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Newly discovered associations between peritrich ciliates (Ciliophora: Peritrichia) and scale polychaetes (Annelida: Polynoidae and Sigalionidae) with a review of polychaete-peritrich epibiosis

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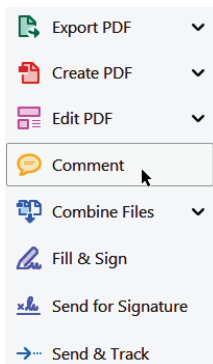
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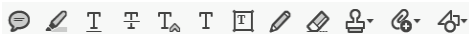
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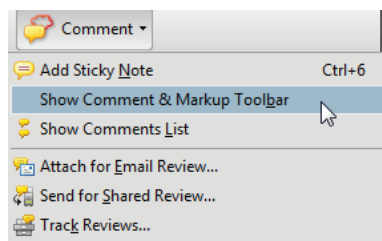


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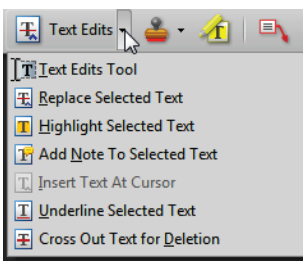


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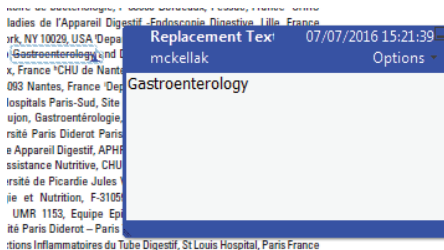
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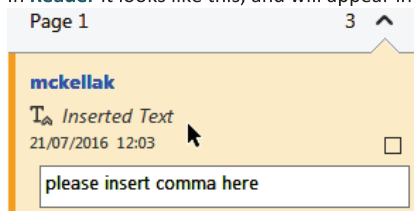


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Newly discovered associations between peritrich ciliates (Ciliophora: Peritrichia) and polychaetes (Polynoidae and Sigalionidae) with a review of polychaete–peritrich epibiosis

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In this research, we report the presence of two ciliate protozoans of the subclass Peritrichia, *Cothurnia amphicteis* and *C. peloscolicis*, as epibionts on the chaetae of scaled polychaetes *Malmgrenia lilianae*, *M. andreapolis* (fam. Polynoidae) and *Sthenelais boa* (fam. Sigalionidae), from the north Adriatic (Mediterranean Sea). Both ciliate species are herein found for the first time after their original description and are redescribed, based on light and scanning electron microscopy analyses. This is the first record of an association between ciliates and polychaetes of the family Sigalionidae. Our results suggest that these host–epibiont relationships might be highly specific. We also present the first review of epibiosis between polychaetes and peritrich ciliates, indicating that this relationship is more diverse than previously thought. Forty taxa of peritrich ciliates from 12 genera and seven families are recorded as epibionts on polychaetes, while 48 polychaete taxa are known as their hosts. The relationship can be considered ectocommensalism, where the ciliates have the advantages of increased food availability. This association might be a more widespread phenomenon than currently known, because it could be easily overlooked or misinterpreted. It, therefore, deserves careful attention and further investigation.

KEYWORDS: Adriatic – Annelida – *Cothurnia* – *Malmgrenia* – Mediterranean – Protozoa – redescription – scanning electron microscopy – *Sthenelais*.

INTRODUCTION

Epibiosis is the ecological association between a substrate organism, a basibiont, and a sessile organism, an epibiont, attached to the outer surface of the basibiont without trophic dependence on it (Wahl, 2009). Ciliate protozoans, particularly from the subclasses Peritrichia and Suctorina, are known

to establish epibiotic relationships with a variety of aquatic metazoans, such as Crustacea, Insecta, Gastropoda, Nematoda, Oligochaeta and Polychaeta (Alvarez-Campos *et al.*, 2014; Ansari *et al.*, 2017; Cabral *et al.*, 2018; Fernandez-Leborans & Tato-Porto, 2000; Sergeeva & Dovgal, 2014; Sartini *et al.*, 2018). Despite the high diversity and wide distribution of polychaetes in marine environments, records of polychaete–peritrich association are rare, and few papers deal with this relationship as their main topic (Magagnini & Verni, 1988; Alvarez *et al.*, 2014; Jankowski, 2014).

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In this paper, we report for the first time the presence of ciliate peritrichs *Cothurnia amphicteis* Lang, 1948 and *Cothurnia peloscolicis* Precht, 1935 on scaled polychaetes *Malmgrenia lilianae* (Pettibone, 1993), *M. andreapolis* McIntosh, 1874 (fam. Polynoidae) and *Sthenelais boa* (Johnston, 1833) (fam. Sigalionidae) from the north Adriatic (Mediterranean Sea). The representatives of the protozoan genus *Cothurnia* are loricate ciliates found in fresh, brackish and marine waters, and have a cosmopolitan distribution. The lorica is attached to aquatic animals, plants, algae or inanimate substrate by a non-contractile external stalk. In many species, the stalk appears to be smooth and comparatively featureless, while others possess lines or stripes that run longitudinally down the stalk (Warren & Paynter, 1991). Transverse folds or furrows may also be present on the stalk surface. The shape of the aperture of the lorica, and sculpture of both lorica and stalk, are the principal characters used for identification of *Cothurnia* species. Most descriptions of cothurnians are based on observations from only one direction, where the shape of the aperture of the lorica is not well visible, and often only drawings are provided, which may be prone to error or misinterpretation. The two *Cothurnia* species reported, were no longer observed after their first descriptions, until the present research (Precht, 1935; Lang, 1948). Moreover, some details, especially concerning the outer sculpture of cothurnian lorica and stalk, are only visible with the use of scanning electron microscopy.

Thus, we give herein a redescription of *C. amphicteis* and *C. peloscolicis* based on light and scanning electron microscopy.

Records of ciliate epibionts may be easily overlooked in papers dealing with polychaetes, thus this association might be more common than it appears to be. In order to give a full picture of the present knowledge of polychaete–peritrich ciliate epibiosis we present the first review of this association, based on a detailed analyses of the existing literature and new data.

MATERIAL AND METHODS

RESEARCH AREA

Samples of benthos were collected by the Centre for Marine Research (Ruđer Bošković Institute, Rovinj, Croatia) at three offshore stations in the north Adriatic Sea, from 2003 to 2008. Stations SJ005 (45°18.4' N; 13°18.0' E; 31 m depth) and SJ007 (45°17.0' N; 13°16.0' E; 31 m depth) are situated on the transect Poreč (Croatia)–Venice Lido (Italy), while station SJ107 (45°02.8' N; 13°19.0' E; 37 m depth) lies on the transect Rovinj (Croatia)–Po River Delta (Italy) (Fig. 1). All three stations are characterized by silty sand substrate.

FIELD AND LABORATORY WORK

Macrofaunal samples were taken with a Van Veen grab, sieved through a 1-mm mesh and fixed in 4% buffered

