Syst Parasitol (2018) 95:625–643
https://doi.org/10.1007/s11230-018-9805-1

Xenoligophoroides cobitis (Ergens, 1963) n. g., n. comb. (Monogenea: Ancyrocephalidae), a parasite of *Gobius cobitis* Pallas (Perciformes: Gobiidae) from the Mediterranean and Black seas

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Received: 18 October 2017 / Accepted: 20 January 2018 / Published online: 11 July 2018

Abstract Based on an integrative taxonomic approach, combining morphological characters and partial sequences of the nuclear 28S rRNA gene, a new genus and combination for the species *Xenoligophoroides cobitis* (Ergens, 1963) is proposed, to accommodate ancyrocephalid monogeneans, parasites on the gills of *Gobius cobitis* Pallas (Gobiidae) from the western Mediterranean Sea and the northern Black Sea. A morphological comparison of newly collected material with the descriptions of *Ancyrocephalus cobitis* Ergens, 1963 and *Haliotrema cupensis* Sasal, Pages & Euzet, 1998, recently synonymised and named as *Haliotrema cobitis* (Ergens, 1963), confirms their similarity and belonging to the same species. However, characters of this species, as the vas deferens not looping the caecal branch, the bilobed base of the male copulatory organ and the marginal hooks with an upright thumb, do not correspond to the diagnosis of *Haliotrema* Johnston & Tiegs, 1922. Morphologically, this species is close to members of *Ligophorus* Euzet & Suriano, 1977 and *Kriboetrema* Sarabeev, Rubtsova, Yang & Balbuena, 2013, but differs from the former in the accessory piece articulated with MCO and two prostatic reservoirs, and from the latter in the uncoiled MCO with bilobed base and the dextral vaginal pore. Moreover, all species of *Ligophorus* and *Kriboetrema* are parasites of grey mullets. A 28S rDNA-based phylogenetic analysis of sequences derived from specimens of *X. cobitis* from the Mediterranean and Black seas, along with sequences from several closely related genera of the Ancyrocephalidae, suggested the occurrence of a new

This article was registered in the *Official Register of Zoological Nomenclature* (ZooBank) as 07D79560-0C71-493A-801C-54ECAC396789. This article was published as an Online First article on the online publication date shown on this page. The article should be cited by using the doi number. This is the Version of Record.

This article is part of the Topical Collection Monogenea.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11230-018-9805-1) contains supplementary material, which is available to authorized users.

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taxonomic unit, which corresponded to the *X. cobitis* specimens. This finding supports the establishment of the new genus for the ancyrocephalid parasites on the gills of *G. cobitis* from the western Mediterranean Sea and the northern Black Sea.

Introduction

The genus *Haliotrema* Johnston & Tiegs, 1922 currently includes 139 nominal species (Cruces et al., 2017; Gibson & Bray, 2017; Mendoza-Franco et al., 2017), parasites of a wide range of fish species, from 33 families of 6 orders of teleosts (Kritsky & Stephens, 2001). Despite the several revisions and the exclusion of many species raised to the generic level, this taxon is still considered as a polyphyletic group, as shown by morphological as well molecular data (Kritsky & Boeger, 2002; Wu et al., 2006, 2007; Dang et al., 2010; Sun et al., 2014, 2015; Kritsky & Bakenhaster, 2016).

Only one species of *Haliotrema*, *Haliotrema cupensis* Sasal, Pages & Euzet, 1998, a parasite on the gills of *Gobius cobitis* Pallas, is so far known in the Mediterranean region; moreover this is the sole representative of this genus parasitising fish of the family Gobiidae. Additionally, there is a number of marine ancyrocephalids, namely *Ancyrocephalus cobitis* Ergens, 1963 from the same host species in the Adriatic and Black seas (Ergens, 1960, 1963; Naidenova, 1970), *Ancyrocephalus littoralis* Paperna & Kohn, 1964 from *Atherinomorus pinguis* (Lacepède) and *Ancyrocephalus salinus* Paperna, 1964 from *Aphanius dispar* (Rüppel) in the Mediterranean sea, off Israel (Paperna, 1964; Paperna, Kohn, 1964), whose generic affiliation is still not clear.

Recently, Merella et al. (2010) synonymised *H. cupensis* with *A. cobitis*, based on the comparison of the morphological descriptions of both species (Ergens, 1963; Sasal et al., 1998), and proposed the new combination *Haliotrema cobitis* (Ergens, 1963).

The aim of the present study is to determine the appropriate generic placement of the ancyrocephalid parasites of *G. cobitis* in the Mediterranean and Black seas, and to clarify their phylogenetic position in relation to other marine genera of Ancyrocephalidae Bychowsky & Nagibina, 1968, based on the nuclear 28S ribosomal sequence analysis. The results of the morphological comparisons and phylogenetic analyses showed that the ancyrocephalid monogenean parasite of *Gobius cobitis* represents an undescribed taxon, therefore a new genus is erected.

Materials and methods

Eighteen specimens of *Gobius cobitis*, 6–15 cm in total length (TL), were caught off northeastern Sardinia (western Mediterranean Sea, 41°08′05″N, 9°06′05″E) in October 2008 (9 specimens) and June 2015 (9 specimens), one further specimen (TL 20 cm) was caught off the western coast of the Caucasus (northeastern Black Sea, 44°34′32″N, 37°58′43″E) in July 2015. Gills were examined under a dissecting microscope for the presence of monogeneans. All monogeneans were collected alive, some of them were immediately mounted in glycerine-jelly (prepared with 0.5 g carbolic acid) after Gusev (1983), and others were stored in absolute ethanol and stored at 5°C for DNA analysis.

Measurements and light micrographs were made with Olympus BX51 and CX41 microscopes, using phase-contrast optics and CellSense digital image analysis software.

The measuring scheme (Fig. 1) mainly followed that suggested for the Dactylogyridae by Gussev (1985). Abbreviations of the linear measurements are as follows: APL, length of the accessory piece of the male copulatory organ (MCO); APW, width of the MCO accessory piece; APDL, length of the distal end of the MCO accessory piece; BLC, length of the lateral chamber of the basal widening of the MCO; BMC, length of the main chamber of the basal widening of the MCO; DA, dorsal anchor sickle aperture distance; DBH, dorsal bar height; DBW, dorsal bar width; DI, dorsal anchor inner length; DIR, dorsal anchor inner root length; DM, dorsal anchor main part length; DO, dorsal anchor outer length; DOR, dorsal anchor outer root length; DP, dorsal anchor point length; DSR, span between dorsal anchor roots; MCO, male copulatory organ tube length; MHL, marginal hook total length; MHSL, marginal hook shaft length; VA, ventral anchor sickle aperture distance; VBHm, ventral bar median part height; VBL, ventral bar lateral part length; VBW, ventral bar total width; VBS, ventral bar median slit height; VBWm, ventral bar median part width; VI, ventral anchor inner length; VIR, ventral anchor inner root length; VL, vagina length; VM,

