

Original Article

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Spiophanes adriaticus, a new species from the Mediterranean Sea

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Abstract

Morphological and genetic investigations have led to the identification of *Spiophanes adriaticus* sp. nov. (Polychaeta: Spionidae) from the North Adriatic Sea (Central Mediterranean). A total of 81 specimens were recorded along the sublittoral zone between 8 and 27.5 m of depth. This species differs from other congeners by having: two pairs of black eyes, a cirriform occipital antenna, dorsal ciliated organs as thin bands usually extending to chaetigers 11–12, dorsal ciliated crests from chaetiger 14–17, undulate glandular opening on chaetigers 5–7, unhooded hooks from the 15th chaetiger and Y shaped tubes. A detailed description and illustrations are provided for the new species. Through DNA barcoding results and comparison of DNA sequences of the new species with those of other congeners available in the GenBank database, the validity of the new finding was confirmed. *Spiophanes adriaticus* sp. nov. represents the eighth species of *Spiophanes* recorded for the Mediterranean Sea. A key for the identification of Mediterranean *Spiophanes* species is also provided.

Introduction

The Spionidae family (Grube, 1850) is represented by 39 checked genera, among which *Spiophanes* (Grube, 1860) comprises 31 named species plus three more species whose nomenclature is still incomplete (Meißner, 2005; Meißner & Blank, 2009). Seven species of *Spiophanes* are currently reported for the Mediterranean Sea: *Spiophanes afer* Meißner, 2005; *Spiophanes algidus* Meißner, 2005; *Spiophanes bombyx* (Claparède, 1870); *Spiophanes kroyeri* Grube, 1860; *Spiophanes mediterraneus* Meißner, 2005; *Spiophanes reyssi* Laubier, 1964; and *Spiophanes wigleyi* Pettibone, 1962. Among these, four species are cosmopolitan (i.e. *S. bombyx*, *S. kroyeri*, *S. reyssi* and *S. wigleyi*). *Spiophanes bombyx* is common in shallow waters up to subtidal depths, usually on sandy bottoms (Lardicci, 1989; Meißner, 2005); this species shows variation in important morphological characters in specimens from different localities of the world, so it might represent a species complex (Meißner & Blank, 2009). *Spiophanes kroyeri* occurs from subtidal to bathyal depths on sandy-muddy bottoms, probably preferring mud (Lardicci, 1989; Meißner, 2005), however the distribution of this species should be revised due to misidentifications in the past. *Spiophanes reyssi* was firstly described from bathyal mud of the French Mediterranean coasts as a subspecies of *S. kroyeri* (Laubier, 1964) and then raised to species level, although its original description shows inconsistency of some characters usually observed in other *Spiophanes* species (Meißner, 2005); it occurs from subtidal to bathyal depths (Lardicci, 1989; Çinar *et al.*, 2014). *Spiophanes wigleyi* occurs from subtidal to bathyal bottoms (Meißner & Hutchings, 2003) although in the Mediterranean it has been found only on subtidal sandy-muddy bottoms (Lardicci, 1989). *Spiophanes mediterraneus* distribution is restricted to the Eastern Basin (Israel) (Meißner, 2005) and the Aegean coasts of Turkey (Çinar *et al.*, 2014) in bathyal soft bottoms while the other two species, *S. afer* and *S. algidus*, have a more recent history in the Mediterranean. *Spiophanes afer* was firstly described from the Mediterranean Spanish coasts and subsequently recorded in Israel, Turkey (Dağlı, 2008; Dağlı *et al.*, 2011), Sea of Marmara (Dağlı, 2013) and Adriatic Sea (Mikac, 2015), although the last author also suggests a possible misidentification with the widespread *S. kroyeri*. *Spiophanes algidus*, originally described from the Southern Ocean, has been recently reported only along the Aegean coasts of Turkey and added to the list of alien species of the Mediterranean Sea (Çinar *et al.*, 2011; Dağlı *et al.*, 2011; Zenetos *et al.*, 2017).

Spiophanes species, like other Spionidae, are typical surface deposit feeders having a pair of long peristomial tentacles or palps that are used for selecting particles (Rouse & Pleijel, 2001). They live in burrows formed by fragile mucoid secretion (Day, 1967). This genus differs from the other genera by having large crook-like hooks in the first neuropodium, body subdivided into three different regions and absence of branchiae.

The classification of the *Spiophanes* species underwent revisions and integrations, with reevaluation of diagnostic characters (Pettibone, 1962; Laubier, 1964; Imajima, 1991; Blake



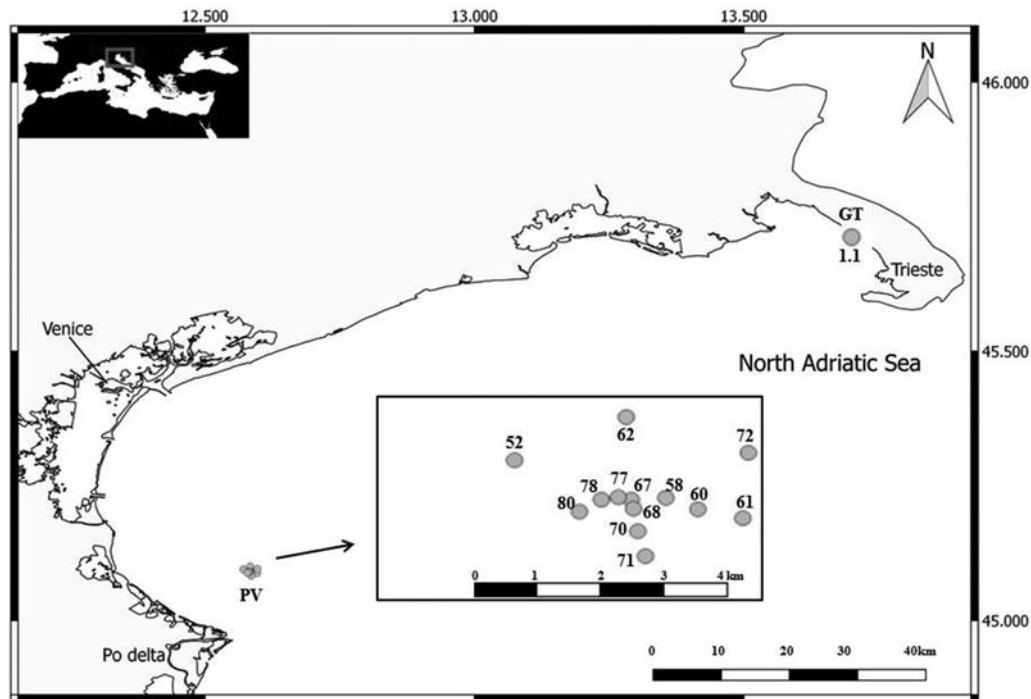


Fig. 1. Maps with sampling points.

et al., 1996; Maciolek, 2000). In a recent review of the genus, Meißner & Hutchings (2003) described a new diagnostic character for the identification of species: the 'chaetal spreader'. This character is related to glandular openings present in the middle body region (usually in chaetiger 4–8/9) of *Spiophanes*. Five different shapes of glandular organs were described: '2 + 3 type' with an undulate glandular opening; the '1 + 2 type' with an undulate glandular opening; the '0 + 1 type' with semicircular or subtriangular glandular opening; the '0 + 1 type' with an undulate glandular opening; and '0 + 1 type' with a simple horizontal glandular opening. The numbers refer to the lobes arising from anterior plus posterior margins of the gland opening (Meißner & Hutchings, 2003). *Spiophanes tcherniai* is actually the only species of the genus where gland opening and chaetal spreader do not appear (Meißner & Hutchings, 2003).

