The LINNEAN SOCIETY of London — ZOOLOGICAL Journal of the Linnean Society

Newly discovered associations between peritrich ciliates (Ciliophora, Peritrichia) and polychaetes Polynoidae and Sigalionidae with the review of Polychaeta – Peritrichia epibiosis

Journal:	*Zoological Journal of the Linnean Society*
Manuscript ID	Draft
Manuscript Type:	Original Article
Keywords:	biodiversity < Ecology, commensalism < Evolution, host utilization < Evolution, Adriatic < Geography, Mediterranean < Geography, Annelida < Taxa, revision < Taxonomy, species redescription < Taxonomy, scanning electron microscopy < Techniques

Abstract:

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 re-described, based on light and scanning electron microscopy analyses. This is the first record of the 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 7 families are up to date recorded as epibionts on polychaetes, while 48 polychaete taxa are known as their hosts. The relationship can be considered ectocommensalism, where ciliates get the advantages of increased food availability. This association might be more widespread phenomenon then currently known, as it could be easily overlooked or misinterpreted, and therefore deserves careful attention and further investigations.

ABSTRACT

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 re-described, based on light and scanning electron microscopy analyses. This is the first record of the 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 7 families are up to date recorded as epibionts on polychaetes, while 48 polychaete taxa are known as their hosts. The relationship can be considered ectocommensalism, where ciliates get the advantages of increased food availability. This association might be more widespread phenomenon then currently known, as it could be easily overlooked or misinterpreted, and therefore deserves careful attention and further investigations.

Key words: Adriatic – Annelida – *Cothurnia* – *Malmgrenia* – Mediterranean – Protozoa – re-description – Scanning Electron Microscopy – *Sthemelais*

INTRODUCTION

Epibiosis is the ecological association between a substrate organism – basibiont – and a sessile organism – epibiont – attached to the basibiont's outer surface without trophic dependence on it (Wahl, 2009). Ciliate protozoans, particularly from the subclasses Peritrichia and Suctoria, 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; Sartini *et al.*, 2018; Sergeeva & Dovgal, 2014). Despite the high diversity and the wide distribution of polychaetes in a marine environment, records of polychaete – peritrich association are rare, and very few papers are dealing with this relationship as the main topic (Alvarez *et al.*, 2014; Jankowski, 2014; Magagnini & Verni, 1988).

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 having 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, which 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 lorica, and sculpture of both lorica and stalk, are the principal characters which are used for identification of *Cothurnia* species. At the same time, most descriptions of cothurnians are based on observations from only one direction, where the shape of lorica's aperture is not well visible, and often, only hand drawings are reported. The two *Cothurnia* species reported, were no longer observed after their first descriptions, until the present research (Lang, 1948; Precht, 1935). Moreover, some details, especially concerning the outer sculpture of corthurnian lorica and stalk are only visible with the use of scanning electron microscopy. Thus, we give herein a re-description of *C. amphicteis* and *C. peloscolicis* based on light and scanning electron microscopy.

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

MATERIALS 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 Van Veen grab, sieved through 1 mm mesh and fixed in 4% buffered formaldehyde–seawater solution. After sorting in the laboratory macrobenthic organisms were preserved in 70% ethanol. Polychaetes were determined 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 Zeiss Axiovert 100 microscope, using 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 SEM analyses fixed specimens were washed in 0.1 M phosphate buffer (pH 7.4), dehydrated in a graded alcohol series until 100% (5 minutes for each solution), then dried with hexamethyldisilazane (HMDS), according to 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* Lang, 1948 were observed on 18 specimens of the polychaete *Malmgrenia lilianae* (Pettibone, 1993) and 2 specimens of *M. andreapolis* McIntosh, 1874 (fam. Polynoidae). Ciliate epibionts *Cothurnia peloscolicis* Precht, 1935 were observed only on one specimen of the polychaete *Sthenelais boa* (Johnston, 1833) (fam. Sigalionidae). *C. amphicteis* was found attached on mid to distal part of *Malmgrenia* chaetae, mostly on notochaetae, more rarely on neurochaetae (Figs 2, 3). *C. peloscolicis* was found attached on lower-basal part of the upper (simple spinous) neurochaetae of *S. boa* (Figs 5, 6A). In general, one ciliate per chaetae was found, only in few cases two. Ciliates were never found attached on polychaetes body surface. Number of epibionts per host ranged from few to about hundred.

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 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 posterior end of lorica; septum connected to base of lorica via mesostyle. Zooid(s) attached to base of lorica (or septum) directly or via endostyle (Warren & Paynter, 1991).

Cothurnia amphicteis Lang, 1948, Figures 2–4

Material examined

Light microscopy measurements based on 6 *Cothurnia* specimens found on 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 (3 polychaetes), 18 October 2006 (1 polychaete); station SJ007 – 15 March 2005 (1 polychaete; mounted for SEM), 21 June 2005 (2 polychaetes), 18 October 2006 (1 polychaete), 13 September 2007 (1 polychaete); station SJ107 – 21 June 2005 (1 polychaete; mounted for SEM), 22 December 2005 (2 polychaetes), 13 September 2007 (2 polychaetes - 1 mounted for SEM), 12 October 2007 (1 polychaete). Additional material examined from the polychaete *M. andreapolis*, North Adriatic Sea: station SJ005 – 22 September 2006 (1 polychaete - mounted for SEM); station SJ107 – 12 October 2007 (1 polychaete).