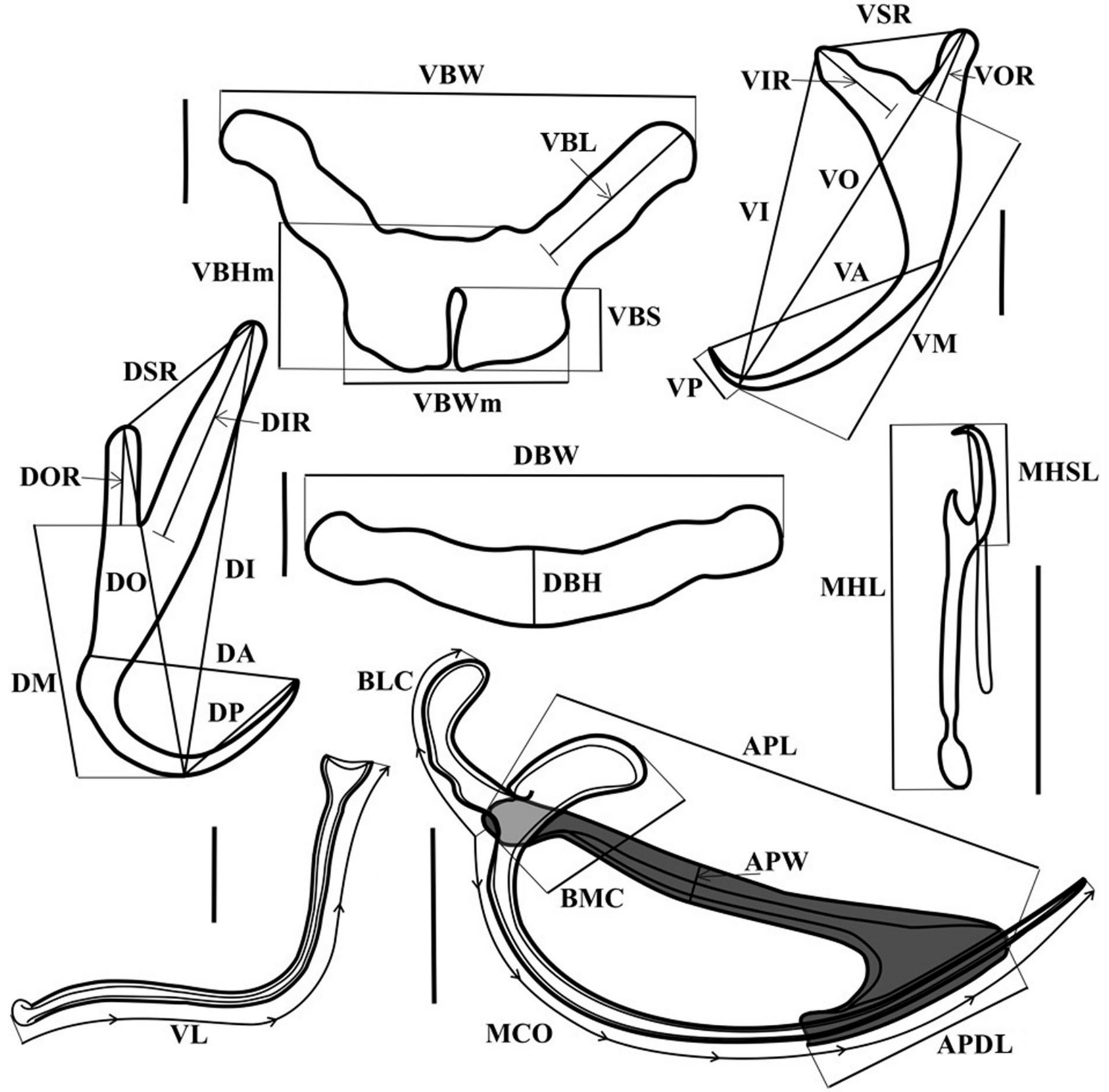

Fig. 1 Drawings and measurement scheme of the haptoral structures, male copulatory organ and vagina of *Xenoligophoroides cobitis* (Ergens, 1963) n. g., n. comb. ex *Gobius cobitis* Pallas. *Abbreviations*: see Materials and methods section. *Scale-bars*: 10 µm

ventral anchor main part length; VO, ventral anchor outer length; VOR, ventral anchor outer root length; VP, ventral anchor point length; VSR, span between ventral anchor roots. Body size is given for mounted and flattened but unbroken worms; width was measured at the level of the vaginal opening. All measurements are given in micrometres, as the range followed by the mean with standard deviation and the number of measurements (in parentheses). Descriptive statistics were produced using the software package Statistica 6 for Windows.

Prior to DNA analysis, each specimen was identified based on the haptoral structures and the MCO morphology. Total DNA was isolated from ethanol-stored single specimens, placing the samples in 200 µl of a 5% suspension of deionised water and ChelexW (BT Chelex® 100 Resin BIO-RAD, USA) containing 0.1 mg/ml proteinase K, followed by incubation at

Fig. 2 *Xenoligophoroides cobitis* (Ergens, 1963) n. g., n. comb. ex *Gobius cobitis* Pallas. Photomicrograph of whole-mount. *Scale-bar*: 20 µm

56°C for 3 h, boiling at 90°C for 8 min, and centrifugation at 14,000 × rpm for 10 min.

The 28S rDNA portion was amplified using the primers C1 (5′-ACC CGC TGA ATT TAA GCA T-3′) and D2 (5′-TGG TCC GTG TTT CAA GAC-3′) as described by Jovelin & Justine (2001), modified as reported in Wu et al. (2005). The amplification procedure involved an initial denaturation of 5 min at 94°C, followed by 30 cycles of 1 min at 94°C, 1 min at 56°C, 1 min at 72°C, and 5 min extension at 72°C. All PCR were carried out in a final volume of 25 µl, using the amplification kit PuRe Taq™ Ready-to-go™ PCR beads GE Healthcare (UK). The reaction was prepared adding 18 µl Milli-Q® (Merck, Germany) water, 10 µM of each primer, and 3 µl of 50 ng/µl of template DNA. Both positive and negative controls were used to test the effectiveness of the PCR reagents. PCR products were purified using the kit NucleoSpin Extract (Macherey-Nagel, Duren, Germany), according to the manufacturer's instructions, and sequenced by an external core service (Macrogen, the Netherlands) for both strands using the PCR primers.

Newly-generated 172 nt long sequences were aligned using the programme CLUSTAL W (Thompson et al., 1994), as implemented in the BioEdit 7.0.5.2 software package (Hall, 1999) and deposited in the GenBank.

The software MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) was employed to perform a phylogenetic Bayesian analysis, specifying a partitioned model; *Pseudomurraytrema* sp. was used as the outgroup. Two independent runs, each consisting of seven Metropolis coupled Markov Chain Monte Carlo (MCMCMC) simulations, were run simultaneously for 5,000,000 generations, sampling trees every 1,000 generations. Each partition was allowed to have its own set of parameters, as retrieved by JModeltest (Posada, 2008). The first 25% of sampled trees were discarded as 'burn-in'. Nodes with a percentage of posterior probability < 0.70 were considered to be not statistically supported. The uncorrected pairwise genetic distances (p-distance) between the taxa were estimated using the software MEGA 7 (Kumar et al., 2016). The analysis involved 56 nucleotide sequences. All positions containing gaps and missing data were eliminated.

Order Dactylogyridea Bychowsky, 1937

Family Ancyrocephalidae Bychowsky & Nagibina, 1968 (*sensu lato*)

Xenoligophoroides n. g.

Diagnosis

Monogenea, Ancyrocephalidae. Body slightly flattened dorsoventrally, comprising cephalic region with 4 lobes, fusiform trunk, short peduncle and trapezoidal haptor. Tegument smooth. Cephalic organs 3 pairs. Eyespots 2 pairs. Mouth subterminal, midventral; pharynx muscular, glandular; oesophagus short; intestinal caeca 2, united posterior to gonads, lacking diverticula. Gonads intercaecal, tandem or slightly overlapping; ovary pretesticular, ventral. Vas deferens sinistral, not looping left intestinal caeca; seminal vesicle large, represents dilation of vas deferens in the middle of its length; prostatic reservoirs 2. Male copulatory organ (MCO) comprising copulatory tube and accessory piece. MCO tube uncoiled, with bilobed base. Accessory piece articulated to MCO base, gutter-shaped, distal end with transverse process, serving as guide for distal portion of MCO. Vagina dextral, sclerotised. Seminal receptacle preovarian. Vitelline follicles scattered throughout trunk. Haptor with well-developed lateral flaps armed with dorsal and ventral anchor/bar complexes and 7 pairs of similar and unmodified marginal hooks. Marginal hooks with upright blunt-pointed thumb, straight shaft with bulb extension at the end separated by well-marked constriction.

Type-species: *Xenoligophoroides cobitis* (Ergens, 1963) n. comb.

Etymology: The genus name refers to its morphological similarity to *Ligophorus* Euzet & Suriano, 1977 and to the different host taxa, from the Greek “xeno” meaning “foreign”.

ZooBank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the *International Code of Zoological Nomenclature* (ICZN, 2012), details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) for *Xenoligophoroides* n. g. urn:lsid:zoobank.org:act:0B5A3C9A-9F85-460F-B91D-E3D3CBA94B81.

Xenoligophoroides cobitis (Ergens, 1963) n. comb.

Syns *Ancyrocephalus* sp. of Ergens (1960); *Ancyrocephalus cobitis* Ergens, 1963; *Haliotrema cupensis* Sasal, Pages & Euzet, 1998; *Haliotrema cobitis* (Ergens, 1963) Merella, Dmitrieva, Piras, Huyse, Gerasev & Garippa, 2010

Type-host: *Gobius cobitis* Pallas (Perciformes: Gobiidae).

Type-locality: Adriatic Sea, off Albania (Gulf of Vlorë).

Other locality: Cerbère Banyuls Marine Reserve (42°27′50″N, 03°09′35″E) (Sasal et al., 1998) and off north-west Sardinia (41°08′05″N, 9°06′05″E) (present study), Mediterranean Sea; off Tuapse (44°05′37″N, 39°02′56″E) (Naidenova, 1972) and off Gelendzhik (44°34′32″N, 37°58′43″E) (present study), off Caucasus coast, Black Sea.

Voucher material: 55 specimens deposited in the collections of the A.O. Kovalevsky Institute of Marine Biological Research (http://marineparasites.org/taxa/?taxon=7), Sevastopol (Nos. 1001.M.8x.v1-1038.M.8x.v54–55).

Site on host: Gills.