Even if phenotypic analysis is the main approach for polychaete taxonomy (Musco *et al.*, 2005; Tovar-Hernández *et al.*, 2007; D'Alessandro *et al.*, 2014), it is currently accepted that, due to polymorphism diversity and high levels of homoplasy, species were often overlooked (Westheide & Schmidt, 2003). For this reason, in the last decades DNA barcode has been considered a well-accepted taxonomic method for discrimination of closely related species (Barroso *et al.*, 2010; Nygren & Pleijel, 2010; Carr *et al.*, 2011). Based on the assumption that intraspecific genetic divergence is always lower than interspecific divergence (Herbert *et al.*, 2003), the widely used mitochondrial Cytochrome c Oxidase Subunit I (COI) facilitates species identification. In this article a combination of morphological and DNA barcoding analysis was carried out to describe the new species *Spiophanes adriaticus* sp. nov. from the Adriatic Sea.

Material and methods

Sampling and taxonomic analyses

Samples of *Spiophanes adriaticus* sp. nov. were collected in November 2016 and July 2017, in two areas of the northern Adriatic Sea: Miramare Marine Reserve (Gulf of Trieste) and Po

River Delta offshore (Figure 1). Coordinates, depth, sediment type and number of specimens belonging to *Spiophanes adriaticus* sp. nov. are reported in Table 1.

Spiophanes adriaticus sp. nov. was found in 14 out of 42 sediments collected in the two sampling areas. Sediments were taken by van Veen grab (0.1 m² sampling surface). Samples were sieved on a 1.0 mm mesh and were immediately fixed in ethanol 95% and subsequently preserved in ethanol 90%. Specimens were observed by means of a Zeiss discovery 2.0 stereomicroscope (8 × 120 magnification) equipped with Zeiss, AxioCam ERC 5s software and a Leica DM 2500 optical microscope (Figures 2 & 3). Methyl green and Shirlastain A were used to observe some diagnostic characters as length of ciliated thin bands, dorsal crests, chaetal spreader and staining pattern. For 20 specimens, the length from the prostomial tip to chaetiger 10 (L10 in mm), the width at chaetiger 10 excluding parapodia (W10 in mm) as well as the main morphological characters were assessed and are reported in Table 2. All the measures were taken with AxioCam ERC 5s. Drawings were made by means of a drawing tablet and Krita software (Figure 4).

Granulometric analysis

At each sampling location, aliquots of top layer sediment (10–15 g) were collected for the grain size analyses. Small pebbles and shell fragments were separated from other fractions by sieving at 2 mm. Each sample was disaggregated and the organic matter oxidized using 10% hydrogen peroxide (Carlo Erba, Rodano, Italy) at 60°C for 24 h. The analyses were performed using a Malvern Mastersizer 2000 equipped with Hydro 2000s (Malvern Instruments Ltd, Malvern, UK). Data are expressed as percentages of sand, silt and clay.

Molecular analyses

Three specimens of *Spiophanes adriaticus* sp. nov. were preserved in EtOH (90%) for DNA extraction. DNA extraction was conducted using PureLink™ Genomic DNA Mini Kit (Invitrogen).

Table 1. Locations, sampling details, sediment characteristics of sampling stations and occurrence of specimens (N)

Area	Site	Latitude (°N)	Longitude (°E)	Site code	Depth (m)	N	%Sand	%Silt	%Clay
Off-shore Po Delta	52	45°05'42.14"	12°34'16.37"	PV_52	26.5	2	78.6	12.0	9.4
Off-shore Po Delta	58	45°05'25.33"	12°35'17.31"	PV_58	27	5	85.9	7.9	6.2
Off-shore Po Delta	60	45°05'20.24"	12°35'30.21"	PV_60	26.9	4	74.6	14.4	11.0
Off-shore Po Delta	61	45°05'16.19"	12°35'48.34"	PV_61	26.8	3	75.5	13.9	10.6
Off-shore Po Delta	62	45°06'01.44"	12°35'01.37"	PV_62	26.9	4	69.1	17.8	13.1
Off-shore Po Delta	67	45°05'24.46"	12°35'03.39"	PV_67	27.5	8	85.5	7.8	6.7
Off-shore Po Delta	68	45°05'20.45"	12°35'04.26"	PV_68	27.1	5	79.5	11.2	9.3
Off-shore Po Delta	70	45°05'10.37"	12°35'06"	PV_70	26.7	7	77.7	12.2	10.1
Off-shore Po Delta	71	45°04'59.23"	12°35'09.10"	PV_71	26.8	2	71.1	15.7	13.2
Off-shore Po Delta	72	45°05'45.46"	12°35'50.56"	PV_72	27.9	3	69.5	17.7	12.8
Off-shore Po Delta	77	45°05'25.58"	12°34'58.15"	PV_77	27.5	5	84.9	8.5	6.6
Off-shore Po Delta	78	45°05'24.50"	12°34'51.32"	PV_78	27.5	15	80.2	11.2	8.6
Off-shore Po Delta	80	45°05'19.12"	12°34'42.46"	PV_80	27	15	72.8	15.1	12.1
Gulf of Trieste	1.1	45°42'16.15"	13°42'38.50"	GT_1.1	8	3	80.1	11.1	8.8

The mitochondrial cytochrome c oxidase I (COI) gene was amplified using LCO1490 and HCO2198 (Folmer *et al.*, 1994). PCR reactions (25 µl) contained 2 µl DNA template, reaction buffer, 1.0 mM MgCl₂, 250 µM of each dNTP, 10 pmol of each primer, and 0.5 U Red TAq Polymerase. The PCR temperature profile consisted of the following steps: initial denaturation at 94°C for 1 min; 35 cycles of 30 s at 94°C, 30 s at 50°C and 1 min at 72°C; and 5 min at 72°C. The amplified products were resolved on a 1% agarose gel purified using a QIAquick PCR purification Kit (QIAGEN) and sequenced on an ABI PRISM 310 automated sequencer (Applied Biosystems). DNA sequences were multiply aligned and compared with *Spiophanes* spp. present in Bold System and GenBank using CLUSTAL X alignment software (Thompson *et al.*, 1994; Larkin *et al.*, 2007) on a default setting and thereafter the sequence alignment was manually rechecked to allow an interpretation of the observed genetic distances. Multiple outgroup COI sequences from GenBank belonging to Spionidae family were analysed in order to select the most closely related taxon to *Spiophanes* genus to be used as outgroup and *Prionospio steenstrupi* was shown to be the most reliable to solve phylogenetic relationships (GenBank accession number GU672396). Mean genetic divergence values within and among different species were calculated using Kimura 2-parameter model as implemented in MEGA vers 6 (Tamura *et al.*, 2013). For phylogenetic tree reconstruction we used Neighbour-joining (NJ) and Maximum parsimony (MP) method. The robustness of the phylogenies obtained by NJ and MP were tested by bootstrap analysis (Felsenstein, 1985) with 1000 replications. All specimens are deposited in the Polychaeta collection of National Institute of Oceanography and Experimental Geophysics (OGS).

Results

Systematics

Class POLYCHAETA Grube, 1850
 Order SPIONIDA sensu Rouse & Fauchald, 1997
 Family SPIONIDAE Grube, 1850
 Genus *Spiophanes* Grube, 1860
Spiophanes adriaticus sp. nov. (Figures 2–4)

Type material

Holotype entire with 70 chaetigers LT: 17.15 mm; L10: 2.25 mm; W10: 0.84 mm, code PV_68.3.