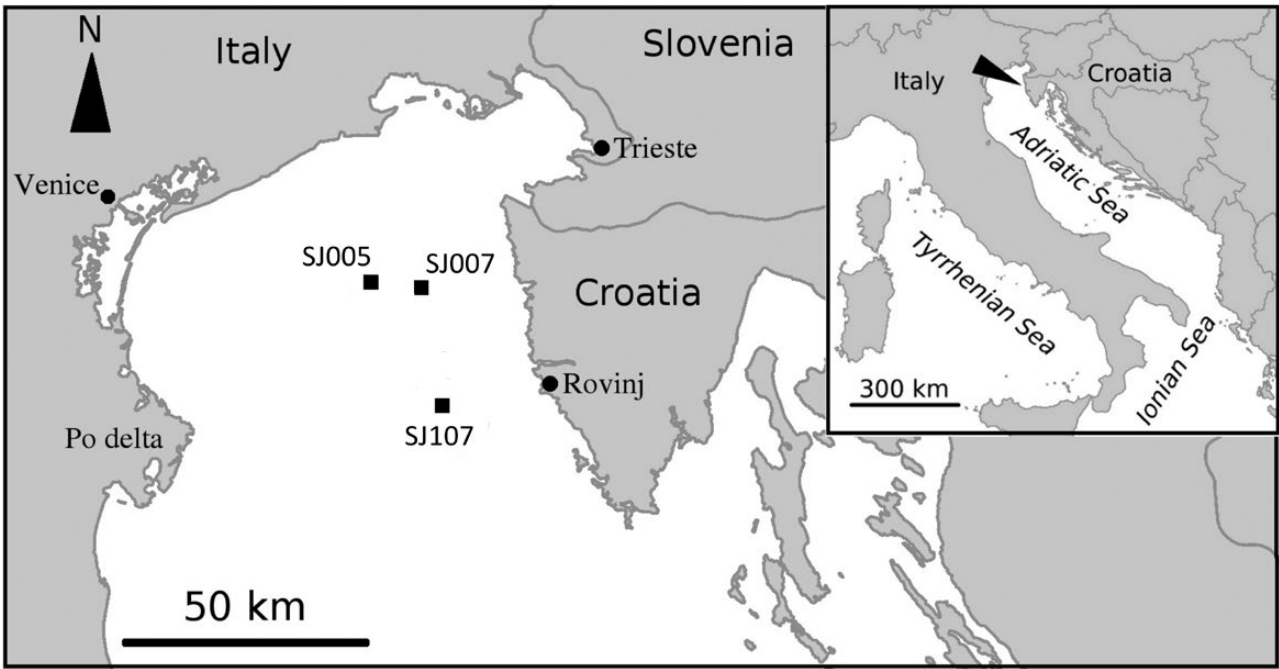


Figure 1. Map of sampling area and stations (SJ005, SJ007 and SJ107).

formaldehyde–seawater solution. After sorting in the laboratory, macrobenthic organisms were preserved in 70% ethanol. Polychaetes were identified to the species level using stereo- and light-microscopes. Those with ciliate epibionts were further studied and photographed by means of light-microscope and Scanning Electron Microscope (SEM, FEI 515). Light micrographs were done under a Zeiss Axiovert 100 microscope, using a Nikon Digital sight DS-Fi2 camera and NIS-Elements D 4.30.02 64-bit programme. Measurements of ciliates were taken from light-microscopy photos. For the SEM analyses, fixed specimens were washed in 0.1 M phosphate buffer (pH 7.4), dehydrated in a graded alcohol series until 100% (5 min for each solution), then dried with hexamethyldisilazane (HMDS), according to the method of [Hochberg & Litvaitis \(2000\)](#). Dry specimens were mounted on aluminium stubs, sputter-coated with gold palladium and finally observed with a Philips 515 SEM.

In order to give a review of polychaete–peritrich ciliate epibiosis, we examined the main literature dealing with polychaetes, ciliates and their association. Classification of peritrich ciliates follows [Lynn \(2008\)](#).

RESULTS

ASSOCIATION OF POLYCHAETES AND CILIATES FOUND IN THIS RESEARCH

Ciliate epibionts *Cothurnia amphicteis* were observed on 18 specimens of the polychaete *Malmgrenia lilianae* and two specimens of *M. andreapolis* (fam. Polynoidae). Ciliate epibionts *Cothurnia peloscolicis* were observed only on one specimen of the polychaete *Sthenelais boa* (fam. Sigalionidae). *Cothurnia amphicteis* was found attached on the middle to distal part of *Malmgrenia* chaetae, mostly on notochaetae, more rarely on neurochaetae ([Figs 2, 3](#)). *Cothurnia peloscolicis* was found attached on the lower-basal part of the upper (simple spinous) neurochaetae of *S. boa* ([Figs 5, 6A](#)). In general, one ciliate per chaeta was found and only in a few cases two. Ciliates were never found attached on body surfaces of polychaetes. Number of epibionts per basibiont ranged from a few to about 100.

TAXONOMIC ACCOUNT OF CILIATES

PHYLUM CILIOPHORA DOFLEIN, 1901

SUBPHYLUM INTRAMACRONUCLEATA LYNN, 1996

CLASS OLIGOHYMENOPHOREA DE PUYTORAC *ET AL.*, 1974

SUBCLASS PERITRICHIA STEIN, 1859

ORDER SESSILIDA KAHL, 1933

FAMILY VAGINICOLIDAE DE FROMENTEL, 1874

GENUS *COTHURNIA* EHRENBERG, 1831

Diagnosis

Marine, brackish or freshwater loricate peritrichs, usually with one or two zooids per lorica. Lorica borne on a stalk and attached to aquatic animals, plants or inanimate objects. Lorica without valves or other means of closing the aperture. Inner layer or septum sometimes present enclosing a space at the posterior end of the lorica; septum connected to the base of the lorica via a mesostyle. Zooid(s) attached to the base of the lorica (or septum) directly or via an endostyle ([Warren & Paynter, 1991](#)).

COTHURNIA AMPHICTEIS LANG, 1948

([Figs 2–4](#))

Material examined

Light-microscopy measurements based on six *Cothurnia* specimens found on the polychaete *Malmgrenia lilianae*, north Adriatic Sea, **station SJ107**, 21 June 2005. Additional material examined from the polychaete *M. lilianae*, north Adriatic Sea: **station SJ005**, 30 August 2005 (three polychaetes), 18 October 2006 (one polychaete); **station SJ007**, 15 March 2005 (one polychaete; mounted for SEM), 21 June 2005 (two polychaetes), 18 October 2006 (one polychaete), 13 September 2007 (one polychaete); **station SJ107**, 21 June 2005 (one polychaete; mounted for SEM), 22 December 2005 (two polychaetes), 13 September 2007 (two polychaetes, one mounted for SEM), 12 October 2007 (one polychaete). Additional material examined from the polychaete *M. andreapolis*, north Adriatic Sea: **station SJ005**, 22 September 2006 (one polychaete, mounted for SEM); **station SJ107**, 12 October 2007 (one polychaete).

Description

Lorica conical, smooth, with thin wall, 57–77 µm long (53–56 µm after [Warren & Paynter, 1991](#)) × 35 µm wide. Aperture circular 30–33 µm (35–37 µm after [Warren & Paynter, 1991](#)) in diameter, the edge of aperture with extremely small, irregular outgrowths ([Figs 2C, 4C](#)). External stalk conical, flexed near substrate ([Fig. 4A](#)), with an annular bulge in connection with lorica base and basal disc, 18–35 µm (54–57 µm after [Warren & Paynter, 1991](#)) long, with conspicuous transverse folds ([Fig. 4D](#)). Endostyle short, broad, with longitudinal striae that are not visible in some cases. Mesostyle absent. Generally, two zooids are present ([Figs 2D, 4B](#)). Zooid conical, 85 × 40 µm, and extends between one third and