Prevalence and intensity: Mediterranean Sea, off Sardinia (TL 6–15 cm), October 2008 [100% of 9 hosts examined, intensity 4–15 (mean 10)] and June 2015 [100% of 9 hosts examined, intensity 30–60 (42)]; Black Sea, off Caucasus (TL 24 cm), July 2015 (1 host examined, intensity 65).

Redescription (Figs. 1–5)

[Based on 49 adult specimens, see metrical data in Table 1.] Small ancyrocephalids, body dorsoventrally flattened, 380–800 × 60–150 (n = 16). Tegument smooth. Cephalic region with 4 well-developed lobes and 3 pairs of cephalic organs; 2 cephalic glands symmetrically lateral to pharynx present. Eyespots 2 pairs, posterior pair larger than anterior. Mouth subterminal, midventral; muscular and glandular pharynx ovoidal, 12–16 (n = 6) in diameter; oesophagus short; intestinal bifurcation just posterior to pharynx; caeca 2, unite just posterior to testis, lacking diverticula.

Body peduncle short. Haptor trapezoidal, well differentiated from body proper, wider than long, 75–100 × 130–180 (n = 18). Dorsal and ventral anchor pairs and bars different in size and shape (Table 1). Dorsal anchors massive, with point longer than shaft, so that blade is sickle-shaped, and inner root twice as long as outer root. Ventral anchors smaller than dorsal, with point distinctly shorter than shaft and almost equal in length short roots. Distal part of blade of both anchors slides within tissue sleeve; thread-like ligaments extend from notch on outer proximal surface of blades to opening through which anchors are projected from the haptor (anchoral openings) (Fig. 3A, B). Dorsal bar slightly bowed in middle, with straightened ends. Ventral bar V-shaped,

Table 1 Comparative metrical data for *Xenoligophoroides cobitis* (Ergens, 1963) n. g., n. comb. from different regions

Region Source	Adriatic Sea Off Albania Ergens (1960, 1963)	Mediterranean Sea Off France Sasal et al. (1998)	Mediterranean Sea Off Sardinia Present study	Black Sea Off Caucasus Present study
Body length	250–620	185–369	380–633 (n = 8)	470–800 (n = 8)
Width	90–170	54–94	60–90 (n = 8)	110–150 (n = 8)
Haptor length	70–80	–	75–98 (n = 10)	75–100 (n = 8)
Width	110–190	67–111	130–150 (n = 10)	130–180 (n = 8)
Dorsal anchor: DI[a]	49–57	37–46 (40 ± 2; n = 30)	39–50 (45 ± 3; n = 28)	52–57 (54 ± 2; n = 15)
DO	–	28–36 (31 ± 2; n = 30)	31–39 (35 ± 2; n = 28)	40–48 (44.5 ± 2; n = 15)
DIR	25–27	17–25 (20 ± 1; n = 29)	18–26 (22 ± 2; n = 28)	26–28 (27 ± 1; n = 15)
DOR	11–13	8–12 (9 ± 1; n = 29)	7–12 (9.8 ± 1.5; n = 28)	11–12 (11 ± 0.5; n = 15)
DM	28.5–32	–	22–26 (24 ± 1; n = 28)	31–36 (33 ± 2; n = 15)
DP	19–21	16–21 (18 ± 1; n = 30)	15–19 (16 ± 1.5; n = 28)	18–19 (18 ± 0.5; n = 15)
DA	–	–	20–25 (21.5 ± 1; n = 28)	26–29 (27 ± 1; n = 15)
DSR	–	–	13–19 (16 ± 2; n = 28)	19–21 (20 ± 0.5; n = 15)
Ventral anchor: VI[a]	–	25–34 (31 ± 2; n = 29)	29–40 (33 ± 3; n = 28)	36–45 (40.5 ± 3; n = 15)
VO	46–51	34–41 (38 ± 1; n = 29)	39–44 (41 ± 2; n = 28)	45–51 (48 ± 2; n = 15)
VIR	11–13	8–11 (9 ± 2; n = 30)	9–12 (10 ± 1; n = 28)	11–14 (12.5 ± 1; n = 15)
VOR	12–13	6–11 (8 ± 2; n = 30)	6–12 (8.5 ± 2; n = 28)	9–14 (12 ± 1.5; n = 15)
VM	34–36	–	29–36 (33 ± 2; n = 28)	32–36 (34 ± 1; n = 15)
VP	5.7	–	4–5 (5 ± 0.5; n = 28)	4–5 (4.5 ± 0.5; n = 15)
VA	–	–	21–23 (22 ± 1; n = 28)	21–23 (22 ± 1; n = 15)
VSR	–	–	14–17 (16 ± 1; n = 28)	13–17 (14.5 ± 1; n = 15)
Marginal hook: MHL[a]	–	12–16 (15 ± 1;n = 30)	15–17 (16 ± 0.5; n = 40)	16–17 (16 ± 0.5; n = 25)
MHSL	–	8–10 (9 ± 1; n = 30)	9–11 (10.5 ± 0.5; n = 40)	9–11 (10 ± 0.5; n = 25)
Dorsal bar: DBH[a]	8–9.5	4–6 (5 ± 1; n = 30)	5–10.5 (7 ± 2; n = 26)	6–10 (8 ± 1; n = 15)
DBW	44–47.5	26–40 (32 ± 3; n = 30)	31–50 (40 ± 6.5; n = 26)	45–53 (48 ± 2; n = 15)
Ventral bar: VBW[a]	51–57	33–45 (39 ± 3; n = 30)	37–55 (45.5 ± 6; n = 26)	52–60 (56 ± 0.5; n = 15)
VBHm	15–17	8–12 (10 ± 1; n = 28)	11–17 (13.5 ± 2; n = 26)	14–18 (16 ± 1; n = 15)
VBL	21–22	–	17–25 (19 ± 2.5; n = 26)	21–25 (23 ± 1; n = 15)
VBWm	21–25	–	20–27 (22 ± 2.5; n = 26)	20–25 (22.5 ± 2; n = 15)
VBS	–	–	5–9 (7 ± 1.5; n = 26)	8–10 (8 ± 1; n = 15)
MCO tube length	40–42	25–36 (30 ± 3; n = 22)	39–44 (42 ± 1.5; n = 20)	52–63 (56 ± 3; n = 12)
MCO accessory piece: APL[a]	–	–	23–31 (27.6 ± 2; n = 20)	34–39 (36 ± 1.5; n = 12)

Table 1 continued

Region Source	Adriatic Sea Off Albania Ergens (1960, 1963)	Mediterranean Sea Off France Sasal et al. (1998)	Mediterranean Sea Off Sardinia Present study	Black Sea Off Caucasus Present study
APW	–	–	2–2.5 (2 ± 0.5; n = 20)	3 (n = 12)
APDL	–	–	12–15 (13 ± 1.5; n = 20)	18–24 (20 ± 1.5; n = 10)
BLC	–	–	8–12 (9.5 ± 1; n = 15)	9–12 (11 ± 1; n = 9)
BMC	–	–	8–10 (9 ± 0.5; n = 15)	15–19 (17 ± 1; n = 9)
Vagina length	38–40	–	31–37 (34 ± 2; n = 22)	44–55 (48 ± 3; n = 12)

[a]For abbreviations see Materials and methods

with massive posterior prominence in middle, divided into 2 parts by deep slit (Fig. 3A). Marginal hooks 14, 15–17 long (n = 65), consist of sickle formed by short base with small upright thumb, slightly curved blade, and straight shaft with relatively flexible constriction located at *c.* 3/4 of shaft length, separating conspicuous oval widening at distal end (Fig. 3C).

Testis elongate-oval, 38, 47 × 30, 45 (n = 2), intercaecal, situated between ovary and caecal union. Male copulatory organ (MCO) consists of copulatory tube and accessory piece. Copulatory tube simple, slightly bent, 39–63 long (n = 32). Accessory piece rod-shaped, with transverse gutter-shaped process at distal end, serving as guide for distal portion of MCO; proximal end connected with MCO base (Fig. 4, Table 1). MCO base 2-chambered, receiving ducts of 2 large prostatic reservoirs. Seminal vesicle large, representing simple dilation of vas deferens, at some distance from MCO base, opening into latter through an elongated narrow duct. Vas deferens sinistral, not looping left intestinal caecum (Fig. 4B).

Ovary elongate-oval, 28, 32 × 20, 30 (n = 2), pretesticular, median. Vaginal canal 31–55 long (n = 34), with thick sclerotised walls; distal part cup-shaped, widened, opens dextrally posterior to copulatory complex (Fig. 4C). Vitellarium follicular, in 2 lateral fields along intestinal caeca. Oviduct, oötype and Mehlis' gland not observed. One ovoid egg observed, 69 × 71.