Paratypes code: Gulf of Trieste, 3 specimens (GT_1.1); Po River Delta, 2 specimens (PV_52); Po River Delta, 5 specimens (PV_58); Po River Delta, 4 specimens (PV_60); Po River Delta, 3 specimens (PV_61); Po River Delta, 4 specimens (PV_62); Po River Delta, 8 specimens (PV_67); Po River Delta, 5 specimens (PV_68); Po River Delta, 7 specimens (PV_70); Po River Delta, 2 specimens (PV_71); Po River Delta, 3 specimens (PV_72); Po River Delta, 5 specimens (PV_77); Po River Delta, 15 specimens (PV_78); Po River Delta, 15 specimens (PV_80).

Description. Prostomium subtriangular, bell-shaped, with short blunt anterolateral projections, two plump palps up to about 10th chaetiger and a cirriform occipital antenna (Figures 2A, B, 4A). Two pairs of black eyes present (Figures 2A, 4A). Peristomium moderately developed as lateral bulges (Figures 2A, 4A). Nuchal organs as two thin bands, divergent in the first parapodium and running parallel along the dorsum, to about chaetigers 11–12 (Figures 2A, 4A). Dorsal ciliated crests from chaetiger 16. Parapodia of chaetigers 1 to 4 oriented dorsolaterally (Figures 2C, 4A). Prechaetal lamellae of chaetiger 1 cirriform oriented dorsally; postchaetal lamellae cirriform with a wide base, prechaetal and postchaetal lamellae equal in length (Figure 2C). Prechaetal lobe of chaetiger 2 triangular with slender tip, postchaetal lamellae sublunate with small cirriform tip (Figure 2C). Prechaetal lobe of chaetigers 3 and 4 triangular with a slender tip, postchaetal lamellae sublunate with a rounded tip (Figure 2C, D). Chaetigers from 5 to 8 with reduced notopodial lamellae triangular with rounded tip, neuropodial lamellae rounded reduced in size (Figure 2C). From chaetiger 9 up to 13, notopodial lamellae with small triangular base and slender tip; neuropodial lamellae rounded (Figures 2E, 3B). In chaetiger 14 notopodial lamellae triangular with slender tip, longer than those of the previous chaetiger, neuropodial lamellae rounded. From chaetiger 15 to 18 notopodial lamellae ovoid with slender tip, longer than those of the previous chaetiger, neuropodia lamellae rounded (Figure 2F). From chaetiger 19 notopodial lamellae smaller than previous ones, ovoid with long slender tip; neuropodial lamellae smaller than previous ones rounded.

Chaetal spreader of the '2 + 3 type' from chaetiger 5 to 7, with undulate glandular opening well developed on chaetigers 5–7 (Figure 2G); glandular organs in chaetigers 9–14 open as lateral vertical slits (Figure 2H). Ventrolateral intersegmental pouches present from between chaetigers 14–15 (Figure 2I). Bacillary chaetae

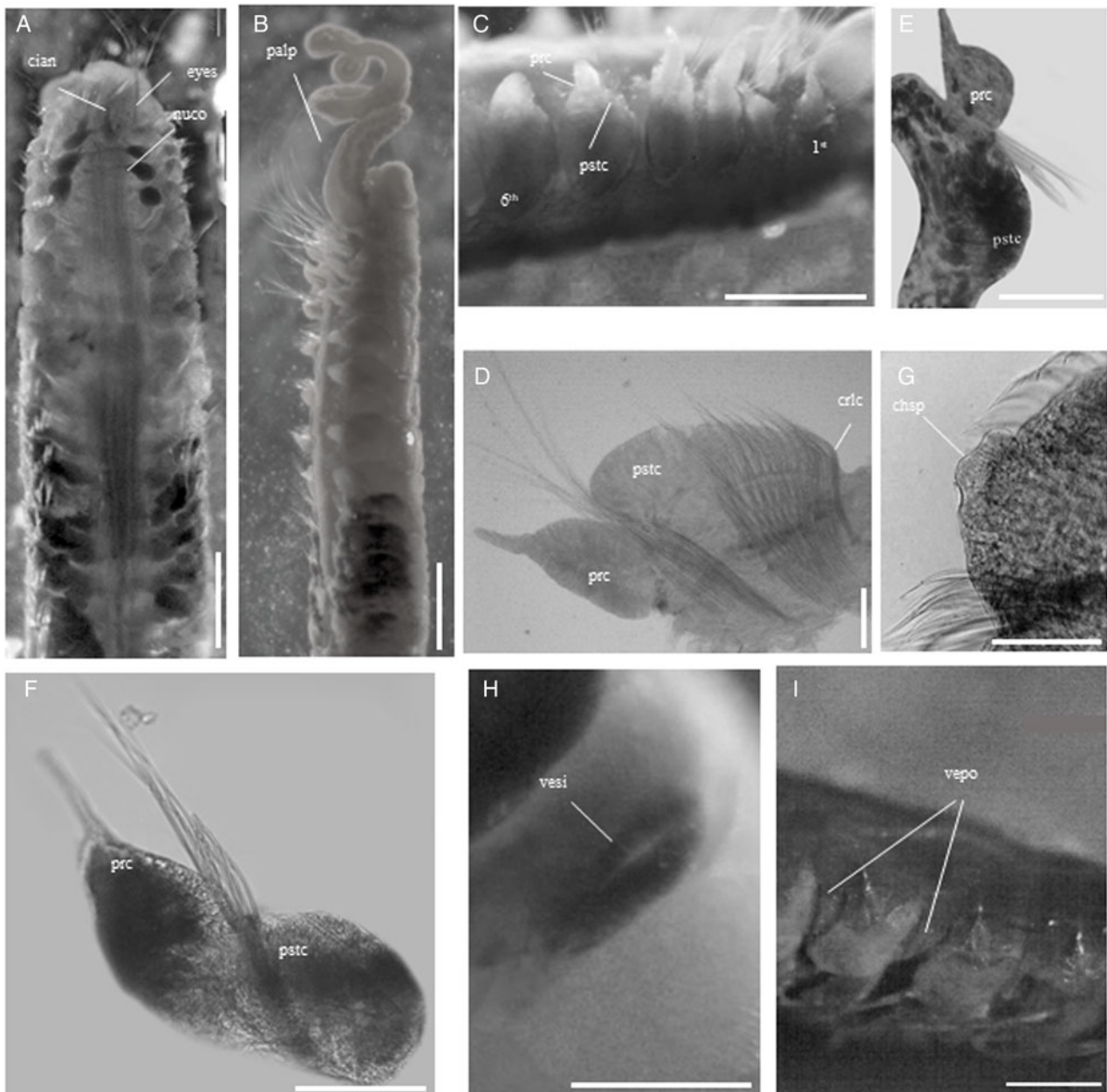


Fig. 2. *Spiophanes adriaticus* sp. nov.: (A) anterior end, dorsal view; (B) anterior end, lateral view; (C) parapodia from chaetigers 1 to 6, lateral view; (D) parapodium 4th, frontal view; (E) parapodium 11th, frontal view; (F) parapodium 16th, frontal view; (G) chaetal spreader of 5th parapodium; (H) vertical slit from 11th parapodium; (I) ventrolateral intersegmental pouches of 16th parapodium, ventral view. Scale bar: (A); (B); (C) 500 μ m; (D); (E); (F) 200 μ m; (G) 50 μ m; (H); (I) 200 μ m. Legend: chsp, chaetal spreader; cyan, cirriform occipital antenna; crlc, crook-like chaeta; nuco, nuchal organs; prc, prechaetal lobe; pstc, postchaetal lobe; vepo, ventrolateral intersegmental pouches; vesi, vertical slit. Holotype (PV_68.3): (B); (C); (D); (E); (F); (G); (H); (I) Paratype (PV_80.1): (A).