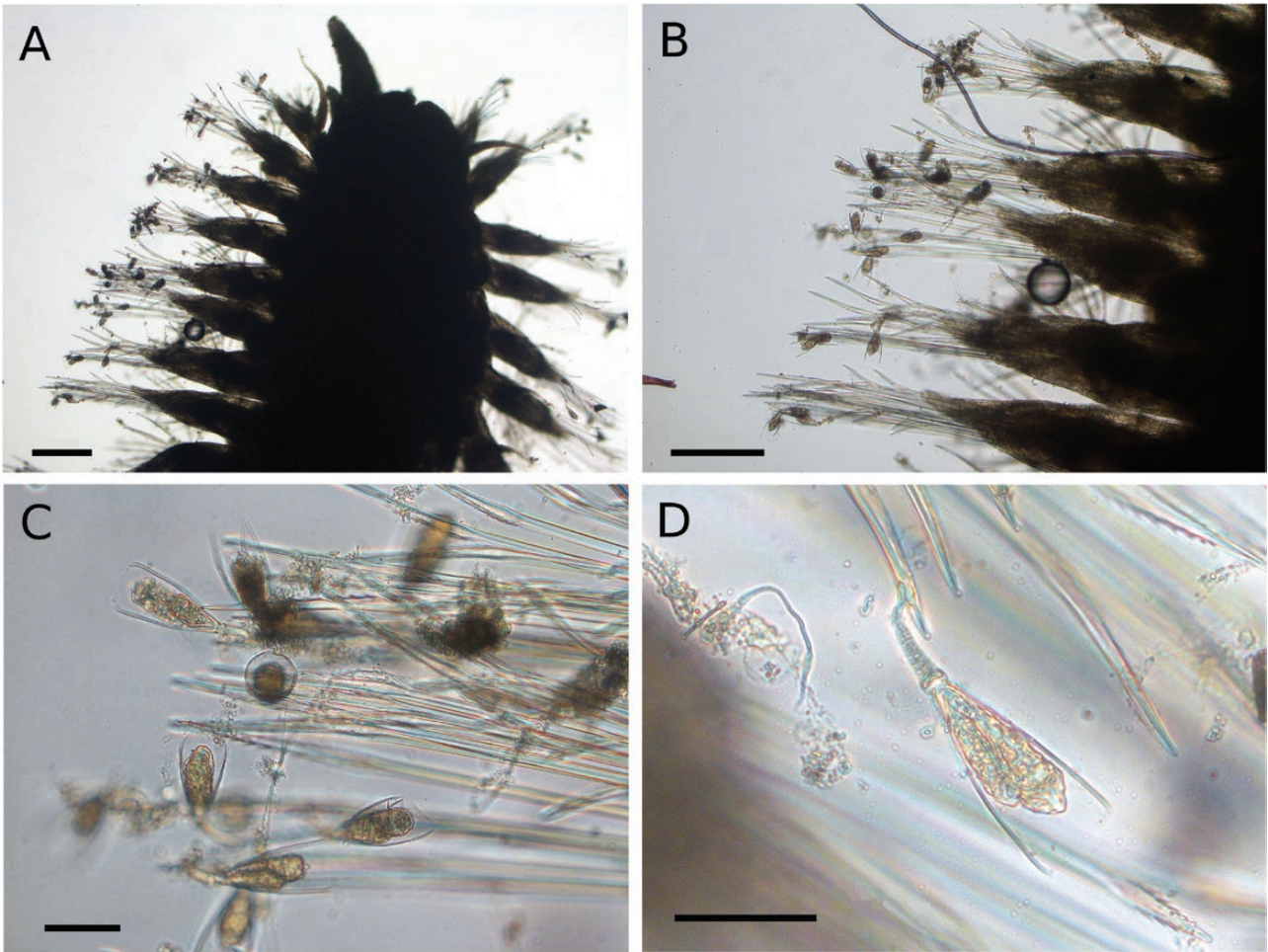


Figure 2. *Cothurnia amphicteis* on the chaetae of *Malmgrenia lilianae* (light microscopy). A, anterior part of *M. lilianae* showing densely attached ciliates. B, anterior chaetigers of *M. lilianae* with ciliates. C, anterior neurochaetae of *M. lilianae* with ciliates showing circular aperture of lorica. D, close-up photography of *C. amphicteis* showing two zooids. Scale bars: A = 250 μm , B = 200 μm and C, D = 50 μm .

one half of its length beyond aperture (Fig. 4B). The length of contracted zooids 31–59 μm , width 7–21 μm . Peristomial lip well-developed, 45 μm in diameter. Disc convex. Contractile vacuole lies just below peristome. Macronucleus elongate slightly curved anteriorly. Pellicular striations conspicuous.

Remarks

New specimens differ from the original description of *C. amphicteis* by the presence of two zooids in the lorica (with one exception) and the greater length of the lorica. Only contracted zooids were measured. The diagnosis of related species *C. acuta* Levander, 1915 (after Warren & Paynter, 1991) is rather similar to *C. amphicteis*. In both diagnoses, the absence of a mesostyle was mentioned. However, on Precht’s

(1935) picture, *C. acuta* has the typical mesostyle with longitudinal striae. In our specimens, the mesostyle was absent, thus we consider them *C. amphicteis*. The suggested synonymy between *C. acuta* and *C. amphicteis* needs further consideration. This is the first finding of the species after its original description by Lang (1948).

Habitat

Marine, originally found attached to the chaetae of polychaete *Amphicteis gunneri* (M. Sars, 1835) (fam. Ampharetidae) (type host) in coastal waters of Sweden, Baltic Sea (type locality) (Lang, 1948). Other locality: north Adriatic Sea, on the chaetae of polychaetes *Malmgrenia lilianae* and *M. andreapolis* (this paper).

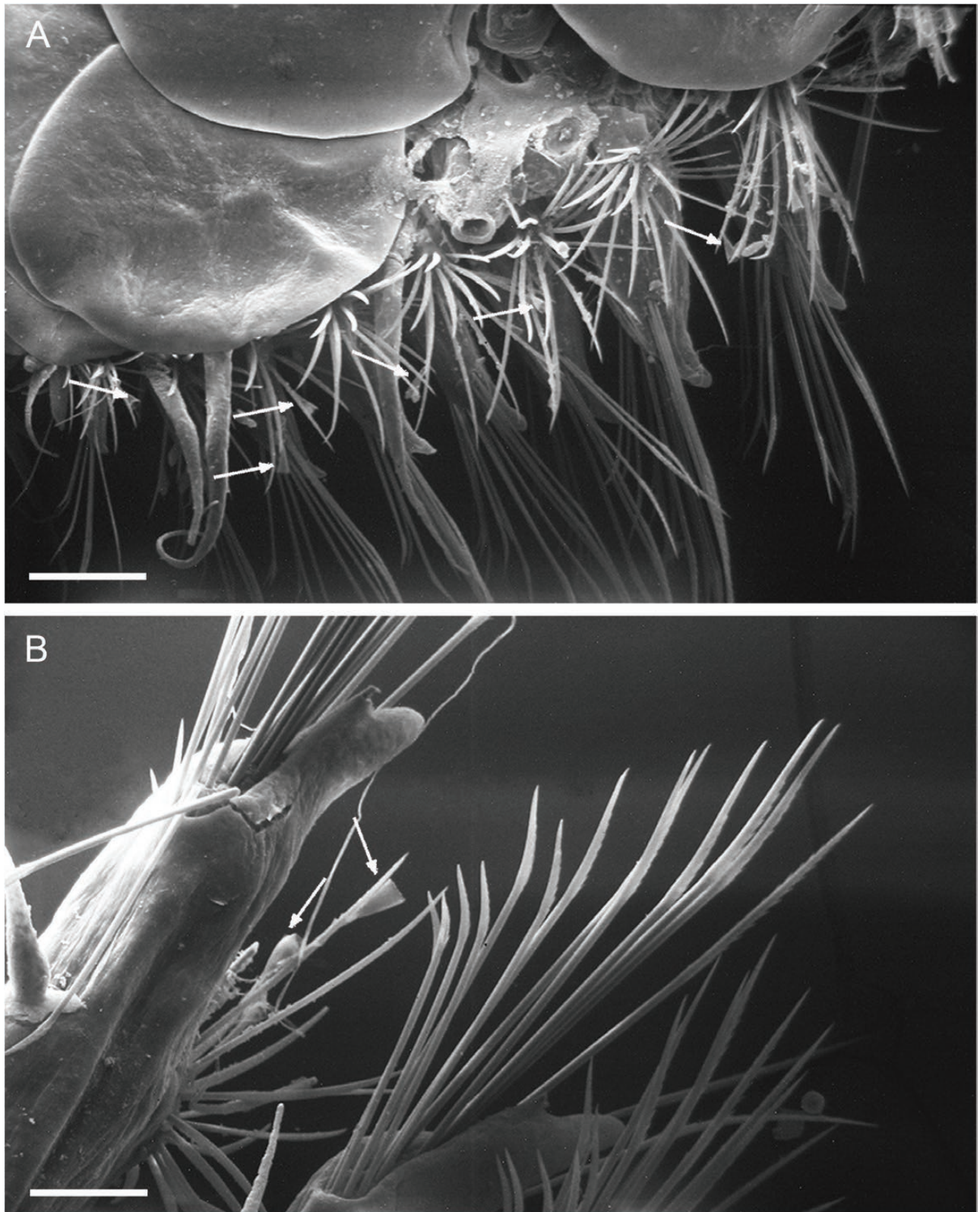


Figure 3. *Cothurnia amphiteis* on the chaetae of *Malmgrenia lilianae* (SEM). A, a part of *M. lilianae* body showing ciliates attached on the chaetae. B, close-up of one chaetiger with two specimens of *C. amphiteis* attached to the notochaeta. Arrows indicating ciliates. Scale bars: A = 280 μ m, B = 140 μ m.

