on pygidium). Thus, with the addition of *S. eridani* n. sp., the number of described species in *Streblospio* would increase to seven. Among the *Streblospio* species previously described, four seem to be native to European waters: the new species herein described, *S. shrubsolii* (Buchanan, 1890), *S. eunateae* Martínez & Adarraga, 2019 and *S. padventralis* Delgado-Blas, Díaz-Díaz & Viéitez, 2018. However, the last two species have not yet been recorded from the Mediterranean Sea. Similarly, despite *S. benedicti* Webster, 1879 having been introduced to European coasts (Carlton 1979; Fonseca-Genevois & Cazaux 1987), it has not been reported from the Mediterranean Sea. Interestingly, *S. japonica* has never been found outside its native biogeographic region.

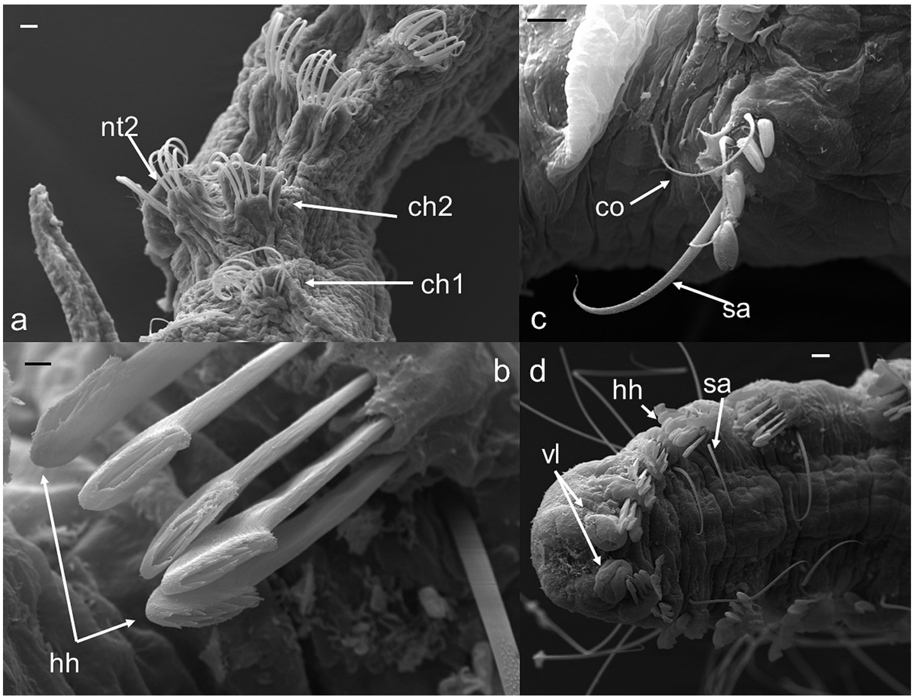

FIGURE 6. *Streblospio eridani* n. sp. **a** anterior chaetigers, left lateral view. **b** hooded hooks with pairs of small teeth distal to the main tooth on posterior chaetigers, ventral view. **c** neuropod of middle chaetiger with granulated sabre chaeta, hooded hooks, and companion chaetae. **d** pygidium with one pair of very short ventral lappets, ventral view. Abbreviations: nt2, notopod of chaetiger 2; ch2, chaetiger 2; ch1, chaetiger 1; co, companion chaetae; sa, sabre chaeta; hh, hooded hooks; vl, ventral lappets. Scale bars: a = 10 µm; b = 2 µm; c-d = 10 µm.