were not observed. Notochaetae of first chaetiger capillary, neurochaetae with 1 stout, crook-like chaeta (Figure 3A) in addition to capillary and long limbate chaetae (Figure 3B). In parapodia 2 and 3, notochaetae capillary and neurochaetae bilimbate. Notochaetae of chaetiger 4 capillary; neurochaetae bilimbate with 1 crook-like chaeta (Figure 2D). Notochaetae of first four parapodia longer than neurochaetae and longer than the following ones (Figure 2B, C). Notochaetae 5–14 of two types: long capillary limbate and short capillary limbate (Figure 3B). Neurochaetae in neuropodia 4–14 bilimbate with one stout curved (90°) crook-like chaeta (Figures 2D, 3C). From chaetiger 14, capillaries with narrow sheath in notopodia, arranged in tufts (Figure 2F). Notopodia of chaetiger 4–18 with long and short notopodial bilimbate chaetae. In neuropodia, from chaetiger 15 neurochaeta presented 7 hooks without hoods, with a broadening in subapical region (Figure 3D), and 1 stout pointed neurochaeta, slightly curved.

Hooks progressively decreasing in number along body, becoming 3 in the second last neurochaeta and 2 in the last one, still accompanied by 1 stout pointed chaeta. From chaetiger 18, capillary chaetae in the notopodia. In the posterior region (from 60° chaetiger), curved notopodial chaetal with slender tip (Figure 2E). Pygidium with 3 pairs of cirri in terminal part (Figure 3F).

Tube formed by an agglomeration of grain sand, Y shaped (Figures 3G, H, 4C). The tube has an internal chitinous structure composed of two pieces: a brownish shield with pointed arched wings and a smaller arched cross (Figures 3G, H, 4D). This particular structure is present along the tube and could have a support function.

Methyl green staining pattern. Inconspicuous.

Distribution and habitat. Currently known only from the North Adriatic Sea, specifically within the Gulf of Trieste and offshore

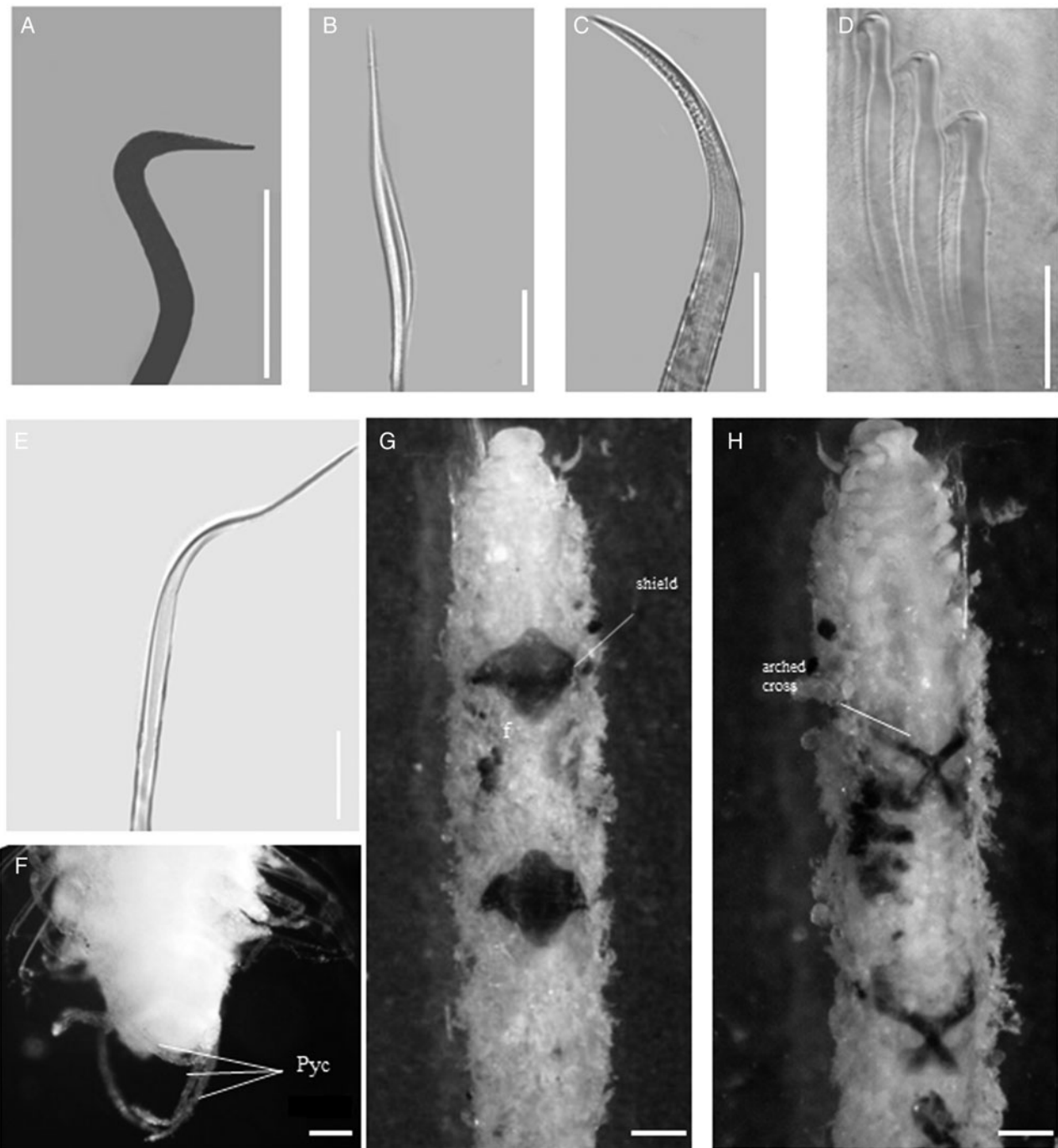


Fig. 3. *Spiophanes adriaticus* sp. nov.: (A) stout, crook-like chaeta from 1st parapodium; (B) short bilimbate chaeta from parapodium 6th; (C) crook-like chaeta from parapodium 4th; (D) hooks without hood from parapodium 15th; (E) capillary chaeta from parapodium 20th; (F) pygidium; (G) tube with shield, ventral view; (H) tube with arched cross, dorsal view. Scale bar: (A, B, C, E): 200 μ m; (D): 50 μ m; (F): 100 μ m; (G, H): 500 μ m. Holotype (PV_68.3): (A, B, C, D, E, F). Paratype (PV_80.1): (G, H). Legend: pyc, pygidial cirri.

the Po delta, in sandy, silty-sandy sediments of the sublittoral zone (between 8 and 27.5 m of depth).

Pigmentation. Conspicuous dark brownish pigmentation on parapodia in chaetigers 9–13 encompasses notopodium and neuropodium, a slightly dark region observable along ventral interramal region in chaetiger 1 to 7 (sometimes to 6).