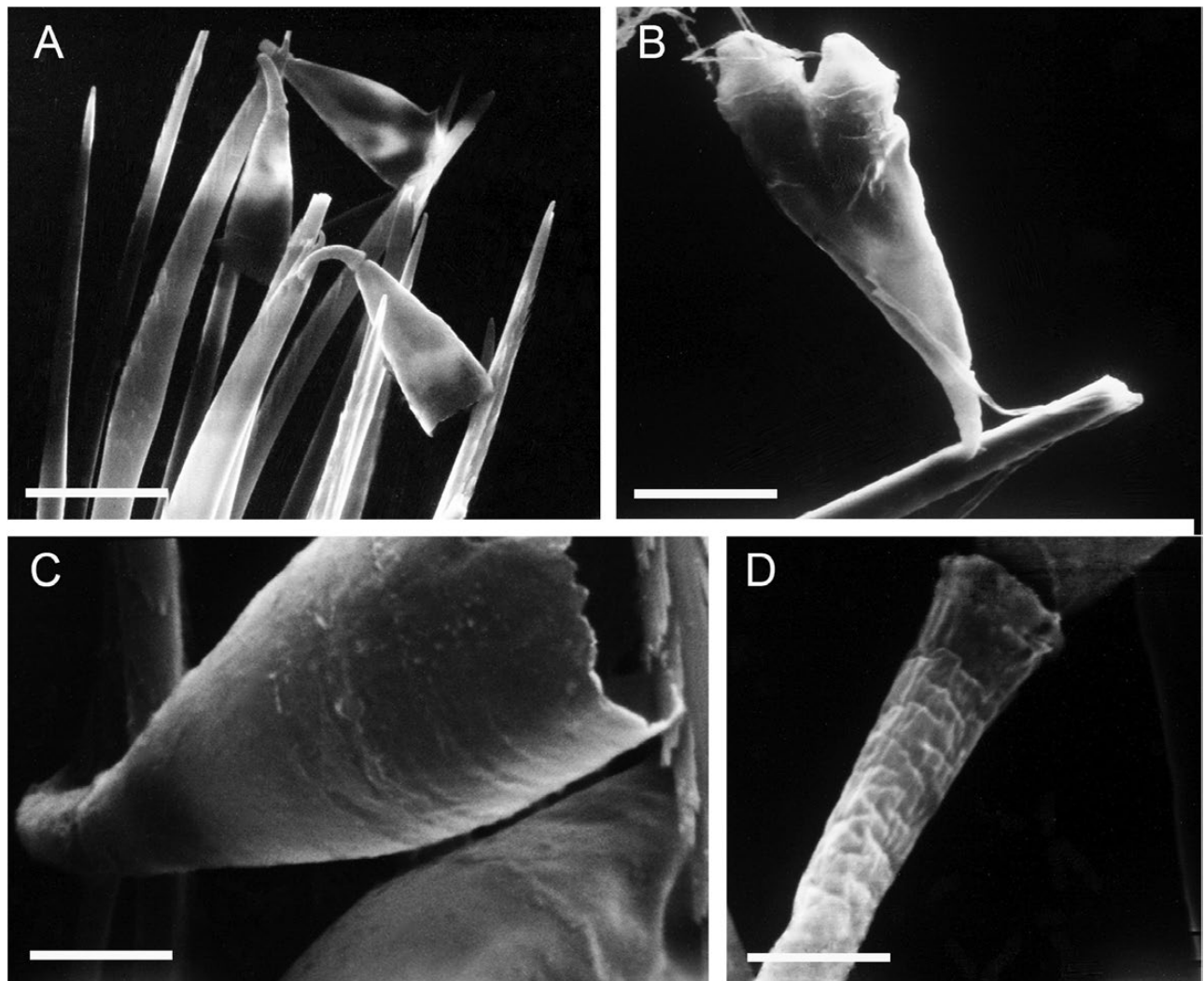


Figure 4. *Cothurnia amphicteis* on the chaetae of *Malmgrenia lilianae* (SEM). A, *C. amphicteis* attached to the notochaetae, showing stalk flexed near substrate. B, one specimen of *C. amphicteis* showing two zooids. C, detail of *C. amphicteis* lorica showing the edge of aperture with small, irregular outgrowths. D, detail of *C. amphicteis* stalk showing transverse folds. Scale bars: A = 60 μm , B = 45 μm , C = 20 μm , D = 12 μm .

COTHURNIA PELOSCOLICIS PRECHT, 1935

FIGS 5–6

Material examined

Material examined by light microscopy (measurements based on six *C. peloscolicis* specimens) and SEM microscopy from one specimen of polychaete *Sthenelais boa*, north Adriatic Sea, station SJ007, 5 March 2004.

Description

Lorica extended near the base and slightly converged toward aperture, smooth, not compressed, 61–86 μm (81 μm after Warren & Paynter, 1991) \times 26–32 μm

(27–41 μm after Warren & Paynter, 1991) (Figs 5B, 6C). Aperture circular when viewed from above, 23–32 μm in diameter (15 \times 33 μm after Warren & Paynter, 1991), the edge of aperture without any outgrow (Fig. 6B). External stalk short, cylindrical, without folds, 10–16 μm long (20 μm after Warren & Paynter, 1991); mesostyle and endostyle absent (Fig. 5B). Stalk with longitudinal striae in some cases. Generally, two zooids present (Fig. 5B). Zooid 80 \times 18–22 μm , extending just beyond aperture. The length of contracted zooid 26–55 μm , width 11–18 μm . Peristomial lip 27 μm in diameter. Contractile vacuole small and situated in the peristomal region. Macronucleus straight, 50 μm long. Pellicular striations inconspicuous.

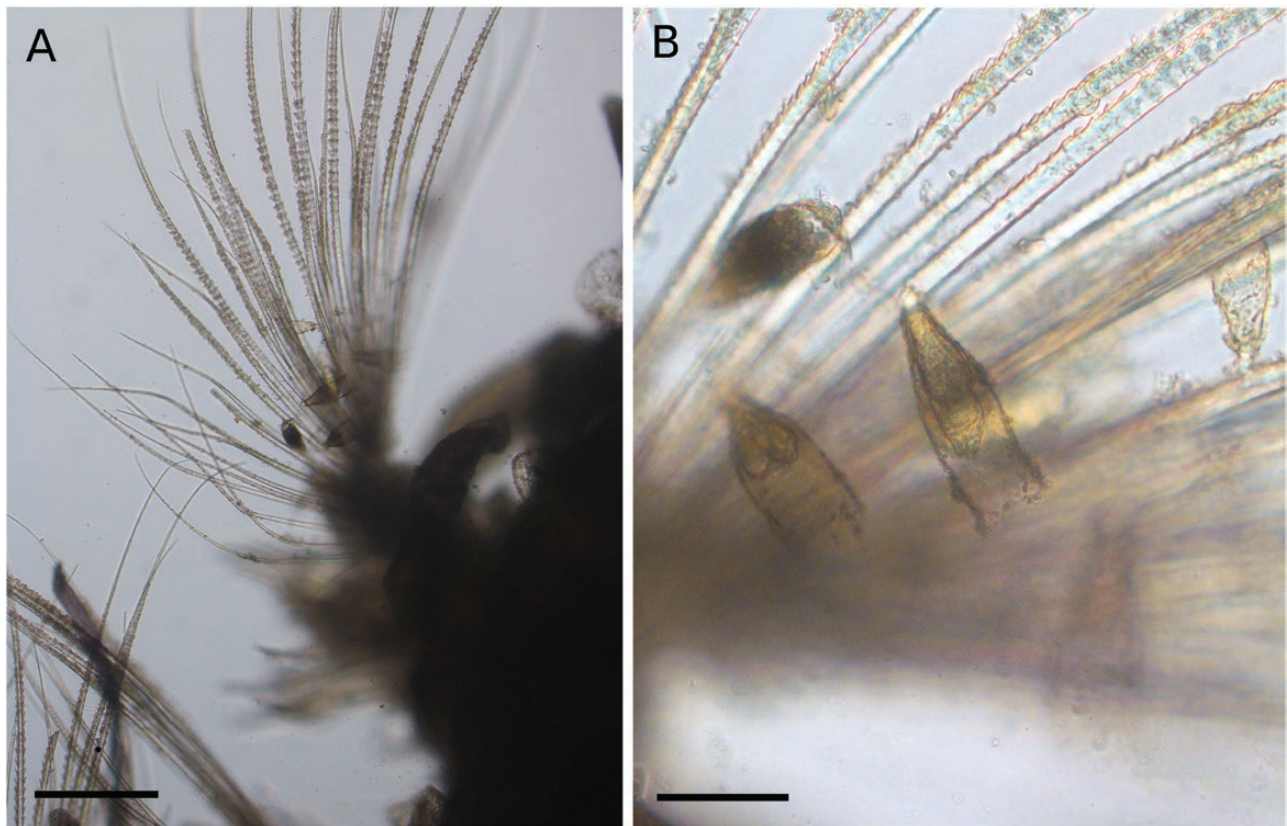


Figure 5. *Cothurnia peloscolicis* on the chaetae of *Sthenelais boa* (light microscopy). A, one chaetiger of *S. boa* with ciliates attached on the neurochaetae. B, close-up of neurochaetae with several ciliates showing short stalk and two zooids. Scale bars: A = 200 μ m, B = 50 μ m.