The species placed within the genus *Streblospio* display slight morphological differences although there are considerable differences in their reproductive biology (Mahon *et al.* 2009). For example, males of *S. gynobranchiata* Rice & Levin, 1998 are morphologically similar to *S. benedicti*, but females differ in reproductive structures, that is for the lateral body wall extensions resembling branchiae in *S. gynobranchiata* and for the specialized dorsal brooding pouches in *S. benedicti* (Rice & Levin 1998). Schulze *et al.* (2000) on the basis of genetic and morphological analyses even hypothesized a historical hybridization between *S. benedicti* and *S. gynobranchiata* followed by repeated backcrossing of the hybrids to *S. benedicti*, resulting in the transfer of *S. gynobranchiata* haplotype(s) to an *S. benedicti* morphology. Moreover, a wide variability in the morphological characters was found in the species of the genus *Streblospio*, and with considerable overlap in ranges. For example, a certain overlap is reported in the first and last gametogenic chaetiger in females and for this reason Schulze *et al.* (2000) suggested using caution when using the reproductive morphology alone to identify the species. Likewise, some morphological characteristics of *S. eridani* n. sp. displayed an intraspecific variability, such as the beginning of gametogenic chaetigers, the number of sabre and capillary chaetae, the number of hooded hooks. In specimens of *S. eridani* n. sp. such a variability of morphological characters was also found between body regions. Further difficulties in the morphological identification derive from the differences in the descriptions of specimens and/or populations from different geographical areas of *S. benedicti* (Webster 1879; Hartman 1936; Foster 1971), *S. gynobranchiata* (Rice 1984) and *S. shrubsolii* (Buchanan 1890; Horst 1909; Fauvel 1927). For example, Horst (1909) described *S. dekhuyzeni* Horst, 1909 with two types of capillary notochaetae and more hooded hooks per ramus than *S. shrubsolii*. However, these differences were minor enough and *S. dekhuyzeni* was considered synonymous with *S. shrubsolii* by Fonseca-Genevois & Cazaux 1987. Hartman (1936) described *Streblospio lutincola* Hartman, 1936 with much smaller ventral peristomial fold, and much larger prostomium than *S. benedicti*, and *S. lutincola* was later synonymized with *S. benedicti* (Hartman 1944).

Streblospio eridani n. sp. is similar to the original description (Rice & Levin 1998) of *S. gynobranchiata* in having branchiae with distal digitiform appendage, branchiate brooding structures, dorsal collar entire on chaetiger 2, presence of a dorsal papilla between branchiae, and beginning from chaetiger 7 of both sabre chaetae and hooded hooks. However, *S. eridani* n. sp. can be distinguished by the shape of posterior dorsal appendages on female, beginning (i.e., first branchiate structures at chaetiger 13 in the new species and chaetiger 20 in *S. gynobranchiata*) and number of paired branchiate structures (i.e., for about 10-12 chaetigers in *S. eridani* n. sp. and for about 15 chaetigers in *S. gynobranchiata*), as well as in that the new species has: 4-5 pairs of teeth in the neuropodial hooks rather than 3-4 rows of teeth, absence of postchaetal lamellae on posterior chaetigers rather than digitiform ones, and pygidium with lappets rather than simple, as specified in the original description of *S. gynobranchiata* by Rice & Levin (1998) and in the subsequent description by Çinar *et al.* (2005a).