Molecular analyses. The sequence size has a variation of 550–600 base pairs (bp). Alignment was conducted with *S. berkeleyorum*, *S. bombyx*, *S. kimballi*, *S. kroyeri*, *S. norrisi* and *S. uschakowi* for a total of 46 sequences. Some sequences ascribed to *S. bombyx* specimens were excluded from our analyses due to a species

misidentification (Meißner & Blank, 2009). Of 536 aligned base pairs, 222 (41%) resulted as variable, of which 178 (33%) were informative and 44 singleton (8%). The mean genetic divergence within species ranged from 0.0–0.56%, while the pairwise genetic divergence between species ranges from 0.021 between *S. bombyx* and *S. uschakowi* and 0.239 between *S. bombyx* and our specimens (Table 3). Phylogenetic tree constructed under NJ and MP showed a similar topology (Figure 5). The NJ tree showed well-supported distinct clades corresponding with discrete species; *Spiophanes* specimens from the Adriatic Sea (present study) grouped together forming a specific distinct unit, close to *S. kimballi* and *S. berkeleyorum* and *S. kroyeri*. From this group, *S. norrisi*, *S. bombyx* and *S. uschakowi* branched off.

Table 2. Comparison of morphological and morphometric characters in 20 specimens of *Spiophanes adriaticus* sp. nov. collected in two areas (PV and GT) of the Adriatic Sea

	L10 (length in mm from prostomium to chaetiger 10)	W10 (width in mm at chaetiger 10 excluding parapodia)	Shape of prostomium	Number of stout crook-like chaetae on 1st chaetiger	Dorsal ciliated crests from chaetiger	Ventrolateral intersegmental pouches from between chaetiger	Pigment colour in chaetigers 9–13	Nuchal organs extending to chaetiger
PV_52.2	2.795	1.150	Dep	1	17	14	Dark brown	12
PV_60.2	3.149	1.045	Bell	1	16	14	Dark brown	11
PV_60.3	2.363	0.661	Bell	1	16	14	Dark brown	12
PV_67.2	2.070	0.785	Bell	1	14	15	Dark brown	12
PV_67.5	2.690	0.835	Bell	1	16	14	Dark brown	12
PV_68.1	2.507	0.786	Bell	1	16	14	Dark brown	9
PV_68.5	2.057	0.753	Bell	1	14	14	Dark brown	11
PV_70.7	3.184	1.064	Dep	1	14	14	Dark brown	12
PV_711	2.021	0.794	Dep	1	14	14	Light brown	11
PV_72.1	2.525	0.770	Bell	1	14	14	Dark brown	11
PV_72.3	2.180	0.599	Bell	2	16	13	Dark brown	12
PV_77.5	2.799	0.823	Bell	1	15	13	Dark brown	12
PV_78.2	2.825	0.873	Dep	1	15	14	Dark brown	–
PV_78.3	1.825	0.619	Dep	–	16	14	Light brown	12
PV_80.1	2.890	0.958	Bell	2	17	14	Dark brown	12
PV_80.2	2.010	0.559	Bell	1	17	14	Dark brown	12
PV_80.3	2.390	0.826	Bell	1	17	15	Dark brown	12
GT_1.1.1	2.809	0.946	Bell	–	15	14	Dark brown	–
GT_1.1.2	2.563	0.715	Bell	1	15	14	Dark brown	12
GT_1.1.3	2.184	0.817	Bell	–	17	15	Dark brown	12

Bell, bell-shaped with short antero-lateral projections; Dep, bell-shaped anteriorly depressed into two frontal lobes; –, not clear visible in the specimen.

Table 3. Estimates of molecular divergence among species calculated with Kimura 2-parameter model

	<i>adriaticus</i>	<i>kroyeri</i>	<i>norrisi</i>	<i>kimballi</i>	<i>berkeleyorum</i>	<i>bombyx</i>	<i>uschakowi</i>
<i>adriaticus</i>							
<i>kroyeri</i>	0.2254						
<i>norrisi</i>	0.2325	0.1842					
<i>kimballi</i>	0.2379	0.1718	0.2107				
<i>berkeleyorum</i>	0.2074	0.1848	0.2062	0.1682			
<i>bombyx</i>	0.2390	0.1872	0.0726	0.2251	0.2273		
<i>uschakowi</i>	0.2383	0.1923	0.0800	0.2378	0.2347	0.0216	

Remarks. The paratypes showed some intraspecific morphological variability. In particular, the prostomium is bell-shaped with short anterolateral projections or anteriorly depressed into two frontal lobes, chaetiger 1 bears 1 or 2 stout crook-like chaetae, the first appearance of dorsal ciliated crests ranged from the 14th to the 17th chaetiger, ventrolateral intersegmental pouches present from between chaetiger 13–14 to between chaetiger 15–16, pigmentation in chaetigers 9–13 varies from light brown to dark brown, nuchal organs extend to chaetigers 9–12. All the specimens showed 4 eyes and chaetal spreader developing from chaetiger 5. No specimen was observed with gametes. As for morphometric characters, L10 ranged from 1.825–3.184 mm with a mean value of 2.480 mm, while W10 ranged from 0.559–1.150 mm with a mean value of 0.820 mm.

Etymology. *Spiophanes adriaticus* sp. nov. refers to the occurrence of the species along Italian coasts and offshore of North Adriatic Sea.

Discussion

Spiophanes adriaticus sp. nov. differs from the other species of *Spiophanes* by having a combination of the following characters: two pairs of black eyes, a cirriform occipital antenna, nuchal organs usually extending to chaetigers 11–12 (rarely to chaetiger 9), dorsal ciliated crests from chaetigers 14–17, chaetal spreader '2 + 3 type', undulate glandular opening, ventrolateral intersegmental pouches from between chaetigers 14–15 (rarely 13–14 or 15–16), hooks without hood from 15th chaetiger and Y shaped tubes.