Remarks

This is the first finding of the species after its original description by [Precht \(1935\)](#).

Habitat

Marine, originally found as epibiont of the oligochaete *Tubificoides benedii* (d'Udekem, 1855) [reported as *Peloscolex benedeni* (d'Udekem, 1855); type host] from the Bay of Kiel, Germany (Baltic Sea) (type locality) ([Precht, 1935](#)). Other locality: north Adriatic Sea on the chaetae of polychaete *Sthenelais boa* (this paper).

EPISTYLIS SP.

Diopatra marocensis Paxton, Fadlaoui & Lechapt, 1995 (Fam. Onuphidae) – on the gills and on the first parapodia; Bay of Biscay, Spain, north-east Atlantic ([Arias et al., 2010](#)).

GENUS RHABDOSTYLA KENT, 1880

RHABDOSTYLA ARENICOLA [FABRE-DOMERGUE, 1888](#) AQ4
Arenicola marina (Linnaeus, 1758) (Fam. Arenicolidae) – on the branchial tufts; Concarneau, France, the Bay of Biscay, north-eastern Atlantic Ocean ([Fabre-Domergue, 1888](#)); on the gills, also on the body surface in the branchial region, especially ventrally, and on the slim posterior part of the body; Bay of Kiel, Germany, Baltic Sea ([Precht, 1935](#)); on the gills; Helgoland, Germany, Nord Sea ([Kahl, 1935](#)). AQ5

RHABDOSTYLA COMMENSALIS MOEBIUS, 1888

Capitella capitata (Fabricius, 1780) (Fam. Capitellidae) – on the body cuticle; Bay of Kiel, Germany, Baltic Sea ([Möbius, 1888](#)).

A REVIEW OF THE PERITRICH CILIATE EPIBIONTS ON POLYCHAETES

SUBCLASS PERITRICHIA STEIN, 1859

ORDER SESSILIDA KAHL, 1933

FAMILY EPISTYLIDIDAE [KAHL, 1935](#)

GENUS EPISTYLIS EHRENBERG, 1830

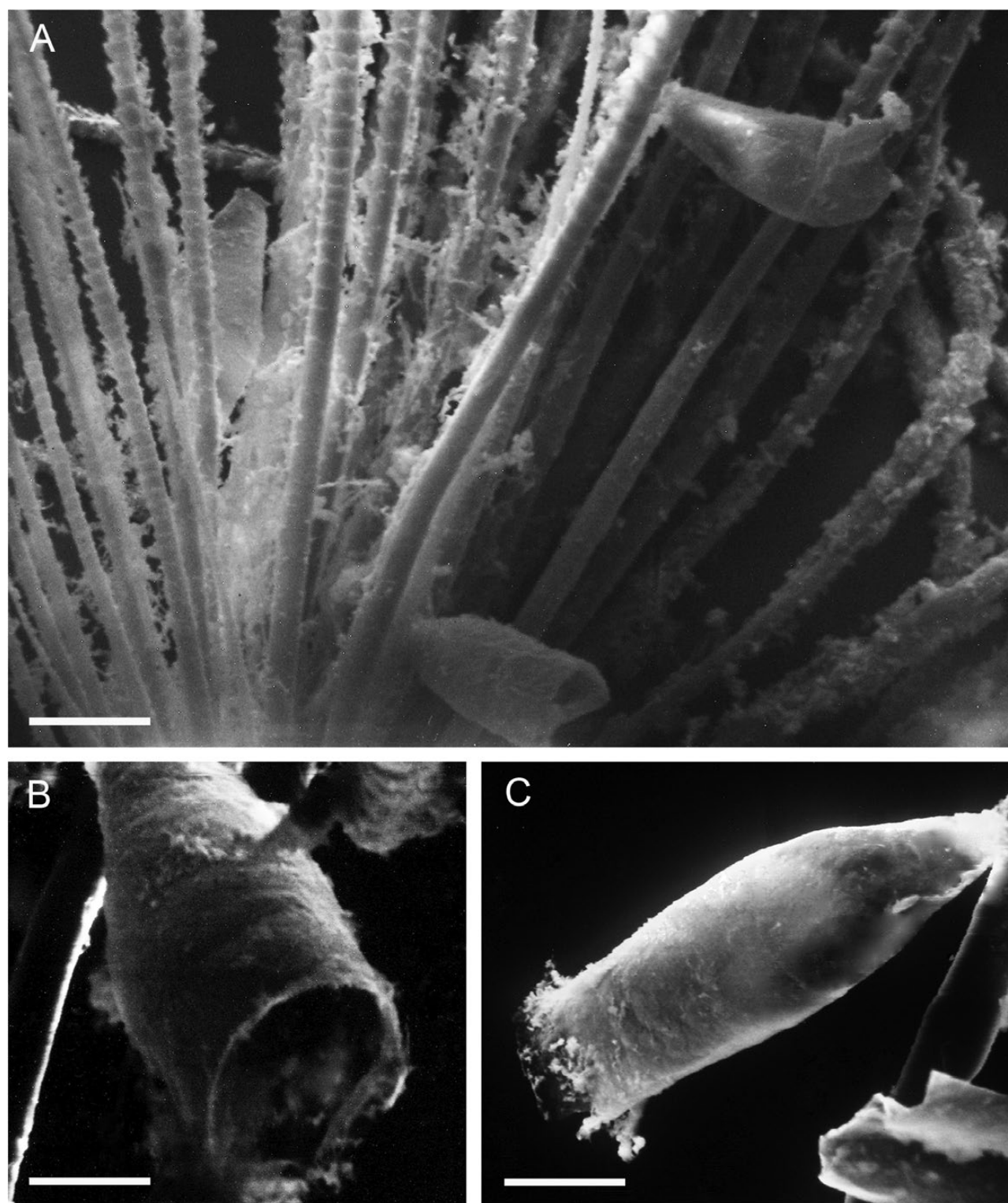


Figure 6. *Cothurnia peloscolicis* on the chaetae of *Sthenelais boa* (SEM). A, neurochaetae of *S. boa* with attached ciliates. B, close-up of ciliate *C. peloscolicis* lorica showing circular aperture. C, one *C. peloscolicis* attached to *S. boa* neurochaeta. Scale bars: A = 35 μm , B = 15 μm and C = 20 μm .

Terebellides stroemi Sars, 1835 (Fam. Trichobranchidae) – on the cirri; Bay of Kiel, Germany, Baltic Sea (Möbius, 1888; Kahl, 1935; Precht, 1935).

Rhabdostyla mapuche Álvarez-Campos, Fernández-Leborans & Verdes, 2014.

Syllis magdalena Wesenberg-Lund, 1962 (Fam. Syllidae) – on the intersegmental furrows, close to parapodial bases and on the prostomium; Las Cruces, Central Chile, south-eastern Pacific Ocean (Álvarez-Campos *et al.*, 2014).

Syllis sp. 1 (Fam. Syllidae) – on the intersegmental furrows, close to parapodial bases; Las Cruces, Central Chile, south-eastern Pacific Ocean (Álvarez-Campos *et al.*, 2014).

Syllis sp. 2 (Fam. Syllidae) – on the intersegmental furrows, close to parapodial bases and on the prostomium; Las Cruces, Central Chile, south-eastern Pacific Ocean (Álvarez-Campos *et al.*, 2014).

Salvatoria concinna (Westheide, 1974) (Fam. Syllidae) – on the intersegmental furrows, close to parapodial bases; Las Cruces, Central Chile, south-eastern Pacific Ocean (Álvarez-Campos *et al.*, 2014).

Salvatoria sp. (Fam. Syllidae) – on the intersegmental furrows, close to parapodial bases; Las Cruces, Central Chile, south-eastern Pacific Ocean (Álvarez-Campos *et al.*, 2014).

RHABDOSTYLA NEREICOLA PRECHT, 1935

Platynereis dumerilii (Audouin & Milne Edwards, 1833) (Fam. Nereididae) – dorsally on the parapodia; Bay of Kiel, Germany, Baltic Sea (Precht, 1935).

Rhabdostyla taboadai Álvarez-Campos, Fernández-Leborans, Riesgo & Martin, 2014.

Syllis prolifera Krohn, 1852 (Fam. Syllidae) – on the intersegmental furrows, close to parapodial bases; Costa Brava, Spain, north-western Mediterranean coast (Álvarez-Campos *et al.*, 2014).