Development of haptoral armament in ontogeny

Different stages of development of the post-larvae were observed (Fig. 5); these showed a sequence of formation of the haptoral armament. The marginal hooks of post-larvae and mature specimens do not change in shape and size (Table 2, Fig. 5). The complex of ventral anchors and bar are formed first. Dorsal anchors appear in the posterior part of the body, and then migrate to the haptor. The dorsal bar, the last formed structure, starts to develop in two parts (Fig. 5B). This sequence of development of the haptoral structures is common for ancyrocephalids and was previously described for many species (e.g. Bychowsky, 1957; Malmberg, 1990).

Remarks

Xenoligophoroides cobitis was first described from *G. cobitis* in the Adriatic Sea as *Ancyrocephalus cobitis*

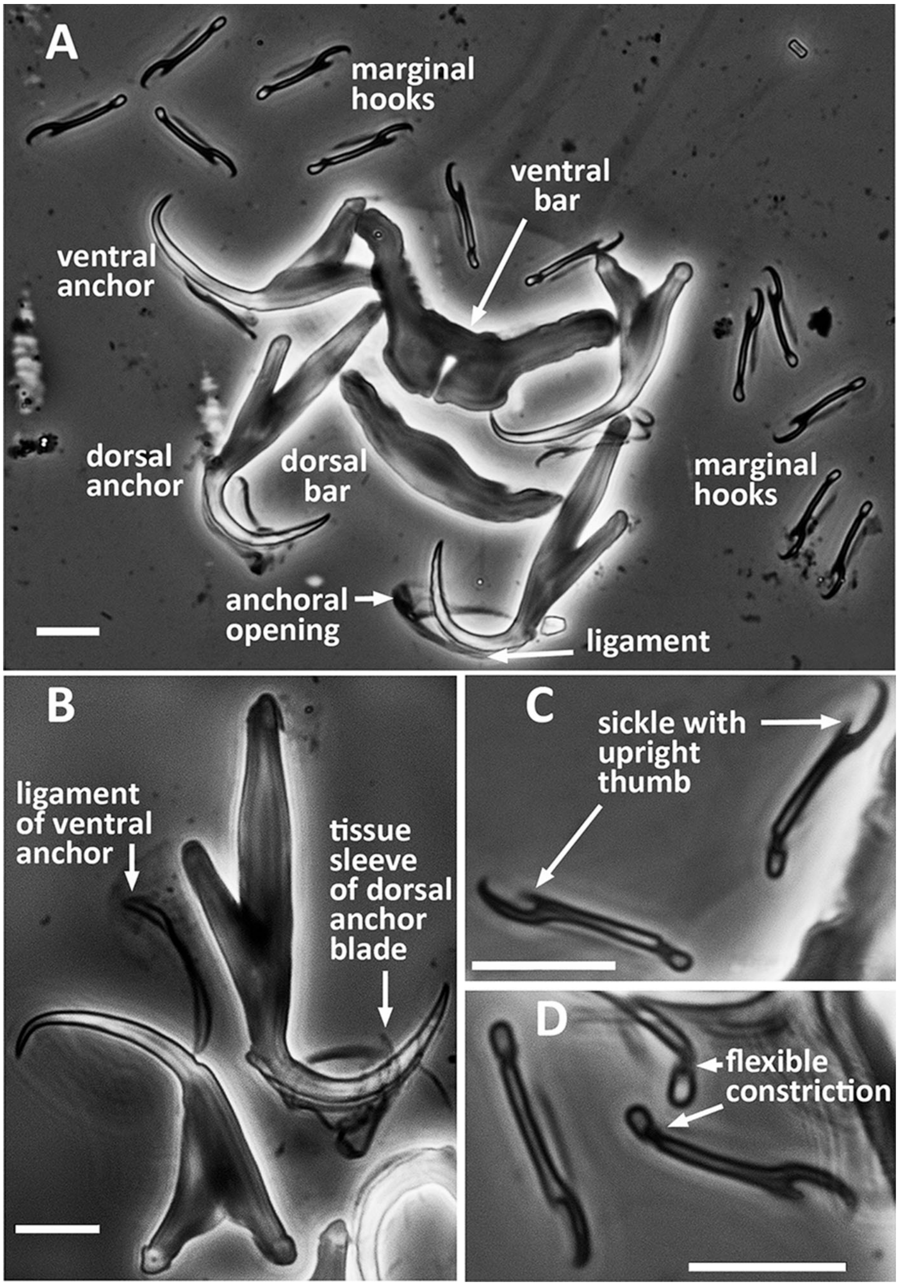

Fig. 3 Photomicrographs of the haptoral structures of *Xenoligophoroides cobitis* (Ergens, 1963) n. g., n. comb. ex *Gobius cobitis* Pallas. A, Haptor armament; B, Ventral and dorsal anchors; C, D, Marginal hooks. *Scale-bars*: 10 µm

(Ergens, 1963). Naidenova (1974) reported this species from the same host in the Black Sea near Tuapse (Caucasus coast), but the publication did not provide any description or drawings. Taking into account that the generic diagnosis of *Ancyrocephalus* Creplin, 1839 has been changed, and now it only includes parasites of the Percidae (see Bychowsky & Nagibina, 1970), *A. cobitis* clearly cannot belong to this genus.

On the other hand, the ancyrocephalid monogenean *Haliotrema cupensis* was described from *G. cobitis* in the Mediterranean Sea off the coast of France (Sasal

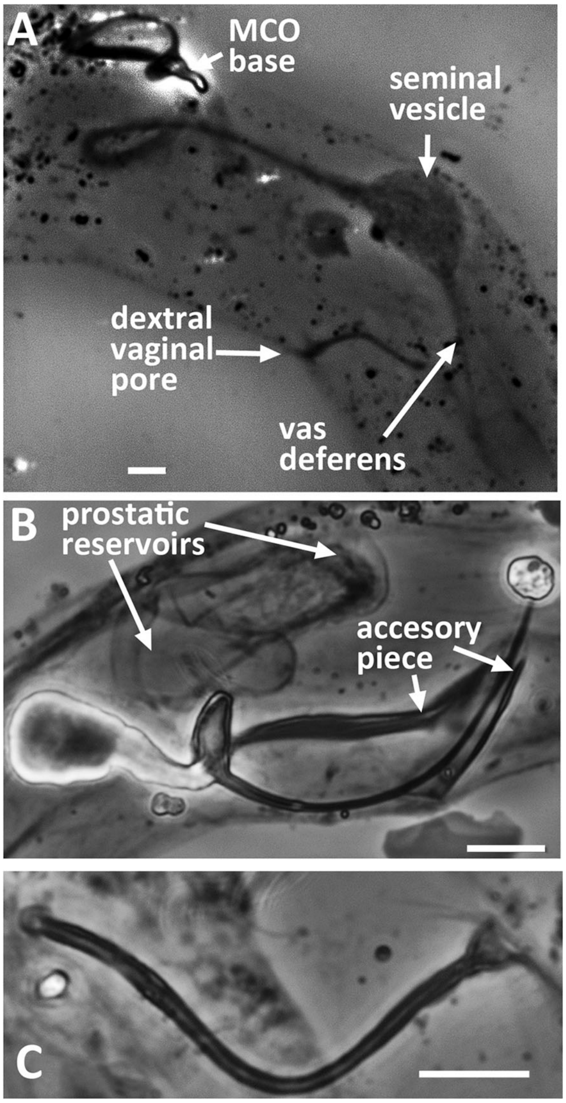

Fig. 4 Photomicrographs of the male reproductive system elements (A, ventral view; B, dorsal view) and vagina (C, dorsal view) of *Xenoligophoroides cobitis* (Ergens, 1963) n. g., n. comb. ex *Gobius cobitis* Pallas. *Scale-bars*: 10 µm

et al., 1998). Comparison of the descriptions of *H. cupensis* with the measurements and the drawings of *A. cobitis* showed that the two sets of specimens belong to the same species, for which a new combination of *Haliotrema cobitis* (Ergens, 1963) was proposed (Merella et al., 2010).