Description

Lorica conical, smooth, with very 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 outgrows (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, which are nor visible in some cases. Mesostyle absent. Generally, two zooids are present (Figs 2D, 4B). Zooid conical, 85 µm long × 40 µm wide, and extends between one third and 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 original description of *C. amphicteis* by the presence of two zooids in lorica (with one exception) and the greater length of 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 mesostyle was mentioned. However, on the Precht's (1935) picture, *C. acuta* presents the typical mesostyle with longitudinal striae. In our specimens mesostyle was absent, thus we consider them *C. amphicteis*. The synonymy between *C.*

acuta and *C. amphicteis* needs further considerations. 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).

Cothurnia peloscolicis Precht, 1935, Figures 5–6

Material examined

Material examined by light microscopy (measurements based on 6 *C. peloscolicis* specimens) and SEM microscopy from 1 specimen of polychaete *Sthenelais boa*, North Adriatic Sea, station SJ007, 05 March 2004.

Description

Lorica extended near base and slightly converged toward aperture, smooth, not compressed, 61–86 µm (81 µm after Warren & Paynter, 1991) long × 26–32 µm (27–41 µm after Warren & Paynter, 1991) wide (Fig. 5B, 6C). Aperture circular when viewed from above, 23–32 µm in diameter (15 × 33 µm after Warren & Paynter, 1991), the edge of aperture without any outgrow (Fig. 6B). External stalk short, cylindrical, without folds, 10–16 µm (20 µm after Warren & Paynter, 1991) long; mesostyle and endostyle absent (Fig. 5B). Stalk with longitudinal striae in some cases. Generally, two zooids present (Fig. 5B). Zooid 80 µm long × 18–22 µm wide, 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 peristomal region. Macronucleus straight, 50 µm long. Pellicular striations inconspicuous.

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 Kiel Bay (Germany, Baltic Sea) (type locality) (Precht, 1935). Other locality: North Adriatic Sea on the chaetae of polychaete *Sthenelais boa* (this paper).

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

***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 arenicole* Fabre-Domergue, 1888**

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)

***Rhabdostyla commensalis* Moebius, 1888**

Capitella capitata (Fabricius, 1780) (Fam. CAPITELLIDAE) - on the body cuticle; Kiel Bay, Germany, Baltic Sea (Möbius, 1888)

Terebellides stroemi Sars, 1835 (Fam. TRICHOBRANCHIDAE) - on the cirri; Kiel Bay, 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 (Alvarez-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 (Alvarez-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 (Alvarez-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 (Alvarez-Campos *et al.*, 2014)

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

***Rhabdostyla nereicola* Precht, 1935**

Platynereis dumerilii (Audouin & Milne Edwards, 1833) (Fam. NEREIDIDAE) - dorsally on the parapodia; Kiel Bay, 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 (Alvarez-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; Kiel Bay, 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 (Alvarez-Campos *et al.*, 2014)

***Rhabdostyla* sp.**

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

Syllis microoculata (Hartmann-Schröder, 1965) (Fam. SYLLIDAE) - on the intersegmental furrows, close to parapodial bases; Maui, Hawaii, northern Pacific Ocean (Alvarez-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; Alvarez-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 terebellae* (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 terebellidis* 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 (Alvarez-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, Norwegean 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 he previously (Dons, 1918) re-described 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**

Spirorbis 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 (Leiotrocba) serpularum*)

Remark: *Urceolaria serpularum* was originally described in the monotypic genus *Leiotrocha* Fabre-Domergue, 1888. Later, Haider (1964) transferred *Leiotrocha serpularum* into the genus *Urceolaria*, which was further 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 *L. serpularum* should be a synonym of *U. serpularum*.

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 & Wiliams, 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 terebellidis* Precht, 1935**

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

Unclassified peritrichous ciliates

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

Ampharete santillani Parapar, Kongsrud, Kongshavn, Alvestad, Aneiros & Moreira, 2017 (Fam. AMPHARETIDAE) - on the abdominal uncini, the branchial surface, the dorso-lateral 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 7 families are up to date recorded as epibionts on polychaetes. Most of them belong to the order Sessilida, while only five taxa to the order Mobilida. The most diverse are the representatives of the genus *Cothurnia* (11 taxa, of which 10 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 8 determined to species level).

DISCUSSION

In this research, 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. As far as the family Polynoidae is concerned, 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 Kiel Bay (Germany, Baltic Sea).