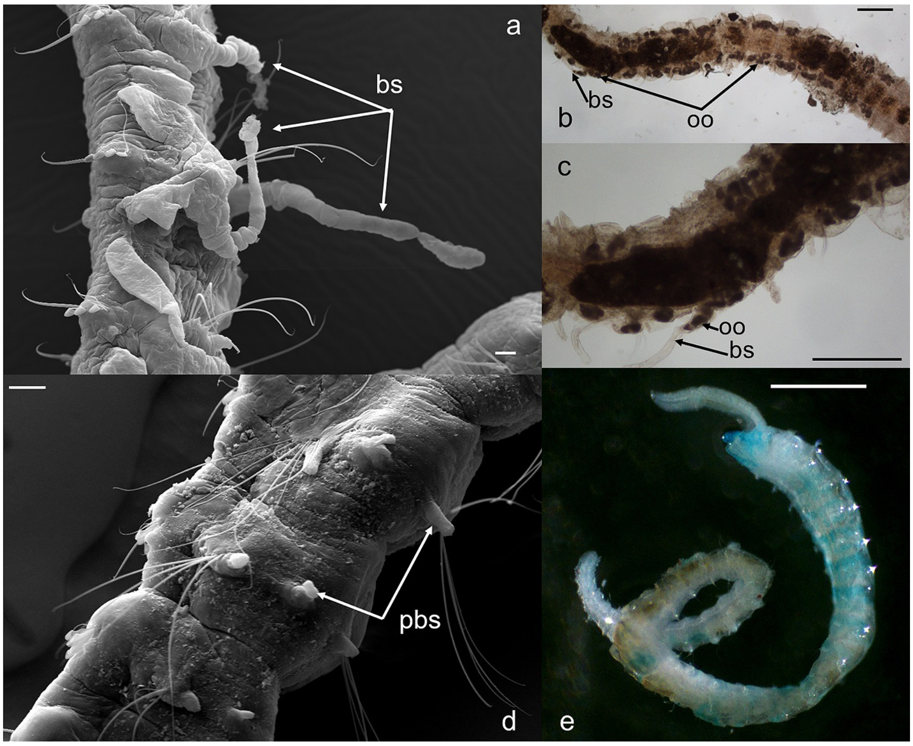

FIGURE 7. *Streblospio eridani* n. sp. **a** brooding structures. **b** oocytes along the body, and in a lateral row at each side of the body on anterior chaetigers. **c** oocytes inside the brooding structures. **d** posterior branched structures. **e** methyl green staining pattern (paratype MNHF I.AL.19.0002-9), ventrolateral view Abbreviations: bs, brooding structures; oo, oocytes; pbs, posterior branched structures. Scale bars: a = 20 µm; b-c = 100 µm; d = 30 µm; e = 0.5 mm.

Besides the presence of branchiate brooding structures (rather than transverse, mid-segmental ridges), the new species is easily distinguished from the other congeneric species from the Mediterranean, that is *S. shrubsolii*, by several characters: shape of pygidium (with lappets rather than simple, that is without such lobes, as in the latter species), beginning of neuropodial hooks from chaetiger 7 rather than from chaetigers 8-10, number of small teeth in the neuropodial hooks, the first gametogenic chaetiger (19 in the latter species, rather than in chaetigers 7-8), presence of dorsal papilla and sabre chaetae, which are both absent in *S. shrubsolii* even though the latter species presents a low raised elevation on the first chaetiger. Finally, *S. eridani* n. sp. differs from *S. shrubsolii* also in the distribution of prostomial peaks: it has such sensory knobs (like papillae) restricted to the fronto-lateral edge of the prostomium, rather than being widely scattered on all surfaces of the prostomium as observed in *S. shrubsolii* by Dauer *et al.* (2003). *Streblospio eridani* n. sp. differs from *S. padventralis* in that in the former species sabre chaetae begin from chaetiger 7 rather than from chaetiger 3, and dorsal (occipital) papilla is present, rather being absent. The shape of prostomium is a further character useful to differentiate this new species from *S. padventralis*, in that the latter species has a subdistal pad surrounding the ventral region. *Streblospio eridani* n. sp. is easily distinguished from the description of *S. eunateae* (Martínez & Adarraga 2019) by its smaller size (4.7 - 14.9 mm, compared to 10 - 25 mm in *S. eunateae*), and the staining pattern with methyl green, in that the latter species shows uniform staining thoroughout the body, rather than a defined pattern (as in the new species herein described). *Streblospio eridani* n. sp. differs from *S. eunateae* also in that the latter has: neuropodial hooded hooks from chaetigers 9-10, rather than from chaetiger 7; oogenesis beginning from chaetiger 20, rather than 7-8; presence of incubator chambers without extensions resembling branchiae, rather than with such structures (as in this new species).

About the term nuchal antenna (or papilla) used by Rice and Levin (1998), in agreement with Delgado-Blas *et* *al.* (2018) and Radashevsky (2012) we believe that it is unsuitable as this appendage is located dorsally on the first chaetiger between the two branchiae. Following these authors (Radashevsky 2012; Delgado-Blas *et al.* 2018) we referred to this structure to as dorsal papilla or occipital antenna.

On the basis of the revision of the genus *Streblospio* provided by Rice & Levin (1998) and the recent descriptions of two new species of *Streblospio* by Delgado-Blas *et al.* (2018), and Martínez & Adarraga (2019), we summarize in Table 2 the main morphological, reproductive and ecological characteristics of the currently known species of *Streblospio*, providing differences between the new species of *Streblospio* and the previously described ones. Females of the *S. eridani* n. sp. also reveal differences from *S. benedicti* (planktotrophs and lecithotrophs), *S. gynobranchiata* and *S. shrubsolii* in egg diameter, location of the first gametogenic chaetiger, as well as brood structures (Table 2).

TABLE 2. Main morphological, reproductive and ecological characteristics of the currently known species of *Streblospio*.