RHABDOSTYLA VARIABILIS DONS, 1918

Scoloplos armiger (Müller, 1776) (Fam. Orbiniidae) – between the parapodia and on the anterior and posterior part of parapodia; Baltic Sea, Germany (Dons, 1918; Precht, 1935).

Phyllodoce laminosa Savigny in Lamarck, 1818 (Fam. Phyllodocidae) – on the posterior part of parapodia; Baltic Sea, Germany (Dons, 1918); on the posterior part of the wide notopodia and on the body, positioned between the notopodia; Bay of Kiel, Germany, Baltic Sea (Precht, 1935).

RHABDOSTYLA SP. 1

Syllis elongata Day, 1949 (Fam. Syllidae) – on the dorsal surface, the nuchal organs, the mouth opening

and the anterior cirri; Tumbes, Peru, south-eastern Pacific Ocean (Álvarez-Campos *et al.*, 2014).

RHABDOSTYLA SP.

Typosyllis macropectinans Hartmann-Schröder, 1982 (Fam. Syllidae) – on the ventral surface; New South Wales, Australia (Álvarez-Campos *et al.*, 2014).

Syllis microoculata (Hartmann-Schröder, 1965) (Fam. Syllidae) – on the intersegmental furrows, close to parapodial bases; Maui, Hawaii, northern Pacific Ocean (Álvarez-Campos *et al.*, 2014).

Dipolydora armata (Langerhans, 1880) (Fam. Spionidae) – described as spermatophores attached to the chaetae of female worms; west coast of Barbados, West Indies, north-western Atlantic Ocean (Lewis, 1998).

PERITRICHIA CF. *RHABDOSTYLA* SP.

Parapionosyllis papillosa (Pierantoni, 1903) (Fam. Syllidae) – described as papillae placed in the interramal furrows and on the parapodia of anterior segments; Gulf of Naples, Italy, Tyrrhenian Sea, Mediterranean Sea (Pierantoni, 1903; Álvarez-Campos *et al.*, 2014).

FAMILY SCYPHIDIIDAE KAHL, 1933

GENUS *PARAVORTICELLA* KAHL, 1933

PARAVORTICELLA LYCASTIS CHAKRAVORTY, 1937

Namalycastis indica (Southern, 1921) (Fam. Nereididae) – on the parapodia; India (Chakravorty, 1937).

PARAVORTICELLA TERESELLAE (FAURÉ-FREMIET, 1920)

Terebella lapidaria Linnaeus, 1767 (Fam. Terebellidae) – forming fluffy spots on the polychaete skin; Germany (Kahl, 1935).

Terebellides stroemii Sars, 1835 (Fam. Trichobranchidae) – on the ventral body side, from the anterior part under gills to the mid body; Bay of Kiel, Germany, Baltic Sea (Precht, 1935).

GENUS *SCYPHIDIA* DUJARDIN, 1841

SCYPHIDIA SPIONICOLA PRECHT, 1935

Pygospio elegans Claparede, 1863 (Fam. Spionidae) – on the tentacles, the body surface, the cirri of posterior

parapodia, and between both the anterior and posterior parapodia; Bay of Kiel, Germany, Baltic Sea (Precht, 1935)

SCYPHIDIA TERESELLIDIS PRECHT, 1935

Terebellides stroemii Sars, 1835 (Fam. Trichobranchidae) – on the branchiae; Bay of Kiel, Germany, Baltic Sea (Precht, 1935).

SCYPHIDIA VARIABILIS DONS, 1922

Terebellidae Johnston, 1846 – Norwegian coast (Kahl, 1935).

SCYPHIDIA SP.

Nerilla antennata Schmidt, 1848 (Fam. Nerillidae) – between the parapodia; Livorno coast, Italy, Tyrrhenian Sea, Mediterranean Sea (Magagnini & Verni, 1988).

FAMILY VAGINICOLIDAE DE FROMENTEL, 1874

GENUS *COTHURNIA* EHRENBERG, 1831

COTHURNIA ACUTA LEVANDER, 1915

Bylgides sarsi (Kinberg in Malmgren, 1866) (Fam. Polynoidae) – on the chaetae, particularly of the anterior parapodia; Tvärminne, Finland, Baltic Sea (Levander, 1915; Kahl, 1935).

Harmothoe imbricata (Linnaeus, 1767) (Fam. Polynoidae) – on the chaetae; Bay of Kiel, Germany, Baltic Sea (Precht, 1935).

COTHURNIA AMPHICTEIS LANG, 1948

Amphicteis gunneri (M. Sars, 1835) (Fam. Ampharetidae) – on the chaetae; coastal waters of Sweden, Baltic Sea (Lang, 1948).

Malmgrenia andreapolis McIntosh, 1874 (Fam. Polynoidae) – on the chaetae; this research, north Adriatic Sea, Mediterranean Sea.

Malmgrenia lilianae (Pettibone, 1993) (Fam. Polynoidae) – on the chaetae; this research, north Adriatic Sea, Mediterranean Sea.

COTHURNIA CERAMICOLA KAHL, 1933

Spirorbis (Spirorbis) spirorbis (Linnaeus, 1758) (Fam. Serpulidae) – on the tube and the operculum; Bay of Kiel, Germany, Baltic Sea (Precht, 1935).

COTHURNIA COMPLANATA PRECHT, 1935

Pherusa plumosa (Müller, 1776) (Fam. Flabelligeridae) – on the chaetae; Bay of Kiel, Germany, Baltic Sea (Precht, 1935).

Cothurnia kiwi Álvarez-Campos, Fernández-Leborans & San Martín, 2014.

Prosphaerosyllis magnoculata (Hartmann-Schröder, 1986) (Fam. Syllidae) – on the intersegmental furrows on the base of parapodia; New Zealand (Álvarez-Campos *et al.*, 2014).

COTHURNIA NEREICOLA PRECHT, 1935

Hediste diversicolor (O. F. Müller, 1776) (Fam. Nereididae) – on both sides of the parapodia; Bay of Kiel, Germany, Baltic Sea (Precht, 1935).

COTHURNIA PEDUNCULATA DONS, 1918

Pherusa plumosa (Müller, 1776) (Fam. Flabelligeridae) – on the chaetae; Trøndelag, Norway, Norwegian Sea (Dons, 1928; Dons 1946); Bay of Kiel, Germany, Baltic Sea (Precht, 1935).

Remark: Dons (1946) described this species as *Cothurnia trophoniae* Dons, 1946, although Dons (1918) previously redescribed the same species as *C. pedunculata* (Warren & Paynter, 1991).

COTHURNIA PELOSCOLICIS PRECHT, 1935

Sthenelais boa (Johnston, 1833) (Fam. Sigalionidae) – on the chaetae; this research, north Adriatic Sea, Mediterranean Sea.

COTHURNIA POLYDORICA JANKOWSKI, 2014

Polydora sp. (Fam. Spionidae) – on the tips of the thick chaetae of two kinds; Sea of Japan (Jankowski, 2014).

COTHURNIA STYLARIOIDES PRECHT, 1935

Pherusa plumosa (Müller, 1776) (Fam. Flabelligeridae) – on the chaetae; Bay of Kiel, Germany, Baltic Sea (Precht, 1935).

COTHURNIA SP.

Dipolydora armata (Langerhans, 1880) (Fam. Spionidae) – on the notopodial capillary chaetae; Ibiza, Spain, Mediterranean Sea (Bick, 2001).

FAMILY VORTICELLIDAE EHRENBERG, 1838

GENUS *VORTICELLA* LINNAEUS, 1767

VORTICELLA OBCONICA KAHL, 1935

SPIROBIS SP. (FAM. SERPULIDAE) – NORWAY (KAHL, 1935)

VORTICELLA SP.

RHAMPHOBRACHIUM MACULATUM ESTCOURT, 1966
 (FAM. ONUPHIDAE) – ON THE DORSAL SURFACE OF
 THE ANTERIOR END OF THE BODY; NEW ZEALAND
 (KNOX & HICKS, 1973).

GENUS PSEUDOVORTICELLA FOISSNER &
 SCHIFFMANN, 1975

PSEUDOVORTICELLA PUNCTATA (DONS, 1918)

Harmothoe imbricata (Linnaeus, 1767) (Fam.
 Polynoidae) – on the chaetae; Bay of Kiel, Germany,
 Baltic Sea (Precht, 1935).