The number of epibionts per host ranged from a few individuals to about hundred. 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 (Lewis, 1998; Precht, 1935; Levander, 1915; Kahl, 1935; Lang, 1948; Dons, 1928; Dons 1946; Jankowski, 2014; Bick, 2001; Wiliams & Radaskevsky, 1999; Parapar *et al.*, 2018). Williams & Radashevsky (1999) found peritrich ciliates attached on 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 dorso-lateral 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 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 and Williams (2012) reported that specimens of *Polydora colonia* hosting 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 21000 individuals belonging to 230 polychaete species were carefully analysed and epibiosis was observed only between ciliate *Cothurnia amphicteis* and polychaetes *Malmgrenia lilianae* and *M. andreapolis*, and between *C. peloscolicis* and polychaete *Sthenelais boa*. This suggests that these host-epibiont relationships might be species-specific. Congruently, Magagnini & Verni (1988) found ciliate *Scyphidia* sp. associated only to 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 and Jones (1991) showed that 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 in the vicinity of Rovinj (North Adriatic Sea) *Cothurnia membranoloricata* Stiller, 1968, attached to the algae *Cladophora coelothrix* Kützing, 1843 and *Cladophora laetevirens* (Dillwyn) Kützing, 1843. 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 amphicteis* and *C. peloscolicis*, newly recorded in the North 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 be easily overlooked in the extensive literature on polychaetes, as already stressed by Jankowski (2014), and this association might be even more common than it actually appears to be. In fact, many papers reporting polychaete – peritrich association are focused on polychaete taxonomy or ecology, and the presence of ciliate is barely mentioned or eventually accompanied by an iconography and a very short description of the association (Arias *et al.*, 2010; Bick, 2001; David & Williams, 2012; Douglas & Jones, 1991; Knox & Hicks, 1973; Parapar *et al.*, 2018; Williams & Radashevski, 1999). 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 (Alvarez *et al.*, 2014; Dons, 1946; Fabre-Domergue, 1888; Jankowski, 2014; Lang, 1948; Levander, 1915; Möbius, 1888; Precht, 1935). Although polychaete – peritrich association appears not to be so frequent and diversified as the epibiosis of peritrichs on some other invertebrates groups, such as Crustaceans (Fernandez-Leborans & Tato-Porto, 2000), our review showed that it is documented in a remarkable number of reports. This epibiosis might be even more widespread phenomenon, due to the possible overlooking or misinterpretations. In fact, ciliate epibionts were previously interpreted as morphological structures of polychaete body (i.e. papillae and reproductive structures). Pierantoni (1903) described 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 inter-ramal furrows and on parapodia. San Martin (2003) suggested that these papillae could actually be parasites and queried the taxonomic validity of the *P. papillosa*. Later analyses by Alvarez-Campos *et al.* (2014) of 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: however, given the distance between the Mediterranean Sea and the species type locality (Hawaii), further studies are needed to consider a possible synonymy. 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 polychaetes 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 "spermatophores" description and light microscopy photographs revealed that they actually were peritrich ciliates, possibly belonging to genus *Rhabdostyla* (Jankowski, 2014).

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

REFERENCES

Álverez-Campos P, Fernández-Leborans G, Verdes A, San Martín G, Martin D, Riesgo A. 2014. The tag-along friendship: epibiotic protozoans and syllid polychaetes. Implications for the taxonomy of Syllidae (Annelida), and description of three new species of *Rhabdostyla* and *Cothurnia* (Ciliophora, Peritrichia), *Zoological Journal of the Linnean Society* 172: 265–281.

Ansari KGMT, Guidi L, Dovgal I, Balsamo M, Semprucci F. 2017. Some epibiont suctorian ciliates from meiofaunal organisms of Maldivian archipelago with description of a new ciliate species, *Zootaxa* 4258(4): 375–387.

Arias A, Anadón N, Paxton H. 2010. New records of *Diopatra marocensis* (Annelida: Onuphidae) from northern Spain, *Zootaxa* 2691: 67–68.

Bianchi CN, Boero F, Fraschetti S, Morri C. 2004. The wildlife of the Mediterranean. In: Minelli A, Chemini C, Argano R, Ruffo S, eds. *Wildlife in Italy*. Milan: Touring Editor and Rome: Italian Ministry for the Environment and Territory, 248–335.

Bick A. 2001. The morphology and ecology of *Dipolydora armata* (Polychaeta, Spionidae) from the western Mediterranean Sea, *Acta Zoologica* 82: 177–187.

Boero F, Bonsdorff E. 2007. A conceptual framework for marine biodiversity and ecosystem functioning, *Marine Ecology-an Evolutionary Perspective* 28 (Supplement 1): 134–145.

Boero F, Féral JP, Azzurro E, Cardin V, Riedel B, Despalatović M, Munda I, Moschella P, Zaouali J, Fonda Umani S, Theocharis A, Wiltshire K, Briand F. 2008. Climate warming and related changes in Mediterranean marine biota. In: Briand F, ed. *Climate warming and related changes in Mediterranean marine biota*. Monaco: CIESM Workshop Monographs, Vol. 35, 5–21.

Cabral AF, Dias RJP, de Oliveira VC, da Gama Alves R, D'Agosto M. 2018. Rhabdostylid ciliates (Ciliophora, Peritrichia, Epistylididae) as epibionts on chironomid larvae: evidence of high specificity and association with organic pollution. *Zoological Science* 35: 514–520.

Campoy A. 1982. *Fauna de España. Fauna de Anélidos Poliquetos de la Península Ibérica*. Pamplona: EUNSA (Ediciones de la Universidad de Navarra), Publicaciones de Biología de la Universidad de Navarra, Serie Zoológica, 7(1), 781 pp.

Chakravorty M. 1937. On *Paravorticella lycastis* n. sp. an ectoparasitic ciliate on the parapodia of an Indian polychaete, *Lycastis indica*, *Journal of the Royal Microscopical Society* 57(2): 71–74.

David AA, Williams, JD. 2012. Morphology and natural history of the cryptogenic sponge associate *Polydora colonia* Moore, 1907 (Polychaeta: Spionidae), *Journal of Natural History* 46(23-24): 1509–1528.

Dons C. 1918. Neue marine Ciliaten und Suctorien, *Tromsø Museums Aarschefter* 38–39: 75–100.

Dons C. 1928. Neue und wenig bekannte Protozoen, *Det Kongelige Norske Videnskabers Selskabs Skrifter* 7: 1–17.