Character	*Streblospio benedicti*	*Streblospio gynobranchiata*	*Streblospio shrubsolii*	*Streblospio japonica*	*Streblospio padventralis*	*Streblospio eunateae*	*Streblospio eridani* sp. nov.
Length of chaetigers	70	70	48	48	54	77	50
Nos of pairs of eyes	2-3	2-3	2-4	2	2, crescent-shaped	2-3	2
Dorsal (occipital) papilla on chaetiger 1	present	present	absent (only a low rised elevation)	present	absent	present	present
Start of sabre chaetae (chaetiger)	6-7	7	apparently absent	2-3	3	6-7	7
Palps (chaetiger that is reached)	8-9	7	3-4 with transverse bands	4	5	9	5-10
Branchiae (chaetiger that is reached)	7-8 with transverse bands	8	5-8	5	6	unknown	4-11 with transverse bands
Shape of prostomium	conical with papillae restricted to the peripheral surface	elongate, rounded anteriorly	blunt conical, with papillae scattered on all surfaces	anteriorly rounded	fusiform, rounded anteriorly, with a subdistal pad	anteriorly rounded, with (eversible) marginal papillae	elongate, anteriorly rounded with papillae along the peripheral surface
Dorsal collar	entire	entire	bilobed	entire	entire	entire	entire
Start of hooded hooks	6-9	7	8	7	7-9	9-10	7
Number of hooks per parapod: anterior end	3-5	3-4	2-3	5-6	unknown	6-8 (central region)	2-4
Posterior end	8-12	8-10	5	unknown	up to 9	8-12	6-10 (2-4 in the last chaetigers)

....*Continued next page*

TABLE 2. (Continued)

Character	*Streblospio benedicti*	*Streblospio gynobranchiata*	*Streblospio shrubsolii*	*Streblospio japonica*	*Streblospio padventralis*	*Streblospio eunateae*	*Streblospio eridani* sp. nov.
Rows of teeth above main tooth	3-4	3-4	2	5 pairs	4-5 pairs	3-5	4-5 pairs
Brood structures	pouches	branchiate	transverse, mid-segmental ridges	unknown	chambers in coelomic cavities	chambers in coelomic cavities	branchiate
First gametogenic chaetiger (mean ± stand. dev.)	9-12 (10.5±1.1 planktotrophs) 11-14 (12.7±1.3 lecithotrophs)	5-10 (7.7±2.0)	18-20 (19±1.1)	unknown	21	20	7-8 (7.5±0.5)
Pygidium	simple or bilobed	simple	simple	with lappets	with lappets	with lappets	with lappets
Colour	colorless, pale greenish gray, light flesh-color	colorless, or pale yellow, or light flesh color	reddish	colorless in alcohol	pale white in alcohol	unknown	pale beige
Habitat	moderately euryhaline; brackish waters and marshes	brackish and marine shallow waters	marine and brackish shallow waters	tidelands	intertidal, river mouth	sections of estuaries, with low salinity, shallow and intertidal waters	brackish and marine waters

Moreover, in the phylogenetic reconstruction based on the COI marker (Fig. 8) the sequence obtained from the Adriatic *S. eridani* n. sp. specimens resulted clearly separated from the other species of the genus and was included in a well-supported clade (100NJ/100MP/99ML) with nine sequences of *Streblospio* sp. from India. The range of nucleotide divergence within the clade was 0-1.20%. *S. gynobranchiata* resulted sister taxon to this group (100NJ/100MP/99ML). The nucleotide divergence calculated between the Adriatic specimens and the other *Streblospio* species ranged from 16.43% to 17.84% (*S. eridani* n. sp. vs *S. benedicti*), from 9.02% to 9.82% (*S. eridani* n. sp. vs *S. gynobranchiata*) and from 23.25% to 23.85% (*S. eridani* n. sp. vs *S. shrubsolii*). These values are significantly higher than the intraspecific range and comparable with the interspecific one reported for the genus by Mahon *et al.* (2009) (0.44-2.60% and 15.51-22.50%, respectively). These molecular results strongly support the erection of a new species, *S. eridani* n. sp., for the Adriatic and the Indian *Streblospio* sp. specimens.

Etymology. The specific name (*S. eridani*) refers to Eridanus, the ancient Latin name of the Po River, which ones flooded in the vicinity of Ravenna town, and whose current course ends creating a wide delta projected into the northern Adriatic Sea and including the Sacca di Goro, the Valle di Gorino, and the Valli di Comacchio from which the specimens of the new species come from.