Spirorbis (Spirorbis) spirorbis (Linnaeus,
 1758) (Fam. Serpulidae) – on the tube; Bay of Kiel,
 Germany, Baltic Sea (Precht, 1935).

FAMILY ZOOTHAMNIIDAE SOMMER, 1951

GENUS HAPLOCAULUS WARREN, 1988

HAPLOCAULUS NICOLEAE PRECHT, 1935

Nicolea zostericola Örsted, 1844 (Fam. Terebellidae) –
 on the posterior slim body part; Bay of Kiel, Germany,
 Baltic Sea (Precht, 1935).

GENUS ZOOTHAMNIUM BORY DE ST. VINCENT, 1826

ZOOTHAMNIUM DUPLICATUM KAHL, 1933

Spirorbis (Spirorbis) spirorbis (Linnaeus, 1758) (Fam.
 Serpulidae) – on the tube; Bay of Kiel, Germany, Baltic
 Sea (Precht, 1935).

ZOOTHAMNIUM VERMICOLA PRECHT, 1935

Lagis koreni Malmgren, 1866 (Fam. Pectinariidae) – on
 the tentacles, the parapodia, the branchiae, the body
 surface; Bay of Kiel, Germany, Baltic Sea (Precht, 1935).

Nephtys sp. (Fam. Nephtyidae) – Bay of Kiel,
 Germany, Baltic Sea (Precht, 1935).

Nereimyra punctata (Müller, 1788) (Fam.
 Phyllodocidae) – on the whole body, including the long
 cirri; Bay of Kiel, Germany, Baltic Sea (Precht, 1935).

Eteone longa (Fabricius, 1780) (Fam. Phyllodocidae)
 – on all parts of the body; Bay of Kiel, Germany, Baltic
 Sea (Precht, 1935).

ZOOTHAMNIUM SP.

Pherusa plumosa (Müller, 1776) (Fam. Flabelligeridae)
 – on the chaetae; Bay of Kiel, Germany, Baltic Sea
 (Precht, 1935).

ORDER MOBILIDA KAHL, 1933

FAMILY URCEOLARIIDAE DUJARDIN, 1840

GENUS URCEOLARIA LAMARCK, 1801

URCEOLARIA CONVEXA HAIDER & DIETRICH 1977

Phyllodoce mucosa Örsted, 1843 (Fam. Phyllodocidae)
 – Büsum, Germany, North Sea (Haider & Dietrich,
 1977).

URCEOLARIA SERPULARUM (FABRE-DOMERGUE, 1888)
 Fam. Serpulidae – on the branchial lamellae;
 Concarneau, France, the Bay of Biscay, north-eastern
 Atlantic Ocean (Fabre-Domergue, 1888, as *Leiotrocha*
serpularum).

Nephtys sp. (Fam. Nephtyidae) – Bay of Kiel,
 Germany, Baltic Sea (Precht, 1935, as *Cyclochaeta*
serpularum).

Phyllodoce laminosa Savigny in Lamarck, 1818
 (Fam. Phyllodocidae) – Bay of Kiel, Germany, Baltic
 Sea (Precht, 1935, as *C. serpularum*).

Serpula sp. (Fam. Serpulidae) – branchiae; Germany
 (Kahl, 1935, as *Cyclochaeta (Leiotrocha) serpularum*).
 Remark: *Urceolaria serpularum* was originally
 described in the monotypic genus *Leiotrocha*
 Fabre-Domergue, 1888. Haider (1964) transferred
Leiotrocha serpularum into the genus *Urceolaria*,
 which was accepted by Xu & Song (2003), but not
 by Lynn (2008), who insisted on validity of the
 monotypic family Leiotrochidae Johnston, 1938.
 Recent morphological and genetic analyses by Zhan
 et al. (2013) support that *Leiotricha* should be placed
 in *Urceolaria*.

URCEOLARIA SP.

Polydora colonia Moore, 1907 (Fam. Spionidae) – on
 the palps, the anterior and the posterior chaetigers
 and the pygidium; Hempstead East Marina, New
 York, north-western Atlantic Ocean (David &
 Williams, 2012).

Polydora cornuta Bosc, 1802 (Fam. Spionidae) – on
 the body surface; Los Angeles Bay, southern California,
 north-eastern Pacific Ocean (Douglas & Jones, 1991).

Family Trichodinidae Claus, 1951.

GENUS TRICHODINA EHRENBERG, 1830

TRICHODINA SCOLOPLONTIS PRECHT, 1935

Scoloplos armiger (Müller, 1776) (Fam. Orbiniidae) –
 on the posterior end of the body; Bay of Kiel, Germany,
 Baltic Sea (Precht, 1935).

TRICHODINA TEREPELLIDIS PRECHT, 1935

Terebellides stroemii Sars, 1835 (Fam. Trichobranchidae) – on the branchiae; Bay of Kiel, Germany, Baltic Sea (Precht, 1935).

UNCLASSIFIED PERITRICHIOUS CILIATES

Polydora neocaeca Williams & Radaskevsky, 1999 (Fam. Spionidae) – on the hoods of the bidentate hooded hooks; State of Rhode Island, North America, north-western Atlantic Ocean (Williams & Radaskevsky, 1999).

Ampharete santillani Parapar, Kongsrud, Kongshavn, Alvestad, Aneiros & Moreira, 2017 (Fam. Ampharetidae) – on the abdominal uncini, the branchial surface, the dorsolateral area behind the branchial surface and the ciliated buttons over the abdominal neuropodia; Galicia, north-western Spain and off Morocco, north-eastern Atlantic Ocean (Parapar *et al.*, 2018).

ANALYSES OF THE POLYCHAETE–PERITRICH CILIATE ASSOCIATION

Forty taxa (30 determined to species level) of peritrich ciliates belonging to 12 genera and seven families are up to date recorded as epibionts of polychaetes. Most of them belong to the order Sessilida, while only five taxa belong to the order Mobilida. The most diverse are representatives of the genus *Cothurnia* (11 taxa, of which ten determined to species level). Forty-eight polychaete taxa (39 determined up to species level) are known as hosts for peritrich ciliate epibionts. They belong to the families Ampharetidae, Arenicolidae, Capitellidae, Flabelligeridae, Nephtyidae, Nereididae, Nerillidae, Onuphidae, Orbiniidae, Pectinariidae, Phyllodocidae, Polynoidae, Serpulidae, Sigalionidae, Spionidae, Syllidae, Terebellidae and Trichobranchidae. Most polychaete basibionts pertain to the family Syllidae (11 taxa, of which eight determined to species level).

DISCUSSION

In this research, the peritrich ciliate *Cothurnia amphicteis* was found on polynoid polychaetes *Malmgrenia lilianae* and *M. andreapolis*, while *Cothurnia peloscolicis* was found on sigalionid polychaete *Sthenelais boa*. This is the first observation of polychaete–ciliate epibiosis from the Adriatic Sea and the first record of epibiosis between peritrich ciliates and polychaetes of the family Sigalionidae.

Other reports of Polynoidae as epibionts of polychaetes are sparse. Levander (1915) described *Cothurnia acuta* from the polychaete *Bylgides sarsi* [reported

as *Harmothoe sarsi* (Kinberg in Malmgren, 1865)] in Tvärminne (Finland, Baltic Sea), while Precht (1935) found *Cothurnia acuta* and *Pseudovorticella punctata* on *Harmothoe imbricata* in the Bay of Kiel (Germany, Baltic Sea).

The number of epibionts per host ranged from a few individuals to about 100. In all analysed specimens, ciliates were localized only on the chaetae (one ciliate per chaeta, rarely two), never on the body surface or other structures. Accordingly, in several previous studies, ciliates, particularly of the genus *Cothurnia*, were found attached on the chaetae (Levander, 1915; Dons, 1928; Kahl, 1935; Precht, 1935; Dons 1946; Lang, 1948; Lewis, 1998; Williams & Radaskevsky, 1999; Bick, 2001; Jankowski, 2014; Parapar *et al.*, 2018). Williams & Radashevsky (1999) found peritrich ciliates attached to the hoods of the bidentate hooded hooks of the spionid *Polydora neocaeca*; the stalks of the peritrich extended dorsally so that its body and oral region were positioned near the branchiae of the worm. Similarly, Bick (2001) found *Cothurnia* sp. attached on the notopodial capillaries of the postbranchiate chaetigers of the spionid *Dipolydora armata*. Ciliates were previously also found in other regions of the polychaete body, such as the body surface, the intersegmental furrows, the parapodia, the branchiae, the cirri, the tentacles, the prostomium, the mouth opening, the palps, the nuchal organs, the pygidium and the tube of the spirorbids. Parapar *et al.* (2018) found peritrich epibionts on different body parts, including the chaetae of the ampharetid *Ampharete santillani*, but noticed that their abundance was higher in ciliated body parts, such as the branchial surface, the dorsolateral area behind them and the ciliated buttons over the abdominal neuropodia.