Dons C. 1946. Zwei neue Cothurnien, *Det Kongelige Norske Videnskabers Selskabs* 12: 119–121.

Douglass TG, Jones I. 1991. Parasites of California Spionid polychaetes, *Bulettin of Marine Science* 48(2): 308–317.

Fabre-Domergue P. 1888. Étude sur l'organisation des Urcéolaires et sur quelques genres d'infusoires voisins de cette famille. *Journal de l'anatomie et de la physiologie normales et pathologiques de l'homme et des animaux* 23: 214–260.

Fauvel P. 1923. *Faune de France 5. Polychètes Errantes*. Paris: Le Chevalier, 488 pp.

Fernandez-Leborans G, Tato-Porto ML. 2000. A Review of the Species of Protozoan Epibionts on Crustaceans. I. Peritrich Ciliates, *Crustaceana* 73(6): 643–683.

Fernandez-Leborans G, Chatterjee T, Grego M. 2012. New records of epibiont ciliates (Ciliophora) on Harpacticoida (Copepoda, Crustacea) from the Bay of Piran (Gulf of Trieste, Northern Adriatic), *Cahiers de Biologie Marine* 53: 53–63.

Haider G. 1964. Monographie der Familie Urceolariidae (Ciliata, Peritricha, Mobilina) mit besonderer Berucksichtigung der im süddeutschen Raum vorkommenden Arten. *Parasitologische Schriftenreihe*, 17: 1–251.

Haider G, Dietrich W. 1977. Das mobile peritriche ciliat *Urceolaria convexa* n. sp, *Hydrobiologia* 52(2–3): 135–141.

Hartmann-Schröder G. 1965. Zur Kenntnis der eulitoralen Polychaetenfauna von Hawaii, Palmira und Samoa. *Abhandlungen und Verhandlungen des Naturwissensschaftlichen Vereins in Hamburg* 9: 81–161.

Hochberg R, Litvaitis MK. 2000. Phylogeny of Gastrotricha: a morphology-based framework of gastrotrich relationships, *The Biological Bulletin* 198: 299–305.

Jankowski AW. 2007. Review of taxa Phylum Ciliophora Doflein, 1901. In: Alimov AF, ed. *Protista: Handbook on Zoology*. St. Petersburg: Nauka, Part 2, 415-993. [In Russian]

Jankowski AW. 2014. *Cothurnia polydorica* sp. nov. (Ciliophora: Peritrichia), a symbiont of shell-boring spionid polychaetes in the Sea of Japan, and a review of ciliates associated with polydorids, *Zoosistematica Rossica* 23(2): 167–173.

Kahl A. 1935. Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria), Eine Bearbeitung der freilebenden und ectocommensalen Infusorien der Erde, unter Ausschluß der marinen Tintinnidae, 4. Peritricha und Chonotricha. In: Dahl F, ed. *Die Tierwelt Deutschlands und der angrenzenden Meeresteile nach ihren Merkmalen und nach ihrer Lebensweise, 30*. Jena: Verlag von Gustav Fischer, 651–886.

Knox GA, Hicks K. 1973. The polychaetes of New Zealand Part 5. Onuphidae, *Journal of the Royal Society of New Zealand* 3(2): 281–294.

Lang K. 1948. On *Rhabdostyla commensalis* Möbius and two new *Cothurnia* species (Ciliata, Peritrichia) from the west coast of Sweden, *Arkiv för Zoologi* 41-B: 1–6.

Levander KM. 1915. Über eine neue marine *Cothumia*-Art, *Meddelanden et Societas Profauna et Flora Fennica* 10: 86–88.

Lewis JB. 1998. Reproduction, larval development and functional relationships of the burrowing, spionid polychaete *Dipolydora armata* with the calcareous hydrozoan *Millepora complanata*, *Marine Biology* 130: 651–662.

Lynn DH. 2008. *The Ciliated Protozoa: Characterization, Classification, and Guide to the Literature*. Dordrecht: Springer, 605 p.

Magagnini G, Verni F. 1988. Epibiosis of *Scyphidia* sp. (Ciliophora, Peritrichida) on *Nerilla antennata* (Archiannelida, Nerillidae), *Bolletino di Zoologia* 3: 185–189.

Möbius K. 1888. Bruchstücke einer Infusorienfauna der Kieler Bucht, *Archiv für Naturgeschichte* 1888: 81–116.

Parapar J, Kongsrud JA, Kongshavn K, Alvestad T, Aneiros F, Moreira J. 2018. A new species of *Ampharete* (Annelida: Ampharetidae) from the NW Iberian Peninsula, with a synoptic table comparing NE Atlantic species of the genus, *Zoological Journal of the Linnean Society* 183(3): 526–555.

Pierantoni U. 1903. La gestazione esterna (Contributo alla biologia ed alla embriologia dei Sillidi), *Archivio Zoologico Italiano* 1: 231–252.

Precht H. 1935. Epizoen der Kieler bucht, *Deutsche Akademie der Naturforscher Nova Acta Leopoldiana (Halle) N.F.* 3: 405–474.

San Martín G. 2003. Annelida Polychaeta II: Syllidae. In: Ramos MA, Alba J, Bellés X, Gosálbez J, Guerra A, Macpherson E, Martín F, Serrano J, Templado J, eds. *Fauna Ibérica. Vol. 21*. Madrid: Museo Nacional de Ciencias Naturales, CSIC, 1–554.