Specimens of *X. cobitis* found on the gills of *Gobius cobitis* off northwestern Sardinia (Mediterranean Sea) and Caucasus (Black Sea) are identical in the general body morphology, internal anatomy and the shape of the haptoral structures, copulatory organ and vagina (Figs. 1–5) with those of *Ancyrocephalus cobitis*, described from the same host in the Adriatic Sea (Ergens, 1963), as well as to those of *Haliotrema cupensis* from the Mediterranean Sea (Sasal et al., 1998). However, specimens from the Black Sea exhibit larger measurements for body size and some lengths of anchors, namely DI, DO, DM, DA and VO, MCO tube and accessory piece and vagina, than specimens from the Mediterranean Sea, previously described (Sasal et al., 1998) and collected off Sardinia in the present study (Table 1). This could be due to both the geographical distance between the sampling sites, and the higher total length of the fish

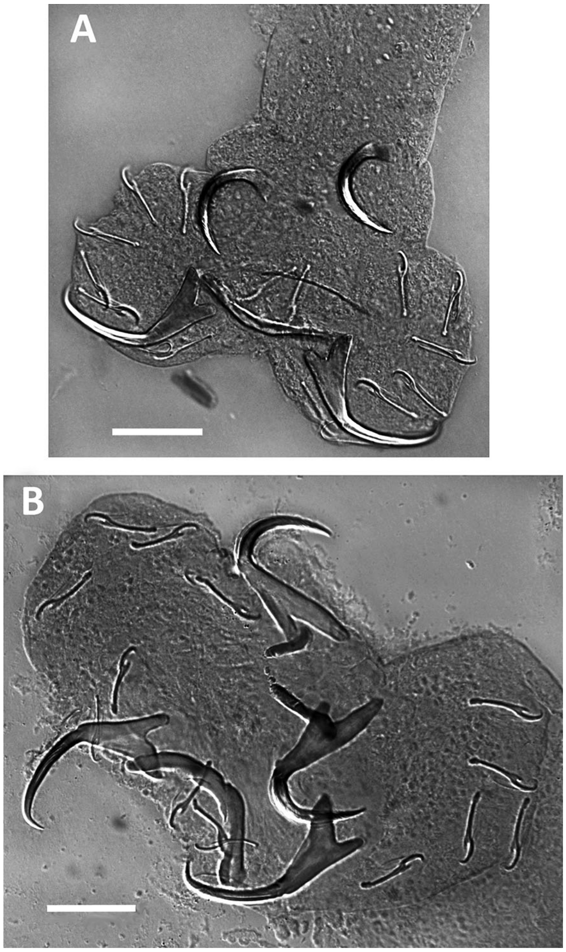

Fig. 5 Photomicrographs of the haptor of the post-larvae of *Xenoligophoroides cobitis* (Ergens, 1963) n. g., n. comb. ex *Gobius cobitis* Pallas, 219 µm (A) and 265 µm (B) in length. *Scale-bars*: 20 µm

Table 2 Comparative data for post-larvae and adults of *Xenoligophoroides cobitis* (Ergens, 1963) n. g., n. comb. from the Mediterranean Sea

	Post-larva with dorsal anchor		Adult
	In peduncle	Migrated into haptor	
No. of specimens	1	5	28
Body size	130 × 35	220–265 × 55–60	350–633 × 65–90
Haptor size	35 × 60	44–71 × 88–124	70–85 × 120–150
Dorsal anchor: DI[a]	–	32–34	39–49
DIR	–	14–15	18–26
DOR	–	6	7–12
DP	–	16–17	15–19
DA	–	20–22	20–25
DSR	–	11–12	13–19
Ventral anchor: VI[a]	–	30–32	29–40
VIR	–	6.5–7	9–12
VOR	–	4–5	6–12
VP	4	4–5	4–5
VA	–	18–19	21–23
VSR	–	10–11	14–17
Marginal hook: MHL[a]	15	15.5–16	15–17
Ventral bar: VBW[a] × VBH	29 × 2	30–37.5 × 3–5	37–55 × 11–17

[a] For abbreviations see Materials and methods

from the Black Sea, 24 cm *vs* less than 15 cm in the both Mediterranean localities.

Moreover, in the description of *H. cupensis* the presence of spirally striated sheath is noted as a specific character of the dorsal anchors of this species (Sasal et al., 1998). This detail is absent in the drawings of the anchors of *A. cobitis* (see Ergens, 1963). The present results show that when the anchor is protruded out from the haptor, the tissue sleeve of the blade and the ligament attached to the anchoral opening can form folds around the blade of both anchors (Fig. 3A, B), that are more prominent in the dorsal anchors, because these possess more massive blades.

In the description of *H. cupensis* vas deferens does not loop around the left caecal branch (Sasal et al., 1998) and this is at odds with the diagnosis of the genus *Haliotrema* Johnston & Tiegs, 1922 *sensu* Young (1968). The present examination of the newly collected specimens confirmed this, and, in addition, showed a bilobed base of the MCO and the marginal hooks with an upright thumb, characters that do not correspond to the emended diagnosis of *Haliotrema* *sensu* Plaisance et al. (2004), as the latter includes characters such as trapezoid base of the MCO and a depressed hook thumb.

Only the representatives of five marine ancyrocephalid genera have the vas deferens not looping the intestinal caecum, namely, *Ergenstrema* Paperna, 1964, *Kriboetrema* Sarabeev, Rubtsova, Yang & Balbuena, 2013, *Ligophorus* Euzet & Suriano, 1977, *Neohaliotrema* Yamaguti, 1965 and *Pseudohaliotrema* Yamaguti, 1953. Among these, *Xenoligophoroides cobitis* is more similar to the species of *Ligophorus* and *Kriboetrema*.

Xenoligophoroides is distinguished from *Ligophorus* by the accessory piece articulated with the MCO base, the presence of two prostatic reservoirs and ventral bar having posterior prominence in the middle, whereas *Ligophorus* spp. have the accessory piece not connected with the MCO base, one prostatic reservoir and an anterior convex middle part of the ventral bar.

The new genus differs from *Kriboetrema* in the slightly bent tube and bilobed base of the MCO and the dextral vaginal pore, as compared with the coiled tube, the bulb-shaped base of the MCO, and the median

Table 3 List of the monogenean species included in the phylogenetic analysis with host, geographical locality and GenBank accession numbers

Monogenean	Host	Geographical locality	GenBank ID	Reference
Haliotrema angelopterum Plaisance, Bouamer & Morand, 2004	*Chaetodon kleinii* Bloch	Philippine Sea, off Palau	AY820620	Plaisance et al. (2005)
H. aurigae (Yamaguti, 1968)	*C. auriga* Forsskål	South China Sea, off Pratas Islands	EU836198	Kritsky et al. (2009)
H. cromileptis Young, 1968	*Epinephelus coioides* (Hamilton)	South China Sea, off Vietnam	EU541306	Dang et al. (2010)
H. ctenochaeti Young, 1968	*Ctenochaetus striatus* (Quoy & Gaimard)	South China Sea, off Pratas Islands	EU836199	Kritsky et al. (2009)
H. johnstoni Bychowsky & Nagibina, 1970	*Upeneus luzonius* Jordan & Seale	South China Sea, off China	DQ157664	Wu et al. (2006)
H. leporinus Sun, Kritsky & Yang, 2007	*Acanthurus nigrofuscus* (Forsskål)	South China Sea, off Pratas Islands	EU836206	Kritsky et al. (2009)
H. macracantha Yamaguti, 1968	*Zebrasoma veliferum* (Bloch)	South China Sea, off Pratas Islands	EU836208	Kritsky et al. (2009)
H. pratasensis Sun, Kritsky & Yang, 2007	*Acanthurus nigrofuscus*	South China Sea, off Pratas Islands	EU836209	Kritsky et al. (2009)
H. scyphovagina Yamaguti, 1968	*Forcipiger flavissimus* Jordan & McGregor	French Polynesia, Pacific Ocean	AY820622	Plaisance et al. (2005)
H. platycephali Yin & Sproston, 1948	*Platycephalus indicus* L.	Yellow Sea, off China	DQ157662	Wu et al. (2006)
Lethrinitrema fleti (Young, 1968)	*Lethrinus nebulosus* (Forsskål)	South China Sea, off China	DQ157661	Wu et al. (2006)
Pseudohaliotrema sphincteroporus Yamaguti, 1953	*Siganus doliatus* Guérin-Méneville	Coral Sea, Green Island, Australia	AF382058	Olson & Littlewood (2002)
Euryhaliotrema anguiformis (Zhang in Zhang, Yang & Liu, 2001)	*Lutjanus monostigma* (Cuvier)	South China Sea, off China	DQ537375	Wu et al. (2007)
E. cribbi (Plaisance & Kritsky, 2004)	*Chaetodon citrinellus*Cuvier	French Polynesia, Pacific Ocean	AY820612	Plaisance et al. (2005)
E. johni (Tripathi, 1959)	*Lutjanus johnii* (Bloch)	South China Sea, off China	EU836193	Kritsky et al. (2009)
E. kurodai (Ogawa & Egusa, 1978)	*Acanthopagrus schlegelii* (Bleeker)	South China Sea, off China	DQ537376	Wu et al. (2007)
E. microphallus (Yamaguti, 1968)	*Heniochus chrysostomus* Cuvier	Philippine Sea, off Palau	AY820617	Plaisance et al. (2005)
E. pirulum (Plaisance & Kritsky, 2004)	*Chaetodon lunula* (Lacépède, 1802)	French Polynesia, Pacific Ocean	AY820618	Plaisance et al. (2005)
E. perezponcei García-Vargas, Fajer-Ávila & Lamothe-Argumedo, 2008	*Lutjanus guttatus* (Steindachner)	Pacific coast of Mexico, Pacific Ocean	KC663669	García-Vásquez et al. (2015)
E. perezponcei	*L. guttatus*	Bay Cerritos, off Mexico, Pacific Ocean	HQ615996	García-Vásquez et al. (2015)
Euryhaliotrema sp. 3 YS-2008	*L. argentimaculatus* (Forsskål)	South China Sea, off China	EU836194	Kritsky et al. (2009)
Haliotrematoides guttati (García-Vargas, Fajer-Ávila & Lamothe-Argumedo, 2008)	*L. guttatus*	Bay Cerritos, off Mexico, Pacific Ocean	HQ615993	García-Vásquez et al. (2015)