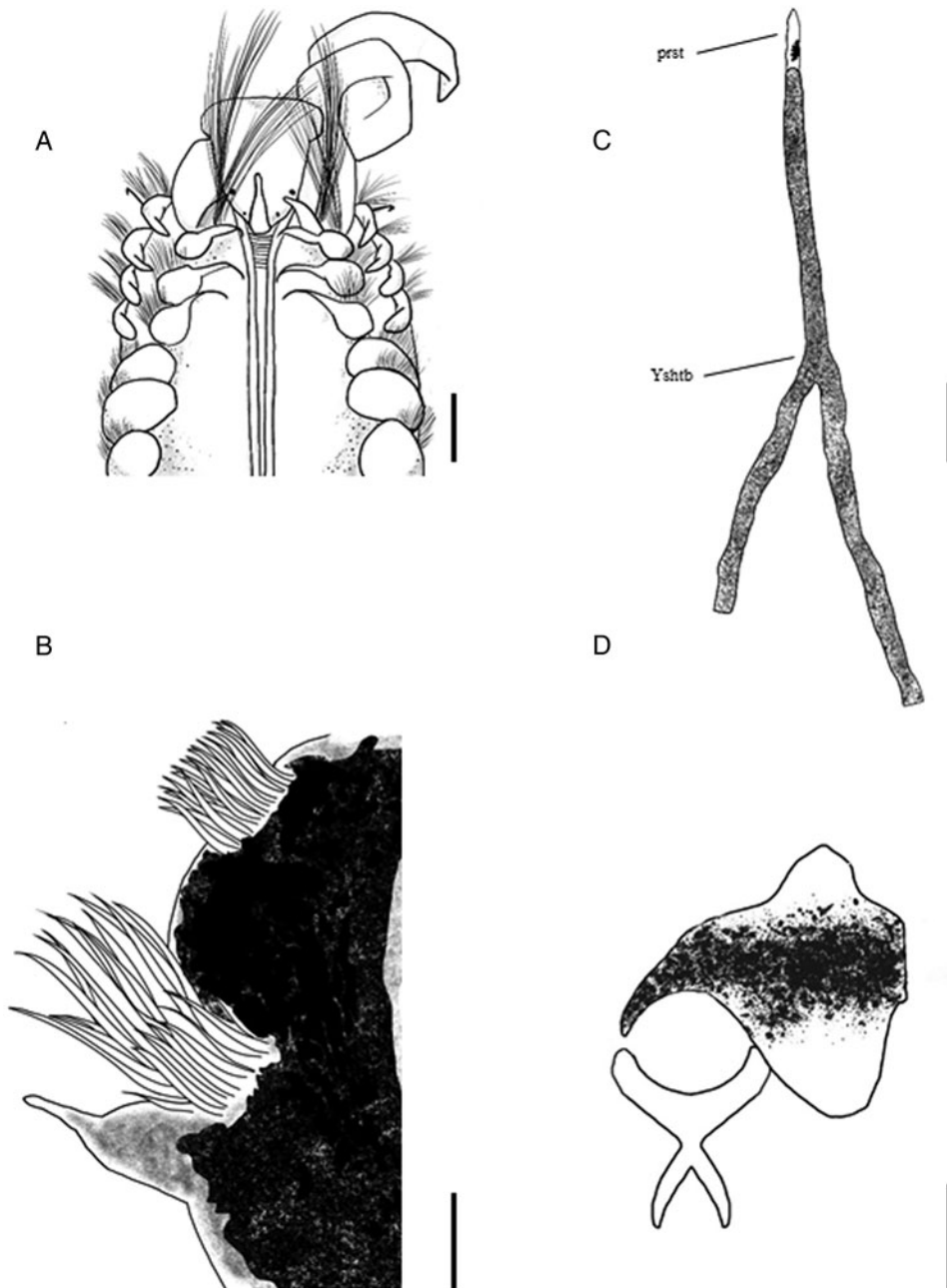


Fig. 4. *Spiophanes adriaticus* sp. nov.: (A) anterior end, dorsal view; (B) parapodia 10th, frontal view; (C) tube, dorsal view; (D) shield, three-dimensional view. Scale bar (A), (B), (C) 200 µm; (D) 500 µm. Legend: prst, prostomium; Yshtb, Y shaped tubes.

There are only five species of *Spiophanes* with chaetal spreader '2 + 3 type': *S. afer*, *S. duplex*, *S. kimballi*, *S. modestus* and *S. viriosus*. *Spiophanes afer* differs from *Spiophanes adriaticus* sp. nov. by the absence of eyes in adult specimens, length of dorsal ciliated organs extending to about chaetigers 13–15 and the shape of parapodia. In addition, in *S. afer* dorsal ciliated crests appear moderately developed from chaetigers 18–19. *Spiophanes afer* also shows fair pigment (pinkish to light brown) developed on chaetigers 14–15 and glandular areas dorsally on parapodia of chaetigers 9–14, more intensively visible after staining with methyl green. *Spiophanes duplex* differs from *Spiophanes adriaticus* sp. nov. in lacking occipital antenna and ventrolateral intersegmental pouches, in having dorsal ciliated organs extending to about chaetigers 13–14 or, alternatively, diverging and converging on chaetigers 15–17 usually diverging at the end, in having dorsal ciliated crests distinct from chaetigers 17–18, pygidium with two lateral anal cirri, and in showing

pigmentation on chaetigers 9–14. *Spiophanes modestus* differs from *Spiophanes adriaticus* sp. nov. for the presence of distinct prostomial horns, short occipital antenna, dorsal ciliated organs becoming divergent and undulate between chaetigers 15–17, dorsal ciliated crests apparent from chaetiger 15, neuropodial hooks without hood from 14th parapodia, for the absence of ventrolateral intersegmental pouches, pygidium with 2 dorsolateral anal cirri and for absence of conspicuous pigmentation. *Spiophanes viriosus* differs from *Spiophanes adriaticus* sp. nov. for the presence of distinct prostomial horns, for nuchal organs as two parallel lines running until parapodia 17–18 and slightly diverging at the end, dorsal ciliated crests apparent from chaetiger 18, and for colouration on vertical slit of parapodia 9–12. *Spiophanes kimballi* differs from *Spiophanes adriaticus* sp. nov. for the presence of only one pair of eyes, for the absence of ventrolateral intersegmental pouches (except in gravid specimens), dorsal ciliated crests visible from chaetiger 17, for

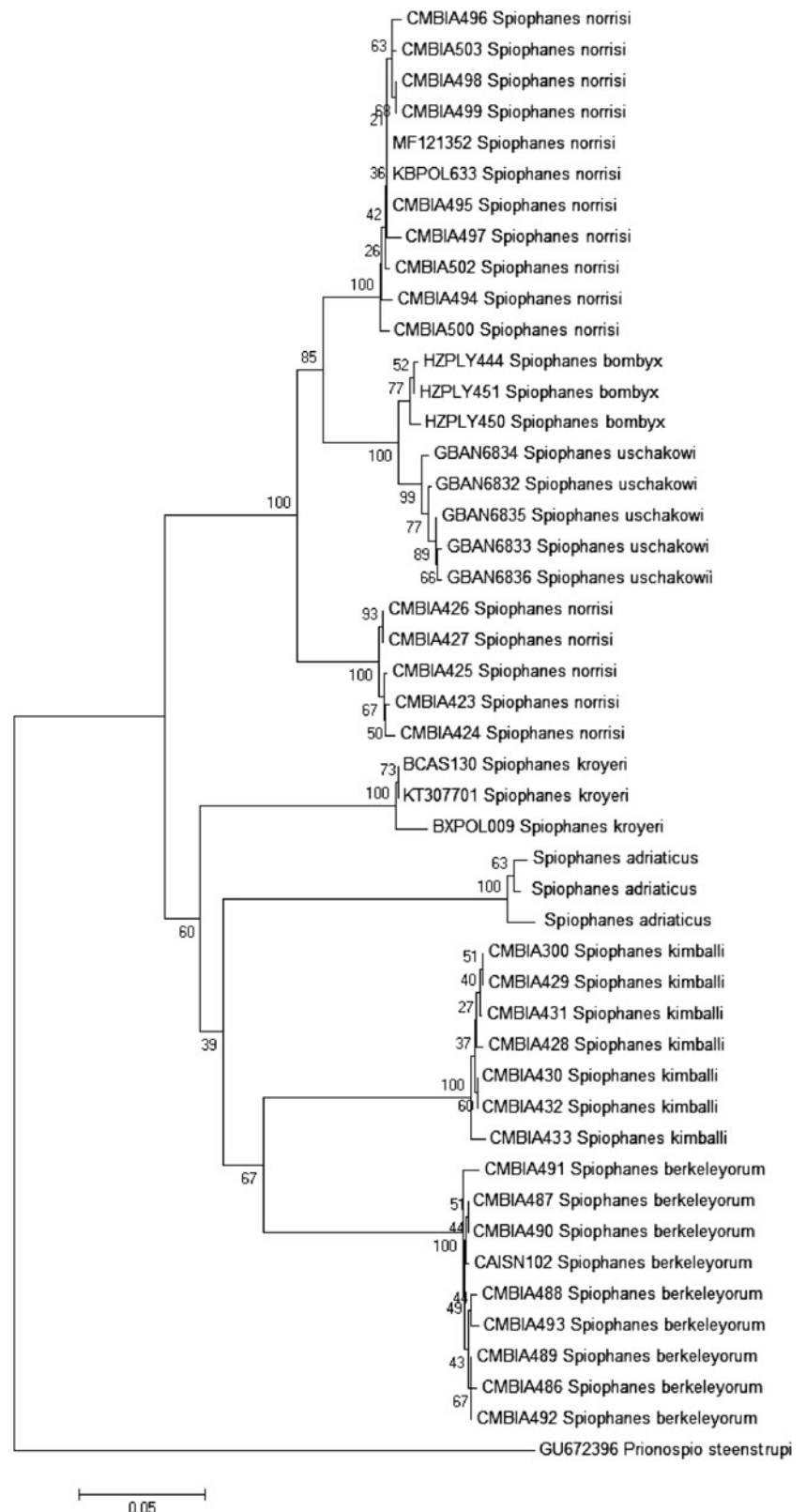


Fig. 5. Neighbour-joining tree based on Kimura 2-parameter distance. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches.

pigmentation more distinct on chaetigers 11–13 and different methyl green staining pattern.