Sartini B, Marchesini R, D´ávila S, D'Agosto M, Dias RJP. 2018. Diversity and distribution of peritrich ciliates on the snail *Physa acuta* Draparnaud, 1805 (Gastropoda: Physidae) in a eutrophic lotic system, *Zoological Studies* 57: 1–12

Sergeeva N, Dovgal I. 2014. First finding of epibiont peritrich and suctorian ciliates (Ciliophora) on oligochaetes and harpacticoid copepods from the deep-water hypoxic/anoxic conditions of the Black Sea, *Ecologica Montenegrina* 1(1): 49–54.

Stiller J. 1968. Peritriche Ciliaten ökologisch verschiedener Biotope von Rovinj und Umgebung, *Acta Zoologica Academiae Scientiarum Hungaricae* 14: 185–211.

San Martín G. 2003. Annelida Polychaeta II: Syllidae. In: Ramos MA, Alba J, Bellés X, Gosálbez J, Guerra A, Macpherson E, Martín F, Serrano J, Templado J, eds. *Fauna Ibérica. Vol. 21*. Madrid: Museo Nacional de Ciencias Naturales, CSIC, 1–554.

Wahl M. 2009. Epibiosis: ecology, effects and defences. In: Wahl M, ed. *Marine Hard Bottom Communities*. *Patterns, Dynamics, Diversity, and Change.* Ecological Studies 206, Springer-Verlag Berlin Heidelberg, 61–72.

Warren A, Paynter J. 1991. A revision of *Cothurnia* (Ciliophora, Peritrichida) and its morphological relatives, *Bulletin of the British Museum (Natural History). Zoology* 57(1): 17–59.

Williams JD, Radashevsky VI. 1999. Morphology, ecology and reproduction of a new *Polydora* species (Polychaeta: Spionidae) from the East Coast of North America, *Ophelia*, 51(2): 115–127.

Xu K, Song, W. 2003. Brief introduction to the mobiline peritrich ciliates with revision of their systematics at the generic level. In: Song W, Zhao Y, Xu K, Hu X, Gong J. *Pathogenic Protozoa in Mariculture*, Beijing: Science Press, 291–314.

Zhan Z, Xu K, Dunthorn M. 2013. Evaluating molecular support for and against the monophyly of the Peritrichia and phylogenetic relationships within the Mobilida (Ciliophora, Oligohymenophorea), *Zoologica Scripta* 42(2): 213–226.

FIGURES LEGENDS

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

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, C,D=50 µm.

Figure 3. *Cothurnia amphicteis* 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. amphicteis* attached to the notochaeta. Arrows indicating ciliates. Scale bars: A=280 µm, B=140 µm.

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 outgrows. D, detail of *C. amphicteis* stalk showing transverse folds. Scale bars: A=60 µm, B=45 µm, C = 20 µm, D=12 µm.

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 µm, B=50 µm.

Fig. 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, C=20 µm.

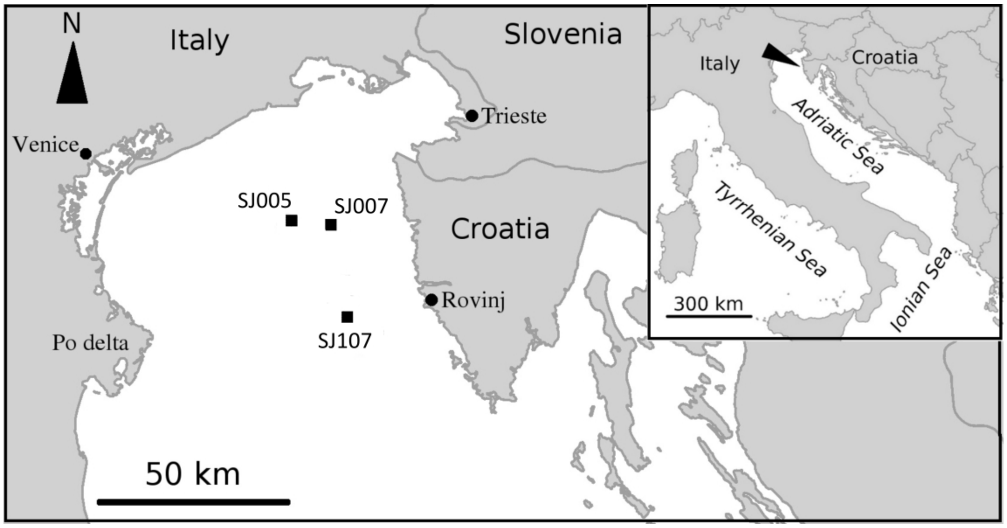

Figure 1. Map of sampling area and stations (SJ005, SJ007 and SJ107).