Using fixed specimens, we could not address the ecological features of the epibiotic relationship. However, some general reflections on the consequences of this epibiosis can be retrieved from the literature. In general, an epibiotic association entails a highly complex suite of advantages and disadvantages for both partners (Wahl, 2009). The major advantage that ciliates gain from being associated with a motile substratum is the increased food availability, assured by the free transport to a variety of habitats, and by increased water flow. In fact, their frequent location on ciliated parts of the polychaete body suggests that they may take advantage of the water currents produced by the polychaete branchiae and cilia for feeding (Bick, 2001; Parapar *et al.*, 2018). Magagnini & Verni (1988) supposed that movement of the polychaete *Nerilla antennata*, determined by its ventral ciliated tract, resuspends the bottom debris containing bacteria and other microorganisms, making it available for the epizoic ciliate *Scyphidia*. In order to assess whether epibiosis interferes with the life cycle of

the polychaete host, the same authors measured for several months various parameters pertaining to the life cycle of *N. antennata*, with and without epibiotic ciliates. Their study indicated that the epibiosis did not appear to affect the life cycle of the polychaete. David & Williams (2012) reported that specimens of *Polydora colonia* hosting the ciliate *Urceolaria* in New York harbour did not appear to be negatively affected by the ciliate. In their study on epibiosis between ciliates and syllid polychaetes, Alvarez-Campos *et al.* (2014) did not notice alteration of swimming efficiency or other external harm in the specimens carrying the ciliate protozoans. Likewise, Parapar *et al.* (2018) did not observe damage caused by peritrich ciliates on the body surface of the polychaete *Ampharete santillani*. All this suggests that in polychaete–peritrich ciliate association, the advantages are limited to the ciliate, while the polychaete gets no harm from the epibiont. This association can, therefore, be considered as ectocommensalism.

Epibiotic relationships are rarely species-specific (Wahl, 2009). During the monitoring study on the three research stations, sampling was done in different seasons from 2003 to 2008; more than 21 000 individuals belonging to 230 polychaete species were carefully analysed and epibiosis was observed only between the ciliate *Cothurnia amphiteis* and the polychaetes *Malmgrenia lilianae* and *M. andreapolis*, and between *C. peloscolicis* and the polychaete *Sthenelais boa*. This suggests that these host–epibiont relationships might be species-specific. Congruently, Magagnini & Verni (1988) found the ciliate *Scyphidia* sp. associated only to the polychaete *Nerilla antennata* in benthic samples from Livorno (Italy), and concluded that the observed epibiosis is likely species-specific. In their survey of symbionts associated with spionid polychaetes from California, Douglass & Jones (1991) showed that a ciliate *Urceolaria* sp. was a specific epibiont of the polychaete *Polydora cornuta* and that its presence on the surface of the polychaete allowed the identification of the worm. They also showed that ciliates tended to have an affinity for polydorids versus other spionids.

Our research adds two more species to the ciliate fauna of the Adriatic Sea. Until today, representatives of the genus *Cothurnia* were reported from the Adriatic Sea only twice. Stiller (1968) found *Cothurnia membranoloricata* Stiller, 1968, in the vicinity of Rovinj (north Adriatic Sea) attached to the algae *Cladophora coelothrix* Kütz. and *C. laetevirens* (Dillwyn) Kütz. (Cladophoraceae). Recently, Fernandez-Leborans *et al.* (2012) reported *Cothurnia triangula* (Precht, 1935) as epibiont of the copepod *Typhlamphiascus* sp. from the Bay of Piran (Slovenia, north Adriatic Sea). *Cothurnia amphiteis* and *C. peloscolicis*, newly recorded in the northern Adriatic, were previously reported only for

the Baltic Sea. These new findings support previous observations showing that the north Adriatic Sea hosts elements of the flora and fauna with cold-temperate water affinities. In fact, the north Adriatic Sea is, together with the Gulf of Lion, the coldest sector of the Mediterranean Sea, showing ecological and biogeographical similarities with the north Atlantic Ocean (Bianchi *et al.* 2004; Boero & Bonsdorff 2007; Boero *et al.* 2008).

Records of ciliate epibionts may easily be overlooked in the extensive literature on polychaetes, as already stressed by Jankowski (2014), and this association might be even more common than it appears to be. Many papers reporting polychaete–peritrich associations are focused on polychaete taxonomy or ecology, and the presence of ciliates is barely mentioned, or eventually accompanied by an iconography and a short description of the association (Knox & Hicks, 1973; Douglas & Jones, 1991; Williams & Radashevski, 1999; Bick, 2001; Arias *et al.*, 2010; David & Williams, 2012; Parapar *et al.*, 2018). On the other hand, several peritrich ciliate species, particularly those of the genus *Cothurnia*, were originally described from the specimens found as epibionts on polychaetes (Fabre-Domergue, 1888; Möbius, 1888; Levander, 1915; Precht, 1935; Dons, 1946; Alvarez *et al.*, 2014; Jankowski, 2014). Although polychaete–peritrich association appears not to be so frequent and diversified as the epibiosis of peritrichs on some other invertebrate groups, such as Crustaceans (Fernandez-Leborans & Tato-Porto, 2000), our review has shown that it is documented in a remarkable number of reports. This epibiosis might even be a more widespread phenomenon, due to the possibility of overlooking or misinterpretations. In fact, ciliate epibionts were previously interpreted as morphological structures of the polychaete body (i.e. papillae and reproductive structures). Pierantoni (1903) described the polychaete *Pionosyllis papillosa* (today acknowledged as *Parapionosyllis papillosa*) from the Gulf of Naples (Italy), emphasizing as the principal diagnostic character of the species, the presence of a high number of large papillae of characteristic shape protruding from the polychaete skin surface of anterior body segments. ‘Papillae’ were gathered in small groups, particularly in interramal furrows and on parapodia. San Martin (2003) suggested that these papillae could actually be parasites and queried the taxonomic validity of *P. papillosa*. Later analyses by Alvarez-Campos *et al.* (2014) of the original description and drawings from Pierantoni (1903) and Fauvel (1923), as well as of the specimens identified and described by Campoy (1982), revealed that papillae were actually ciliate epibionts of the genus *Rhabdostyla*. The same authors analysed museum specimens of the species *Syllis microoculata*, originally collected in Manui, Hawaii. Structures of *S. microoculata* reported by

Hartmann-Schröder (1965) as papillae placed on intersegmental furrows, close to parapodial bases, were found to be misinterpreted ciliate epibionts of the genus *Rhabdostyla*. Alvarez-Campos *et al.* (2014) stated that, except for the ‘papillae’, *S. microoculata* is identical to the Mediterranean *S. prolifera* Krohn, 1852 but, given the distance between the Mediterranean Sea and the species type locality (Hawaii), further studies are needed to consider if they are the same species or a case of convergence. These later findings of overlooked epibiosis of ciliates on syllids have important taxonomic implications, since the papillae have been considered as a diagnostic character to distinguish among species or to erect new species (Alvarez-Campos *et al.*, 2014). The fixation method for polychaete conservation provokes contraction of the ciliate epibionts, which, together with their small size, causes difficulties in distinguishing them from papillae. Similar misinterpretations might have happened in other polychaete families bearing papillae (Alvarez-Campos *et al.*, 2014). Lewis (1998) described small, oval spermatophores produced by males and found attached by a stalk to the capillary chaetae or, occasionally, to the body wall on the genital segments of the females of the spionid polychaete *Dipolydora armata*, boring in the calcareous hydrozoans *Millepora complanata* Lamarck, 1816 on fringing reefs on the western coast of Barbados (West Indies, north-western Atlantic Ocean). Adult females commonly carried two or three, but up to a dozen of ‘spermatophores’ each. Careful analyses of the ‘spermatophores’ description, and light-microscopy photographs, revealed that they actually are peritrich ciliates, possibly belonging to *Rhabdostyla* (Jankowski, 2014).

Together with novel redescrptions of two *Cothurnia* species and new discoveries of polychaete–peritrich epibiosis, our analyses and results suggest that this association warrants further investigations, both to elucidate its real diversity and to deepen our knowledge on its ecological peculiarities.

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