Table 3 continued

Monogenean	Host	Geographical locality	GenBank ID	Reference
H. spinatus Kritsky & Mendoza-Franco in Kritsky, Yang & Sun, 2009	*L. guttatus*	Bay Cerritos, off Mexico, Pacific Ocean	HQ615995	García-Vásquez et al. (2015)
H. nagibinae Kritsky, Yang & Sun, 2009	*L. argentimaculatus*	South China Sea, off China	EU836211	Kritsky et al. (2009)
H. noncalcaroides Kritsky, Yang & Sun, 2009	*L. argentimaculatus*	South China Sea, off China	EU836212	Kritsky et al. (2009)
H. brachyflagellocirrus (Wang, Liu & Zhou, 2003)	*L. argentimaculatus*	South China Sea, off China	EU836213	Kritsky et al. (2009)
Ergenstrema mugilis Paperna, 1964	*Chelon ramada* (Risso)	Mediterranean Sea, Ebro Delta, Spain	JN996800	Blasco-Costa et al. (2012)
Ligophorus acuminatus Euzet & Suriano, 1977	*C. saliens*(Risso)	Mediterranean Sea, Ebro Delta, Spain	JN996816	Blasco-Costa et al. (2012)
L. angustus Euzet & Suriano, 1977	*C. labrosus* (Risso)	Mediterranean Sea, off Cullera, Spain	JN996805	Blasco-Costa et al. (2012)
L. cephali Rubtsova, Balbuena, Sarabeev, Blasco-Costa & Euzet, 2006	*Mugil cephalus* L.	Mediterranean Sea, off Cullera, Spain	JN996830	Blasco-Costa et al. (2012)
L. chabaudi Euzet & Suriano, 1977	*M. cephalus*	Mediterranean Sea, Ebro Delta, Spain	JN996834	Blasco-Costa et al. (2012)
L. confusus Euzet & Suriano, 1977	*Chelon ramada*	Mediterranean Sea, off Cullera, Spain	JN996811	Blasco-Costa et al. (2012)
L. heteronchus Euzet & Suriano, 1977	*C. saliens*	Mediterranean Sea, Ebro Delta, Spain	JN996812	Blasco-Costa et al. (2012)
L. imitans Euzet & Suriano, 1977	*C. ramada*	Mediterranean Sea, Ebro Delta, Spain	JN996815	Blasco-Costa et al. (2012)
L. llewellyni Dmitrieva, Gerasev & Pronkina, 2007	*Planiliza haematocheila* (Temminck & Schlegel)	Sea of Azov, Utlyuksky Estuary, Ukraine	JN996823	Blasco-Costa et al. (2012)
L. mediterraneus Sarabeev, Balbuena & Euzet, 2005	*Chelon saliens*	Mediterranean Sea, Ebro Delta, Spain	JN996829	Blasco-Costa et al. (2012)
L. minimus Euzet & Suriano, 1977	*Mugil cephalus*	Mediterranean Sea, off Cullera, Spain	JN996818	Blasco-Costa et al. (2012)
L. pilengas Sarabeev & Balbuena, 2004	*Planiliza haematocheilus*	Sea of Azov, Utlyuksky Estuary, Ukraine	JN996826	Blasco-Costa et al. (2012)
L. szidati Euzet & Suriano, 1977	*Chelon aurata*(Risso)	Mediterranean Sea, Ebro Delta, Spain	JN996806	Blasco-Costa et al. (2012)
L. leporinus (Zhang & Ji, 1981)	*Mugil cephalus*	South China Sea, off China	DQ537380	Wu et al. (2007)
L. vanbenedenii (Par. et. Per., 1810)	*Chelon aurata*	Mediterranean Sea, Ebro Delta, Spain	JN996801	Blasco-Costa et al. (2012)
L. vanbenedenii sensu (Wu et al., 2006)	*Mugil cephalus*	South China Sea, off China	DQ157655	Wu et al. (2006)
Protogyrodactylus hainanensis Pan, Ding & Zhang, 1995	*Terapon jarbua* (Forsskål)	South China Sea, off China	DQ157653	Wu et al. (2006)
P. alienus Bychowsky & Nagibina, 1974	*Gerres filamentosus* Cuvier	South China Sea, off China	DQ157650	Wu et al. (2006)
Pseudodactylogyrus anguillae (Yin & Sproston, 1948)	*Anguilla japonica* Temminck & Schlegel	South China Sea, off China	DQ157666	Wu et al. (2006)

Table 3 continued

Monogenean	Host	Geographical locality	GenBank ID	Reference
Metahaliotrema mizellei Venkatanarasaiah, 1981	*Scatophagus argus* (L.)	South China Sea, off China	DQ157647	Wu et al. (2006)
Pseudomurraytrema sp.	*Catostomus ardens* Jordan & Gilbert	Snake River, Idaho, USA	AF382059	Olson & Littlewood (2002)
Xenoligophoroides cobitis n. comb. (MEDIT1)	*Gobius cobitis* Pallas	Mediterranean Sea, off NE Sardinia, Italy	MG194736	This study
X. cobitis (MEDIT2)	*G. cobitis*	Mediterranean Sea, off NE Sardinia, Italy	MG194737	This study
X. cobitis (MEDIT4)	*G. cobitis*	Mediterranean Sea, off NE Sardinia, Italy	MG194738	This study
X. cobitis (MEDIT5)	*G. cobitis*	Mediterranean Sea, off NE Sardinia, Italy	MG194739	This study
X. cobitis (MEDIT6)	*G. cobitis*	Mediterranean Sea, off NE Sardinia, Italy	MG194740	This study
X. cobitis (BLACK5)	*G. cobitis*	Black Sea, off Caucasus, Russia	MG194741	This study
X. cobitis (BLACK7)	*G. cobitis*	Black Sea, off Caucasus, Russia	MG194742	This study
X. cobitis (BLACK8)	*G. cobitis*	Black Sea, off Caucasus, Russia	MG194743	This study
X. cobitis (BLACK9)	*G. cobitis*	Black Sea, off Caucasus, Russia	MG194744	This study

position of vagina in two known species of *Kriboetrema*.

Xenoligophoroides cobitis differs from *Ergenstrema* spp. in the presence of two bars and uncoiled vagina, as opposed to only one bar and strong coiled vagina in the compared species.

Moreover, all species of *Ligophorus*, *Kriboetrema* and *Ergenstrema* are only known as parasites of fishes of the family Mugilidae.

The new genus also differs substantially from the representatives of *Pseudohaliotrema* and *Neohaliotrema*. *Xenoligophoroides cobitis* can be easy distinguished from *Pseudohaliotrema* spp. in the male copulatory complex, which serves for copulation rather than for formation of spermatophores as in the latter, and the attachment of the accessory piece to MCO base with narrow strip as opposite to broad laminal bridge connected the accessory piece with MCO in the *Pseudohaliotrema*. In relation to *Neohaliotrema*, *X. cobitis* is distinguished by the lack of the additional haptoral structures and the unmodified standard distributed marginal hooks, whereas anchors have inner root sclerites and marginal hooks morphometrically modified in the first genus.

Thus, *X. cobitis* does not correspond to the diagnosis of any of the known Ancyrocephalidae genera for the set of the following characters: (i) vas deferens not looping the intestinal caecum; (ii) two prostatic reservoirs; (iii) uncoiled MCO; (iv) bilobed base of MCO; (v) accessory piece articulated with MCO through rod-shaped process; (vi) uncoiled dextral vagina; (vii) paired anchors and bars, without additional sclerites; (viii) unmodified normal distributed marginal hooks with upright thumb; (viii) parasitic in the Gobiidae.