288x150mm (300 x 300 DPI)

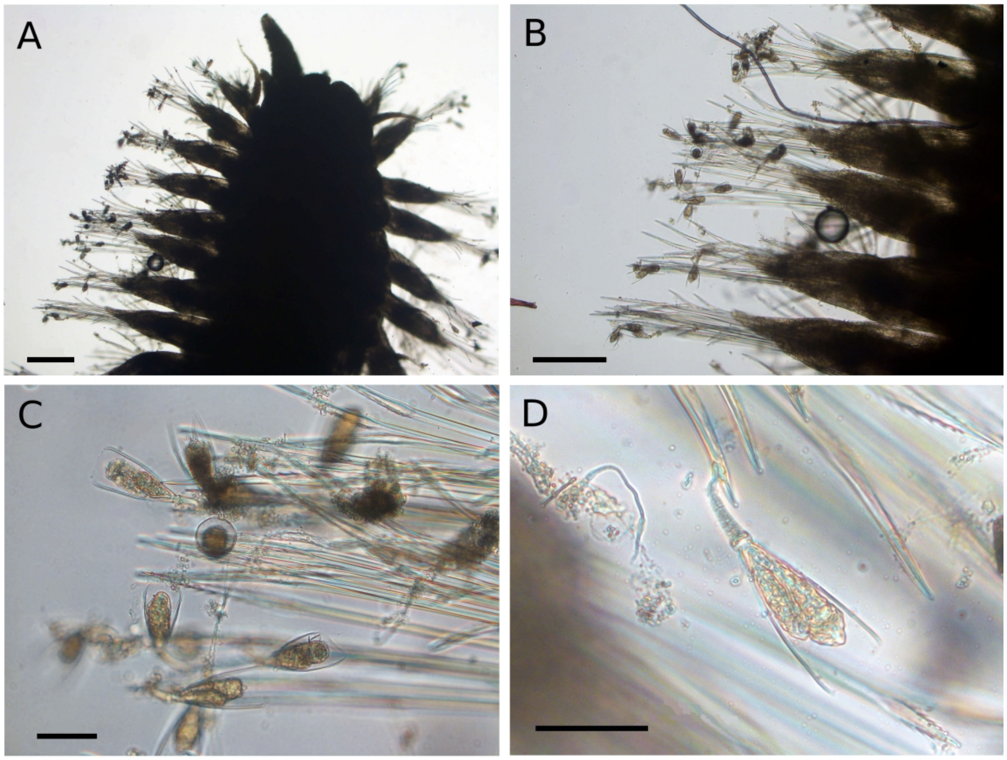

Figure 2. *Cothurnia amphicteis* on the chaetae of *Malmgrenia lilianae* (light microscopy). A, anterior part of *M. lilianae* showing densely attached ciliates. B, anterior chaetigers of *M. lilianae* with ciliates. C, anterior neurochaetae of *M. lilianae* with ciliates showing circular aperture of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

272x205mm (300 x 300 DPI)