From an ecological point of view, *Spiophanes adriaticus* sp. nov. inhabits subtidal sandy and silty-sandy bottoms, but we cannot exclude its occurrence deeper and in other substrates. Among the *Spiophanes* species found in the Mediterranean, *Spiophanes adriaticus* sp. nov. shares its depth range with *S. afer*, *S. bombyx*, *S. wigleyi*, as well as *S. kroyeri* and *S. reysyi*, although the last two species can also be found down to bathyal bottoms.

Species belonging to *Spiophanes* genus are difficult to identify and many possible mistakes were recently reported by several studies, underlining that some intraspecific variations, linked to diagnostic features, could lead to erroneous classification (Meißner & Blank, 2009; Mikac, 2015). Our data indicate that the values of sequence divergence within and between species agree with the assumption of the barcode gap, strengthening the effectiveness of COI for species identification. Further in all the pairwise comparisons of *S. adriaticus* sp. nov. the

sequence divergence values are the highest ones obtained; this result, together with the monophyletic clade shown in the phylogenetic tree and the morphological features, support the validity of the new species *Spiophanes adriaticus* sp. nov.

With this new finding the number of *Spiophanes* species recorded for the Mediterranean Sea increases to eight, emphasizing that the species richness within polychaetes is ever-increasing, thanks both to the improvement of observation techniques and the use of molecular analyses (Dağlı *et al.*, 2011; Mikac, 2015; D'Alessandro *et al.*, 2016).

An integrated taxonomic approach based on traditional morphological characters together with molecular marker species identification is needed to fully delineate species boundaries,

quantifying diversity, and clarifying geographic distributions in the polychaetes considered (Di Camillo *et al.*, 2018). DNA barcoding has proved to be a useful method for species identification and consequently for rapid global biodiversity assessment (Hebert *et al.*, 2003). Regarding polychaetes, Meyer & Paulay (2005) and Carr *et al.* (2011) supported the strength of COI for species identification as well as for the detection of overlooked species, underlining the importance of molecular analyses as a support to morphological taxonomic research. A broader application of the integrated approach in polychaetes taxonomy with the subsequent implementation of a DNA barcode biodiversity library could rapidly increase taxonomic and biodiversity knowledge on the group, identifying cryptic species as well as solving species complexes.

Key to Mediterranean species of *Spiophanes*

1. Occipital antenna absent, neuropodial hooks from chaetiger 15 with reduced hood, ventrolateral intersegmental pouches absent, dorsal ciliated organs short at most to chaetiger 4 2
 - Occipital antenna present, neuropodial hooks from chaetiger 15 without hood, ventrolateral intersegmental pouches present or absent, ciliated organs long 3
2. Prostomium with long anterolateral horns. Usually 2 pairs of eyes present. Dorsal ciliated organs as two wide short dorsal bands to the end of chaetiger 2. Dorsal ciliated crests from chaetiger 3. Reddish pigment in neuropodia of the middle body region sometimes in chaetigers 15–18 *Spiophanes bombyx* (Claparède, 1870)
 - Prostomium without horns. Usually 2 pairs of eyes present. Dorsal ciliated organs as a pair of dorsal loops extending to chaetiger 4. Dorsal ciliated crests from chaetigers 15 or 16. Notopodial postchaetal lamellae of chaetigers 9–15 with reddish-brown pigment in proximal region, darkest on chaetigers 12–15 *Spiophanes wigleyi* Pettibone, 1962
3. Chaetal spreader of the '0 + 1 type' 4
 - Chaetal spreader of the '2 + 3 type' 7
4. Presence of glandular organ opening (vertical slit) in chaetiger 8. Up to 4 red eyes present in juvenile specimens. Dorsal ciliated crests distinct from chaetigers 15–19 *Spiophanes reyssii* Laubier, 1964
 - Absence of glandular organ opening (vertical slit) on chaetiger 8 opening 5
5. Eyes absent. Dorsal ciliated organs as two thin bands to about chaetiger 13. Ventrolateral intersegmental pouches present from between chaetigers 14–15. Dorsal ciliated crests from about chaetiger 18. Dark brown pigment around glandular opening on parapodia of chaetigers 9–14 *Spiophanes mediterraneus* Meißner, 2005
 - Ventrolateral intersegmental pouches present from between chaetigers 15–16 6
6. Eyes absent. Dorsal ciliated organs to chaetigers 14–16. First four parapodia oriented dorsally, anterior postchaetal lamellae foliaceous. Dorsal ciliated crests from chaetigers 16–18. Light brown pigment in 1–7 postchaetal lamellae; brown pigment in neuropodia of chaetigers 7–14, most conspicuous in chaetigers 8–11 *Spiophanes kroyeri* Grube, 1860
 - Eyes absent. Dorsal ciliated organs to about chaetigers 14–15. Only first parapodium dorsally oriented, anterior neuropodial postchaetal lamellae subulate to subtriangular, notopodial postchaetal lamellae long cirriform. Dorsal ciliated crests distinct from chaetigers 17–18. Grey to brownish pigment laterally on parapodia of chaetigers 9–15, best seen on chaetigers 12–14 *Spiophanes algidus* Meißner, 2005
7. Eyes usually absent. Dorsal ciliated organs to chaetigers 13–15. First and second parapodium in dorsal position, 3rd and 4th in dorsolateral to lateral position. Fully developed ventrolateral intersegmental pouches present from between chaetigers 15–16. Dorsal ciliated crests distinct from chaetigers 18–19. Dark brown pigment on parapodia of chaetigers 9–13, faint brownish or pink pigment on chaetigers 14–15 *Spiophanes afer* Meißner, 2005
 - Two pairs of black eyes present. Dorsal ciliated organs to chaetigers 9–12 divergent in first two parapodia. Parapodia 1 to 4 oriented dorsolaterally. Ventrolateral intersegmental pouches present from between chaetigers 14–15, rarely 13–14 or 15–16. Dorsal ciliated crests from chaetiger 14–17. Dark brown pigment on neuropodia of chaetigers 9–13, dark pigment along ventral interramal region in chaetigers 1–7. *Spiophanes adriaticus* sp. nov. D'Alessandro *et al.*, 2018

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References

Barroso R, Klautau M, Solé-Cava AM and Paiva PC (2010) *Eurythoe complanata* (Polychaeta: Amphinomidae), the 'cosmopolitan' fireworm, consists of at least three cryptic species. *Marine Biology* 157, 69–80.