Phylogenetic analysis

In order to investigate phylogenetic relationships among species and test the monophyly of the taxa analysed, an outgroup-rooted tree based on Bayesian

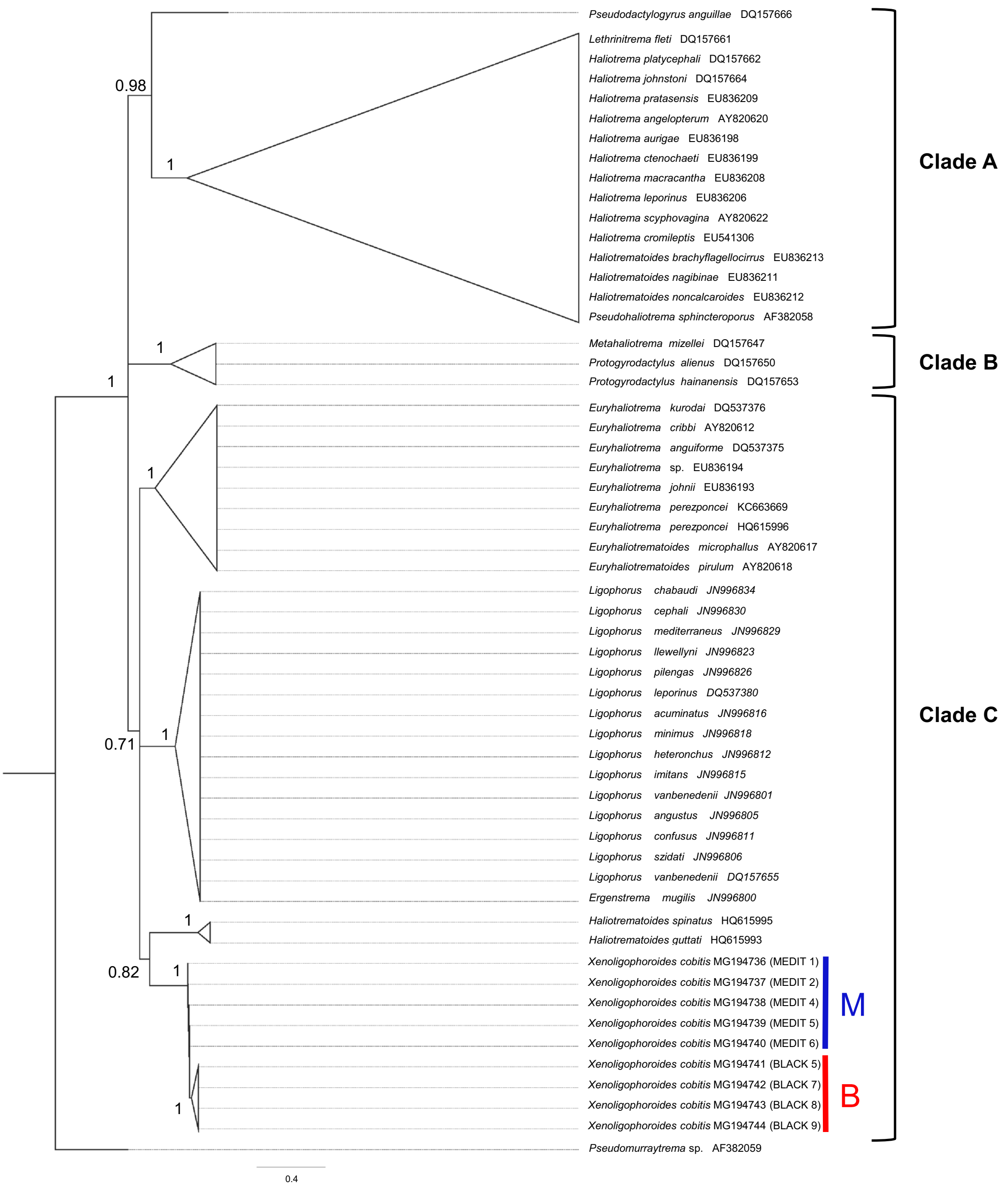

Fig. 6 28S rDNA-based rooted tree obtained by Bayesian inference analysis. In the tree, branch length scale refers to the number of substitutions per site. Nodal support is indicated at the nodes (posterior probabilities >0.70 are shown). *Abbreviations*: M, *Xenoligophoroides cobitis* samples from the Mediterranean Sea; B: *X. cobitis* samples from the Black Sea

inference (BI) was generated. The dataset included 56 sequences which corresponded to 45 species belonging to 10 genera of the Ancyrocephalidae, and nine sequences obtained in this study, five of which were from the Mediterranean Sea and four from the Black Sea. The diplectanid *Pseudomurraytrema* sp. was used as the outgroup (Table 3).

The Bayesian analysis (Fig. 6) provided evidence for the occurrence of three main clades (A, B, C in Fig. 6). Clade A comprised, in a monophyletic cluster, all sequences for species of the genera *Haliotrema*, *Haliotrematoides* Kritsky, Yang & Sun, 2009 [except for *H. guttati* (García-Vargas, Fajer-Ávila & Lamothe-Argumedo, 2008) and *H. spinatus* Kritsky & Mendoza-Franco in Kritsky, Yang & Sun, 2009)], *Pseudohaliotrema* and *Lethrinitrema* Lim & Justine, 2011. *Pseudodactylogyrus anguillae* (Yin & Sproston, 1948) showed a sister taxon relationship with Clade A. Clade B comprised the sequences for three species, *Metahaliotrema mizellei* Venkatanarasaiah, 1981, *Protogyrodactylus hainanensis* Pan, Ding & Zhang, 1995 and *P. alienus* Bychowsky & Nagibina, 1974. Clade C included three well-supported internal monophyletic clusters. The first cluster contained sequences for species belonging to the genus *Euryhaliotrema* Kritsky & Boeger, 2002 and the second cluster contained sequences for species belonging to the genus *Ligophorus* plus *Ergenstrema mugilis* Paperna, 1964. Within the third cluster, sequences split into two highly supported sister groups: one including the specimens of *X. cobitis* from the Mediterranean and Black seas analysed in the present study, and one including sequences for *Haliotrematoides guttati* and *H. spinatus*. Within the *X. cobitis* group, specimens from Black Sea clustered together in a well-supported internal sub-group.

The highest genetic p-distances (see Supplementary Table S1) for *X. cobitis* correspond to the comparisons between *X. cobitis* and some species belonging to the genera *Haliotrema* and *Haliotrematoides* included in Clade A in the Bayesian analysis (Fig. 6). The lowest distances for *X. cobitis* were found between *X. cobitis* and the species included in Clade C (Fig. 6), in particular those belonging to the genus *Ligophorus* and *Ergenstrema mugilis*.

Discussion

After the revisions and emending of generic diagnosis published by Yamaguti and Young (Yamaguti, 1963; Young, 1967), *Haliotrema* comprised 35 species, many of which were transferred from other genera. Subsequently, many marine ancyrocephalids from very different hosts were described as *Haliotrema* spp., so Klassen (1994), after counting 116 species, named this genus as a taxonomic waste-basket. Despite that four additional genera (*Ligophorus*, *Euryhaliotrema*, *Haliotrematoides* and *Lethrinitrema*) have been established for the dozens of species embedded in *Haliotrema*, currently the latter includes 139 species, and is still an unnatural and polyphyletic taxon (Sun et al., 2014).

Xenoligophoroides cobitis n. comb. previously attributed to *Haliotrema*, was the only member of this genus reported from a host belonging to Gobiidae. Moreover, more than 100 known species of *Haliotrema* have been described from coral reef fishes occurring in tropical and subtropical waters (Young, 1968; Bychowsky & Nagibina, 1970; Klassen, 1994; Plaisance et al., 2004, 2005; Wu et al., 2006; Dang et al., 2010; Sun et al., 2007, 2011, 2015; Cruces et al., 2017). Thus, the host and the locality of the findings of *X. cobitis* were both unusual for *Haliotrema*.

Previously, hosts were used, together with morphological characters, for grouping closely related species within *Haliotrema* (see Young, 1968; Bychowsky & Nagibina, 1971). Subsequently, some of these groups were considered to represent distinct genera, e.g. *Ligophorus* erected for two species from grey mullets (Mugilidae) and *Lethrinitrema* for species from representatives of *Lethrinus* Cuvier (Lethrinidae) (see Euzet & Suriano, 1977; Lim & Justine, 2011). Recently, Sun et al. (2015) described nine species of *Haliotrema* from acanthurid fishes and suggested that considering their host specificity, the features of hooks, and rDNA sequences, all *Haliotrema* spp. from acanthurids possibly form a monophyletic group, and a new genus should be proposed for them. Similarly, Kritsky & Bakenhaster (2016) supposed that *Haliotrema* spp. from drepaneid fishes (Drepaneidae) should be reassigned to *Parancylodiscoides* Caballero & Bravo Hollis, 1961. Likewise, *Pseudohaliotrema*, originally established for the monogeneans from *Siganus* spp., was replenished by many species described from hosts belonging to other fish genera, and after a few revisions, the content of this genus was again reduced only to the species specific to the Siganidae (see Lim, 2002; Kritsky & Galli, 2007).

Thus, the specificity of *X. cobitis* to a host species of the family Gobiidae, which is unusual and rather phylogenetically distant from the fish species infected with other marine ancyrocephalids, is an additional evidence for the erection of a new genus for this monogenean.