Discussion

Levin (1984) described the occurrence of multiple patterns of development (i.e., poecilogony) in *S. benedicti* Webster, 1879, and the associated variations in egg size, fecundity, and length of planktonic larval life, and stated that in *S. benedicti* oogenesis begins in anterior chaetigers (chaetigers 7-11 in planktotrophic populations and chetigers 12-14 in lecithotrophic populations). Oocytes develop within paired ovaries attached to genital blood vessels which extend into the coelomic space; when oocytes are fully developed they move into posterior segments, are fertilized

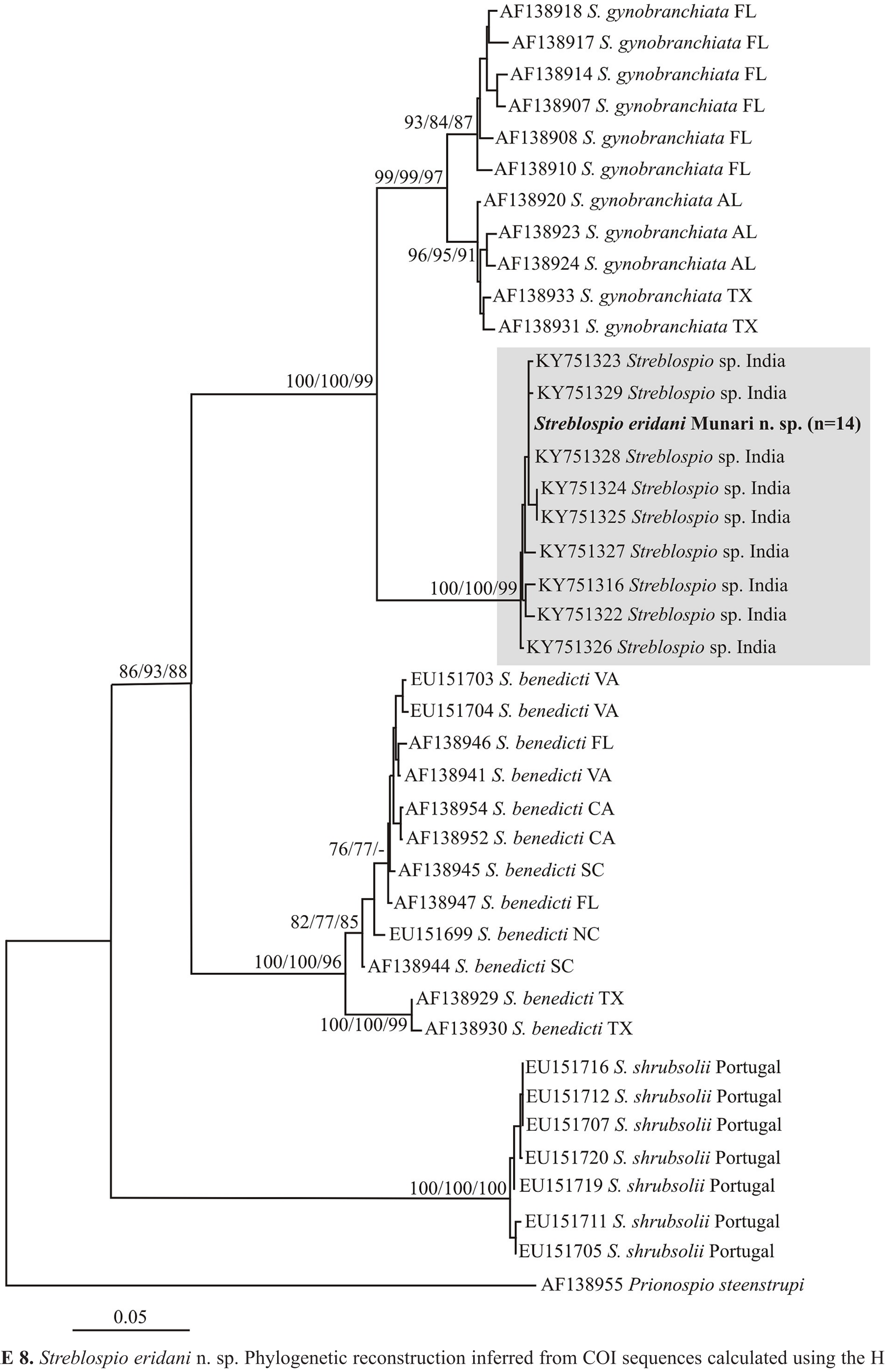

FIGURE 8. *Streblospio eridani* n. sp. Phylogenetic reconstruction inferred from COI sequences calculated using the HKY+G evolutionary model. The numbers near the nodes indicate bootstrap values (> 50) (NJ/MP/ML). In bold is shown the sequences obtained in this study. The clade including *S. eridani* n. sp. sequences is marked in grey. Abbreviations: AL, Alabama USA; CA, California; FL, Florida USA; NC, North Carolina USA; SC, South Carolina USA; TX, Texas USA; VA, Virginia USA.