Blake JA, Hilbig B and Scott PH (eds) (1996) *Taxonomic Atlas of the Benthic Fauna of the Santa Maria Basin and the Western Santa Barbara Channel*. Volume 6. The Annelida Part 3. Polychaeta: Orbiniidae to Coszuridae. Santa Barbara, CA: Santa Barbara Museum of Natural History, 418 pp.

Carr CM, Hardy SM, Brown TM, Macdonald TA and Hebert PD (2011) A tri-oceanic perspective: DNA barcoding reveals geographic structure and cryptic diversity in Canadian polychaetes. *PLoS ONE* 6, e22232.

Çinar ME, Bilecenoglu M, Ozturk B, Katagan T, Yokes M, Aysel V, and Erdogan H (2011) An updated review of alien species on the coasts of Turkey. *Mediterranean Marine Science* 12, 257–315.

Çinar ME, Dağlı E and Şahin GK (2014) Checklist of Annelida from the coasts of Turkey. *Turkish Journal of Zoology* 38, 734–764.

Claparède É (1870) Les annélides chétopodes du Golfe de Naples. *Memoires de la Société de physique et d'histoire naturelle de Genève* 20, 365–542.

D'Alessandro M, Cosentino A, Giacobbe S, Andaloro F and Romeo T (2014) Description of a new species of Abyssoninoe (Polychaeta:

- Lumbrineridae) from north-east Sicily (central Mediterranean Sea). *Journal of the Marine Biological Association of the United Kingdom* **94**, 747–752.
- D'Alessandro M, Romeo T, Castriota L, Cosentino A, Perzia P and Martins R** (2016) New records of Lumbrineridae (Annelida: Polychaeta) in the Mediterranean biogeographic province, with an updated taxonomic key. *Italian Journal of Zoology* **83**, 233–243.
- Dağlı E** (2008) *Taxonomy and Ecology of Spionidae (Polychaeta- Anneliida) Distributed Along the Sublittoral Area of the Turkish Coast of the Aegean Sea* (PhD thesis). Ege University, İzmir, Turkey.
- Dağlı E** (2013) Two new records for the Polychaeta fauna of the Sea of Marmara: *Laubieriellus salzi* and *Spiophanes afer* (Polychaeta: Spionidae). *Turkish Journal of Zoology* **37**, 376–379.
- Dağlı E, Çınar ME and Ergen Z** (2011) Spionidae (Annelida: Polychaeta) from the Aegean Sea (eastern Mediterranean). *Journal of the Marine Biological Association of the United Kingdom* **78**, 49–64.
- Day J** (1967) *A Monograph on the Polychaeta of Southern Africa: Part 2. Sedentaria*. London: Trustees of the British Museum (Natural History), no. 656, 878 pp.
- Di Camillo CG, Gravili C, De Vito D, Pica D, Piraino S, Puce S and Cerrano C** (2018) The importance of applying Standardised Integrative Taxonomy when describing marine benthic organisms and collecting ecological data. *Invertebrate Systematics* **32**, 794–802.
- Felsenstein J** (1985) Confidence limits on phylogenies: an approach using bootstrap. *Evolution* **39**, 783–791.
- Folmer O, Black M, Hoen W, Lutz R and Vrijenhoek R** (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**, 294–299.
- Grube AE** (1850) Die Familien der Anneliden. *Archiv für Naturgeschichte, Berlin* **16**, 249–364.
- Grube AE** (1860) Beschreibung neuer oder wenig bekannter Anneliden. Fünfter Beitrag. *Archiv für Naturgeschichte, Berlin* **26**, 71–118, plates III–V.
- Hebert PDN, Cywinska A, Ball SL and de Waard JR** (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London* **270**, 313–321.
- Imajima M** (1991) Spionidae (Annelida, Polychaeta) from Japan. VII. The Genus *Spiophanes*. *Bulletin of the National Science Museum, Tokyo, Series A* **17**, 115–137.
- Lardicci C** (1989) Censimento dei policheti dei mari italiani. Spionidae Grube, 1850. *Atti della Società Toscana di Scienze Naturali, Memorie, Serie B* **96**, 121–152.
- Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H and Thompson JD** (2007) Clustal W and Clustal X version 2.0. *Bioinformatics (Oxford, England)* **23**, 2947–2948.
- Laubier L** (1964) Un spionidien des vases bathyales de Banyuls-sur-Mer, *Spiophanes kroyeri reysii* ssp. n. *Bulletin de la Société zoologique de France* **89**, 562–577.
- Maciolek NJ** (2000) New species and records of *Aonidella*, *Laonice*, and *Spiophanes* (polychaeta: Spionidae) from shelf and slope depths of the Western North Atlantic. *Bulletin of Marine Science* **67**, 529–547.
- Meißner K** (2005) Revision of the genus *Spiophanes* (Polychaeta, Spionidae); with new synonymies, new records and descriptions of new species. *Zoosystematics and Evolution* **81**, 3–65.
- Meißner K and Blank M** (2009) *Spiophanes norrisi* sp. nov. (Polychaeta: Spionidae) a new species from the NE Pacific coast, separated from the *Spiophanes bombyx* complex based on both morphological and genetic studies. *Zootaxa* **2278**, 1–25.
- Meißner K and Hutchings PA** (2003) *Spiophanes* species (Polychaeta: Spionidae) from Eastern Australia with descriptions of new species, new records and an emended generic diagnosis. *Records of the Australian Museum* **55**, 117–140.
- Meyer CP and Paulay G** (2005) DNA barcoding: error rates based on comprehensive sampling. *PLOS Biology* **3**, 2229–2238.
- Mikac B** (2015) A sea of worms: polychaete checklist of the Adriatic Sea. *Zootaxa* **3943**, 1–172.
- Musco L, Çınar ME and Giangrande A** (2005) A new species of *Sphaerosyllis* (Polychaeta, Syllidae, Exogoninae) from the coasts of Italy and Cyprus (eastern Mediterranean Sea). *Italian Journal of Zoology* **72**, 161–166.
- Nygren A and Pleijel F** (2010) From one to ten in a single stroke – resolving the European *Eumida sanguinea* (Phyllocladidae, Annelida) species complex. *Molecular Phylogenetics and Evolution* **58**, 132–141.
- Pettibone MH** (1962) New species of polychaete worms (Spionidae: *Spiophanes*) from the east and west coast of North America. *Proceedings of the Biological Society of Washington* **75**, 77–88.
- Rouse GW and Pleijel F** (2001) *Polychaetes*. Oxford: Oxford University Press, 354 pp.
- Tamura K, Stecher G, Peterson D, Filipksi A and Kumar S** (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* **30**, 2725–2729.
- Thompson JD, Higgins DG and Gibson TJ** (1994) Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**, 4673–4680.
- Tovar-Hernández MA, Licciano M and Giangrande A** (2007) Revision of *Chone Krøyer*, 1856 (Polychaeta: Sabellidae) from the eastern central Atlantic and Mediterranean Sea with descriptions of two new species. *Scientia Marina* **71**, 315–338.
- Westheide W and Schmidt H** (2003) Cosmopolitan vs cryptic meiofaunal polychaete species: an approach to a molecular taxonomy. *Helgolander Marine Research* **57**, 1–6.
- Zenetos A, Çınar ME, Crocetta F, Golani D, Rosso A, Servello G, and Verlaque M** (2017) Uncertainties and validation of alien species catalogues: the Mediterranean as an example. *Estuarine, Coastal and Shelf Science* **191**, 171–187.