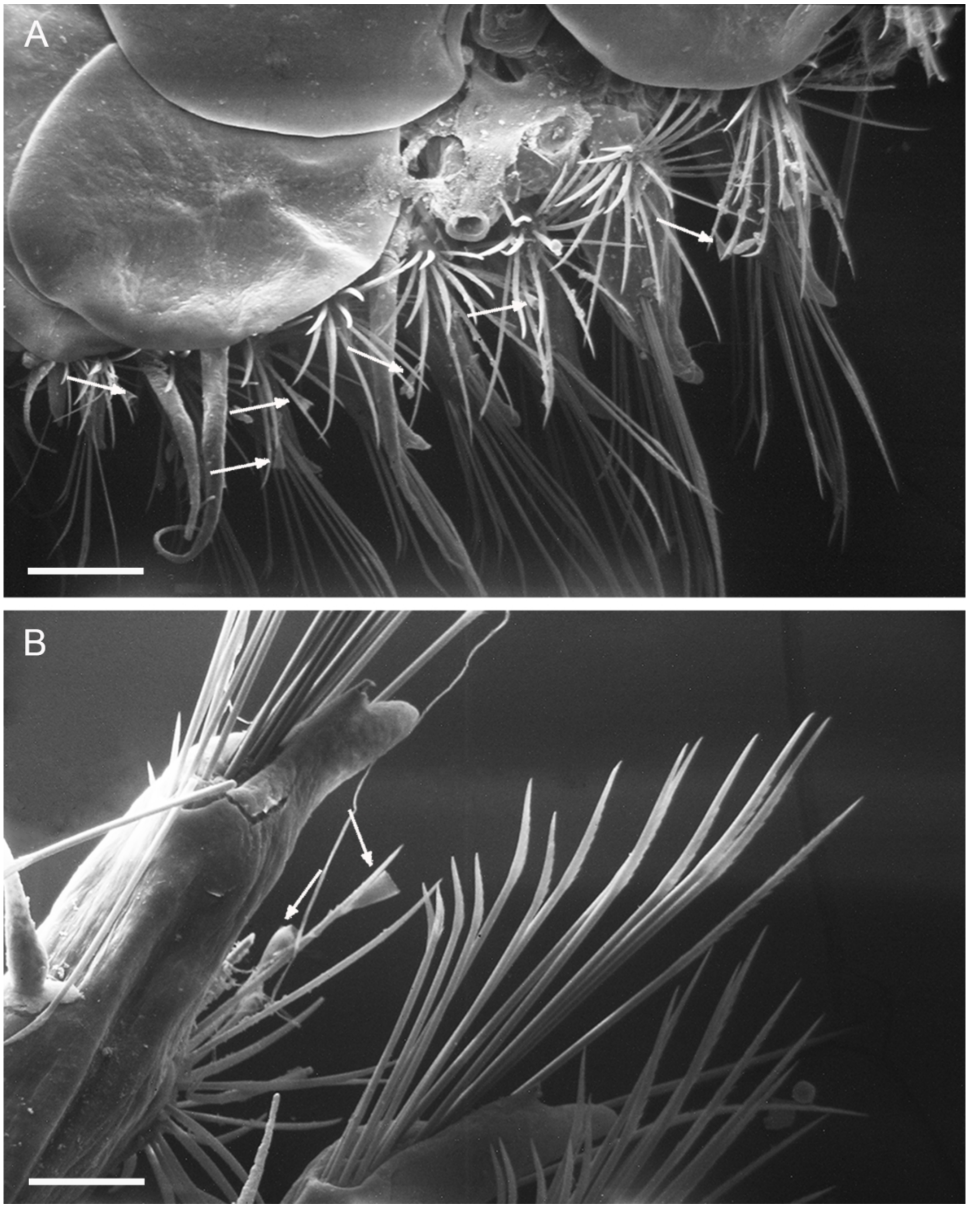

Figure 3. *Cothurnia amphicteis* on the chaetae of *Malmgrenia lilianae* (SEM). A, a part of *M. lilianae* body showing ciliates attached on the chaetae. B, close-up of one chaetiger with two specimens of *C. amphicteis* attached to the notochaeta. Arrows indicating ciliates. Scale bars: A=280 μm, B=140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

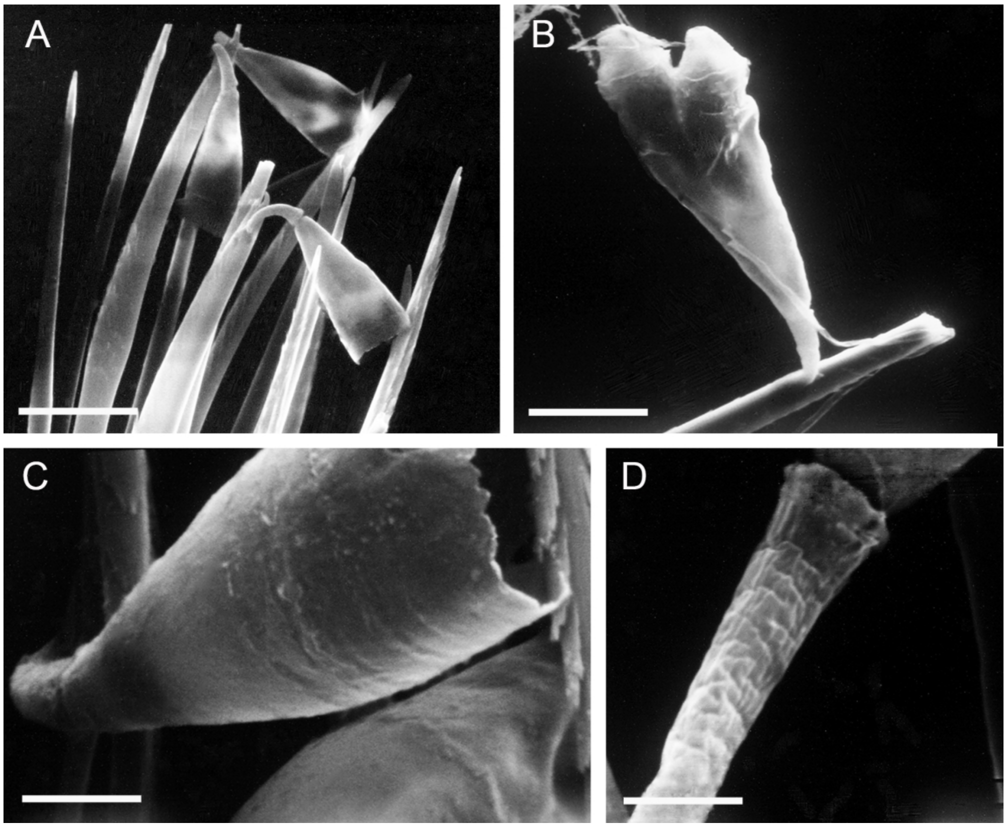

Figure 4. *Cothurnia amphicteis* on the chaetae of *Malmgrenia lilianae* (SEM). A, C. amphicteis attached to the notochaetae, showing stalk flexed near substrate. B, one specimen of *C. amphicteis* showing two zooids. C, detail of *C. amphicteis* lorica showing the edge of aperture with small, irregular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