by sperm stored in spermatophores, and enter brood structures (Levin 1984). Similarly, in *S. eridani* n. sp. oogenesis seems to begin in anterior chaetigers (i.e., chaetigers 7-8) and oocytes seem to move out into the branchiate structures as they become more developed. In specimens of *S. eridani* n. sp. oocytes were variable in size (25.8 – 135.6 µm); maximum diameter size of the oocytes was large compared to those reported by Rice & Levin (1998) for *S. gynobranchiata* Rice & Levin, 1998 (70-90 µm) and plaktotrophic forms of *S. benedicti* (diameter is 70-90 µm in), and to those reported by Mccain (2008) for obligates planktotrophic populations of both *S. gynobranchiata* and *S. benedicti* (80–95 µm), as well as compared to those described by Çinar *et al.* (2005a), and Radashevsky & Selifonova (2013) for specimens of *S. gynobranchiata* (75-88 µm in diameter) from the eastern Mediterranean Sea. However, oocytes of the new species herein described were small compared to those of lecitotrophic forms of *S. benedicti* (100-220 µm) and *S. shrubsolii* (Buchanan, 1890) (200-230 µm in diameter) reported by Rice & Levin (1998), to those found by Mccain (2008) for the facultative planktotrophic *S. benedicti* (180–190 µm) and to those of *S. eunateae* Martínez & Aderraga, 2019 (125-250 µm in diameter) described by Martínez & Aderraga (2019).

Therefore, the occurrence of small oocytes in *S. eridani* n. sp. suggests that they are probably immature or not fertilized. Dorsal appendages occurr on posterior chaetigers on females of *S. gynobranchiata* (Rice & Levin 1998) and, in the same way, of *S. eridani* n. sp., however in the latter such structures do not seem to be as ciliated as in the former.

The Mediterranean Sea is a hotspot of biodiversity with a high rate of endemism (Bianchi & Morri 2000; Coll *et al.* 2010). The current estimated number of polychaete species in the Mediterranean is about 1100, which contribute to 10% of worldwide biodiversity, and count 210 endemisms (Coll *et al.* 2010). However, the species richness of invertebrates is still underestimated (Coll *et al.* 2010), as demonstrated by the current increasing number of new species described (see Çinar *et al.* 2011b, 2015; Dagli & Çinar 2011; Çinar & Dagli 2013; Lezzi 2017). The risk is that undescribed invertebrate species may become extinct before we even become aware of their existence. In fact, Mediterranean marine biodiversity is undergoing rapid alteration (Bianchi 2007) due to habitat loss and degradation, anthropogenic impacts, climate change, eutrophication, and the establishment of alien species (Coll *et al.* 2010; Micheli *et al.* 2013). The description of *S. eridani* n. sp. contributes to improve our knowledge on the diversity of Mediterranean invertebrates, although much further efforts remain to be done in order to obtain a realistic estimate of the number of taxa occurring in such a threatened marine system.

Identification key to *Streblospio* species (adapted from Delgado-Blas *et al*. 2018)

1. Dorsal papilla on chaetiger 1 present 2
- Dorsal papilla on chaetiger 1 absent. 6
2. Sabre chaetae beginning on chaetigers 2–3; hooks with five pairs of teeth above main tooth; pygidium with two lappets. . . . . *Streblospio japonica* Imajima 1990
- Sabre chaetae beginning on chaetigers 6-7 3
3. Hooks with 3-4 pairs of teeth above main tooth; pygidium simple 5
- Hooks with five pairs of teeth above the main tooth; pygidium with lappets 4
4. Hooks beginning on chaetiger 7 . . . . *Streblospio eridani* **n. sp.**
- Hooks beginning on chaetiger 9-10 . . . . *Streblospio eunateae* Martínez & Adarraga 2019
5. Neuropodial lamellae on segment 2 rounded; brooding structures are pouches. . . . . *Streblospio benedicti* Webster, 1879
- Neuropodial lamellae on segment 2 flap-like; brooding structures are branchiate. . . . . *Streblospio gymnobranchiata* Rice & Levin, 1998
6. Dorsal membranous collar on chaetiger 2 bilobed; hooks with two pairs of teeth above main tooth; sabre chaetae absent; pygidium simple. . . . . *Streblospio shrubsolii* (Buchanan, 1890)
- Dorsal membranous collar on chaetiger 2 entire; hooks with five pairs of teeth above main tooth; sabre chaetae beginning on chaetigers 3; pygidium with lappets. . . . . *Streblospio padventralis* Delgado-Blas, Díaz-Díaz & Viéitez, 2018

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