Although Sasal et al. (1998) pointed out that the presence of a non-looping vas deferens in *H. cupensis* (syn. of *X. cobitis*) differentiates this species from other congeners, he suggested that this character is insufficient for the erection of a new genus. The morphological analysis of newly collected specimens allowed us to identify additional diagnostic characters, e.g. the shape of the marginal hooks, which have a sickle with an upright thumb, rather than depressed one as in the most of *Haliotrema* spp. This last character has been added to the diagnosis of *Haliotrema* by Plaisance et al. (2004), but Sun et al. (2015) noticed the presence of a few exceptions, e.g. *Haliotrema geminatohamula* Bychowsky & Nagibina, 1971, which have an upright thumb in the marginal hook sickle. However, practically all *Haliotrema* spp. with this shape of the marginal hook have already been transferred to other genera, i.e. *Euryhaliotrema*, *Haliotrematoides* and *Ligophorus* (see Cruces et al., 2017). So, the shape of the thumb in the marginal hook sickle may be considered a useful tool at the generic level for ancyrocephalids, and additionally distinguishes the members of *Haliotrema* from other genera, including *Xenoligophoroides* n. g.

Some of the currently recognised genera within *Haliotrema*-like ancyrocephalids have been established based on the specific characters of the MCO. For example, *Volsellituba* Řehulková, Justine & Gelnar, 2010 and *Pennulituba* Řehulková, Justine & Gelnar, 2010 were separated from other dactylogyrids and among themselves almost exclusively on the basis of the differences in the morphology of the male copulatory complex (Řehulková et al., 2010). Similarly, *Euryhaliotrema* was initially established for monogeneans having a coiled MCO with a bulbous base (Kritsky & Boeger, 2002), and subsequently, *Euryhaliotrematoides* Plaisance & Kritsky, 2004 and *Aliatrema* Plaisance & Kritsky, 2004 were defined for similar species, but possessing a funnel-shaped base of MCO for *Euryhaliotrematoides* spp., and lacking an MCO accessory piece for *Aliatrema* spp. (see Plaisance & Kritsky, 2004). However, Kritsky (2012) after a re-examination of additionally collected samples of dactylogyrids from snappers, suggested recombination of these three genera in *Euryhaliotrema*, based on a mix of the abovementioned diagnostic characters. Therefore, differences in the structure of the male copulatory complex alone are obviously not sufficient for the definition of generic-level taxa, especially in the case of monotypic genera, as in the present case.

We would like to note that the combination of characters, such as uncoiled narrow tubular MCO with bilobed base and simple articulated accessory piece, which is characteristic for the new genus, is not present in the other marine ancyrocephalids, but *Xenoligophoroides* also distinguished by the set of the characters of the haptoral structures and internal anatomy.

It should be noted that most genera and species among Monopisthocotylea are satisfactorily differentiated by morphology of the attachment sclerites, male copulatory organ and vagina. However, there may be exceptions; for example, the validity of *Ergenstrema* is questioned by the results of previous (Blasco-Costa et al., 2012) and present (Fig. 6) phylogenetic analyses, based on the sequences of the 28S rRNA gene. Despite the great morphological differences in the haptoral armament, shape of the male copulatory organ and vagina between *Ergenstrema* and *Ligophorus*, the monophyly of *E. mugilis* and 14 species of *Ligophorus* seems to be significantly supported. As an alternative example, Mendoza-Palmero et al. (2017) proposed the new dactylogyrid genus *Parasciadicleithrum* Mendoza-Palmero, Blasco-Costa, Hernández-Mena & Pérez-Ponce de León, 2017, based only on the results of molecular phylogenetic analyses, despite the morphological similarity with representatives of *Sciadicleithrum* Kritsky, Thatcher & Boeger, 1989. Apparently, combination of the morphological characters related to various anatomical structures and molecular data must be considered as the best approach to correctly define new taxa among numerous marine ancyrocephalids, some of which are morphologically similar though belonging to different genera, whereas others show significant intrageneric morphological variability.

The results of the Bayesian phylogenetic analysis were overall consistent with the phylogenetic reconstructions based on 28S rDNA sequences obtained in previous studies on ancyrocephalids (Plaisance et al., 2005; Wu et al., 2006; Kritsky et al., 2009; Dang et al, 2010; Blasco-Costa et al., 2012; Sun et al., 2014; García-Vásquez et al., 2015). *Haliotrema* spp. were resolved into monophyletic clades closely related to representatives of other genera (i.e. *Pseudohaliotrema* and *Haliotrematoides*). The species recently transferred from *Aliatrema* and *Euryhaliotrematoides* to *Euryhaliotrema*, namely *E. cribbi* (Plaisance & Kritsky, 2004) Kritsky, 2012 (syn. *Aliatrema cribbi* Plaisance & Kritsky, 2004), *E. microphallus* (Yamaguti, 1968) Kritsky, 2012 [syn. *Euryhaliotrematoides microphallus* (Yamaguti, 1968) Plaisance & Kritsky, 2004] and *E. pirulum* (Plaisance & Kritsky, 2004) Kritsky, 2012 (syn. *Euryhaliotrematoides pirulum* Plaisance & Kritsky, 2004), showed a strong monophyly with other *Euryhaliotrema* spp., thus supporting the revision of this taxon based on morphology (Kritsky et al., 2012).

The specimens of *X. cobitis* analysed in the present study represent a well-supported taxonomic entity, highly divergent from *Haliotrema* spp. Such a finding is consistent with the occurrence of a new, undescribed taxon. Within the *Xenoligophoroides* group, the occurrence of a cluster exclusive to the specimens from Black Sea likely reflects the correlation between genetic structuring and the geographic distance between Mediterranean and Black seas. Such a genetic divergence is also consistent with the above-mentioned differences in the measurements of the haptoral structures, MCO and vagina in the specimens from different seas, along with the possible influence of host length.

The genetic similarity between *X. cobitis* and the species of the genus *Ligophorus*, evidenced by molecular analyses, is consistent with the morphological similarity between the genera *Ligophorus* and *Xenoligophoroides.* On the contrary, the genetic affinity between *X. cobitis* specimens and *Haliotrematoides guttati* and *H. spinatus* was quite unexpected, considering the marked morphological, host and geographical differences between them.

However, all monogeneans in Clade C of the Bayesian phylogenetic tree in Fig. 6 have the same shape of the marginal hooks, with an upright thumb, unlike most of the genera in Clade A, excluding only three species of *Haliotrematoides*, possessing hooks with a depressed thumb. Similarly, Sun et al. (2014) showed, based on 28S rDNA sequence analysis, that members of one lineage contained *Euryhaliotrema* and *Haliotrematoides* spp., whereas a second lineage included species of *Lethrinitrema*, *Bravohollisia* Bychowsky & Nagibina, 1970, *Caballeria* Bychowsky & Nagibina, 1970 and *Haliotrema*, with depressed thumb in the hooks. In such a context, the congruence of phylogenetic analysis with this finding suggests the usefulness of the marginal hook shape for ancyrocephalid taxonomy at the generic level.

Overall, the 28S rDNA-based phylogenetic analysis of 46 species from 11 genera suggested that previous allocation of *Xenoligophoroides cobitis* from *Gobius cobitis* to *Haliotrema* is misleading, since the specimens of this species belong to an independent highly supported monophyletic lineage that justifies the distinct status of the new genus.

Another two marine ancyrocephalids with an unclear taxonomic position are known in the Mediterranean Sea, namely *Ancyrocephalus littoralis* and *A. salinus*. These species were described from *Atherinomorus pinguis* (Lacépède) and *Aphanius dispar* (Rüppell), respectively, which are widely distributed in the Indian Ocean and migrated into the south-eastern Mediterranean Sea through the Suez Canal. *Ancyrocephalus salinus* was also found in the Red Sea. Information on these species are limited to three publications (Paperna, 1964, 1972; Paperna & Kohn, 1964), which contain very brief descriptions (and schematic drawings), mainly concerning the haptoral structures and MCO. This makes the determination of their true generic affinity impossible. However, the figures of the MCO with accessory piece of *A. littoralis* (figure 2 in Paperna & Kohn, 1964) and *A. salinus* (figures 7, 8 in Paperna, 1964) are practically identical with those of *X. cobitis* (Fig. 1). The composition of the haptoral armament is similar in all three species, but measurements of anchors and bars are rather different. It is possible that these two species, *A. littoralis* and *A. salinus*, may also belong to *Xenoligophoroides*; however, to confirm this a detailed study of the internal organs and molecular analysis based on new samples is required.

Acknowledgements We would like to express our gratitude to Dr Fabio Scarpa for his valuable help during the phylogenetic elaboration of the data.

Funding Investigation of the first author was supported by the Russian Foundation for Basic Research, Grant No. 15-29-02684ofi-m and a Visiting Professor/Scientist Programme Grant of the University of Sassari, 2014.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable institutional, national and international guidelines for the care and use of animals were followed.

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