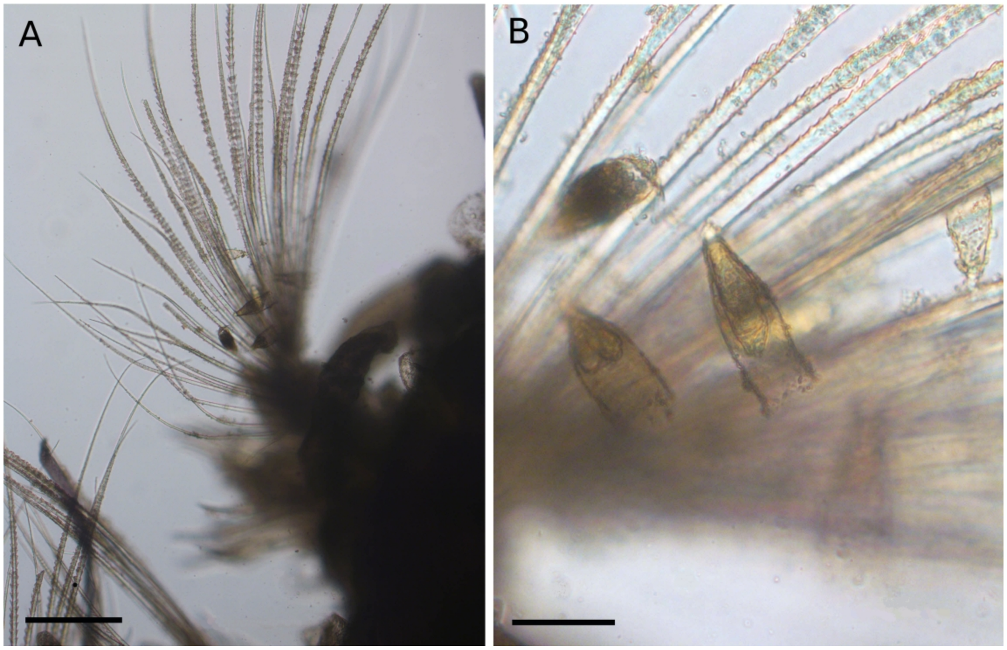

Figure 5. *Cothurnia peloscolicis* on the chaetae of *Sthenelais boa* (light microscopy). A, one chaetiger of *S. boa* with ciliates attached on the neurochaetae. B, close-up of neurochaetae with several ciliates showing short stalk and two zooids. Scale bars: A=200 μm, B=50 μm.

289x186mm (300 x 300 DPI)

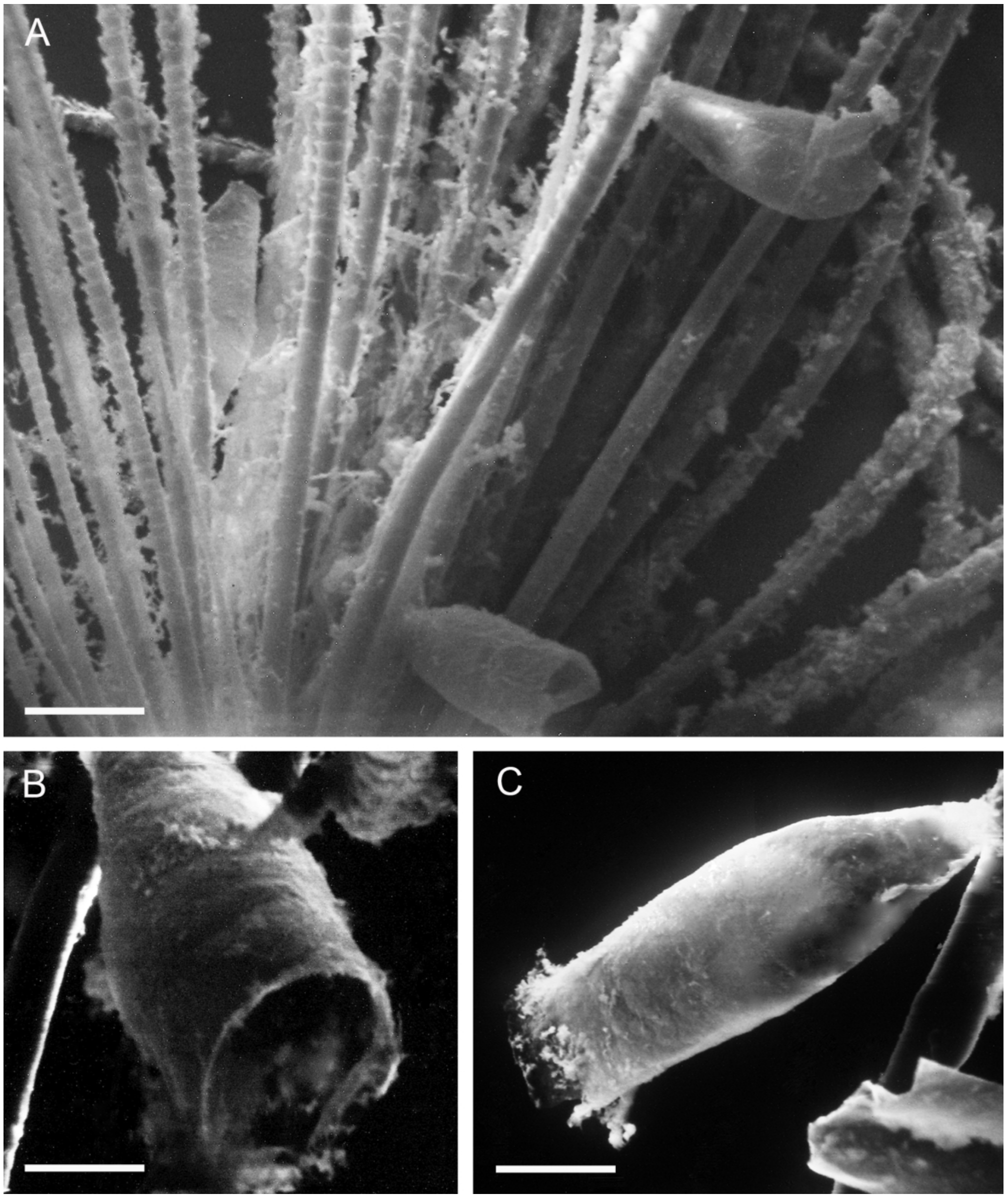

Fig. 6. *Cothurnia peloscolicis* on the chaetae of *Sthenelais boa* (SEM). A, neurochaetae of *S. boa* with attached ciliates. B, close-up of ciliate *C. peloscolicis* lorica showing circular aperture. C, one *C. peloscolicis* attached to *S. boa* neurochaeta. Scale bars: A=35 μm, B=15 μm, C=20 μm.