# *Glyphidohaptor safiensis* n. sp. (Monogenea: Ancyrocephalidae) from the white-spotted rabbitfish *Siganus canaliculatus* (Park) (Perciformes: Siganidae) off Oman, with notes on its phylogenetic position within the Ancyrocephalidae Bychowsky & Nagibina, 1968 (*sensu lato*)

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**Abstract** A new ancyrocephalid monogenean is described from the gills of wild White-spottedrabbit-fish *Siganus canaliculatus* (Park) based on morphological and molecular analyses. *Glyphidohaptor safiensis* n. sp. can be distinguished from other members of the genus by the shape of the accessory piece of the male copulatory organ (MCO). Unlike its congeners, the rod-shaped accessory piece of *G. safiensis* n. sp. is distally broad and flattened. The MCO of *G. safiensis* n. sp. is curved, enclosed in a heavy sheath with a terminal flap. Partial large subunit (LSU), partial small subunit (SSU) and the partial SSU, entire internal transcribed spacer region 1 (ITS1) and partial 5.8S rDNA of the new species and two species of *Tetrancistrum* Goto & Kikuchi, 1917 from the same host and locality were sequenced and subjected to phylogenetic analysis. The LSU rDNA analysis grouped *G. safiensis* n. sp. with *Tetrancistrum* sp. from the gills of *Siganus fuscescens* Houttuyn from Australia, indicating a possible misidentification of the latter. Sequences of the SSU rDNA of the new species were most similar to those for *Pseudohaliotrema sphincteroporus* Yamaguti, 1953, demonstrating the close relatedness of the genera *Pseudohaliotrema* Yamaguti, 1953 and *Glyphidohaptor* Kritsky, Galli & Yang, 2007 within the Ancyrocephalidae. The comparison of the partial SSU (424 bp) and entire ITS1 and partial 5.8S rDNA (246 bp) sequences obtained for *G. safiensis* n. sp. with the only available sequence of another member of *Glyphidohaptor* Kritsky, Galli & Yang, 2007, *G. pletocirra* Paperna, 1972 (HE601931-HE601933) yielded on average 1.08% dissimilarity (a difference of 7 bases), with a p-distance of $0.010 \pm 0.004\%$. This is the first record of a species of *Glyphidohaptor* from *S. canaliculatus* and from the Persian Gulf, the Gulf of Oman and the Arabian Sea.

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## Introduction

The Siganidae is a monotypic family of inshore and reef-associated tropical and subtropical fishes consisting of 29 valid species, all from the Indo-Pacific region (Froese & Pauly, 2018). Several authors have investigated the parasite fauna of siganids reporting high parasite diversity (e.g. Paperna, 1972; Diamant & Paperna, 1986; Martens & Moens, 1995; Geets & Ollivier, 1996; Geets et al., 1997; Shih & Jeng, 2002; Aloo et al., 2004; Hassanine & Al Jahdali, 2007). To date, a total of 18 siganid species were investigated for monogenean parasites, resulting in identification of species of the polyopisthocotylean genus *Polylabris* Euzet & Cauwet, 1967 (see Tingbao et al., 2007; Sailaja & Madhavi, 2010) and four monopisthocotylean genera, including three genera of ancyrocephalids (Goto & Kikuchi, 1917; Young, 1967; Paperna, 1972; Lim, 2002; Kritsky et al., 2007a, b; Kritsky & Galli, 2007) and one viviparous gyrodactylid (Ernst et al., 2001). The ancyrocephalid genus *Glyphidohaptor* Kritsky, Galli & Yang, 2007 was erected by Kritsky et al. (2007a) to accommodate *Glyphidohaptor plectocirra* (Paperna, 1972) which was initially described as *Pseudohaliotrema plectocirra* Paperna, 1972 (syn. *Tetrancistrum plectocirra* (Paperna, 1972) Lim, 2002) from *Siganus luridus* (Rüppell) and *Siganus rivulatus* (Forsskål & Niebuhr) from the Gulf of Aqaba (off Eilat, Red Sea). Two further species were described from the Great Barrier Reef from five siganid hosts, *Glyphidohaptor phractophallus* Kritsky, Galli & Yang, 2007 from *Siganus fuscescens* (Houttuyn) and *G. sigani* Kritsky, Galli & Yang, 2007 from *S. doliatus* Guérin-Méneville, *S. lineatus* (Valenciennes), *S. punctatus* (Schneider & Forster) and *S. corallinus* (Valenciennes) (see Kritsky et al., 2007a). Other reports include an unidentified *Glyphidohaptor* sp. from the gills of an unknown *Siganus* sp. from Macassar, Sulawesi (Indonesia) (Kritsky et al., 2007a).

The class Monogenea has been subject of several major molecular phylogenetic studies (Justine et al., 2002). Molecular-based phylogenetic relationships of the Platyhelminthes, including the Monogenea, were carried out utilising RNA gene sequences (Baverstock et al., 1991; Mollaret et al., 1997; Campos et al., 1998; Littlewood et al., 1999; Olson & Littlewood, 2002). Mollaret et al. (2000) investigated the phylogenetic relationships between the two main subclasses of the Monogenea (Polyopisthocotylea and Monopisthocotylea) using the large subunit (LSU) of the ribosomal RNA gene. Furthermore, a number of authors evaluated the phylogenetic position of the ancyrocephalid genera (e.g. *Haliotrema* Johnston & Tiegs, 1922; *Euryhaliotrema* Kritsky & Boeger, 2002; *Haliotrematoides* Kritsky, Yang & Sun, 2009; and *Pseudempleurosoma* Yamaguti, 1965) through analyses of the small subunit (SSU) and LSU of the rRNA gene (Plaisance et al., 2005; Wu et al., 2007; Dang et al., 2010; Garcia-Vasquez et al., 2015; Mendoza-Palmero et al., 2015; Theisen et al., 2017, 2018). The internal transcribed spacer region 1 (ITS1) was also used to explore the intraspecific variability (Šimková et al., 2004; Stefani et al., 2012; Šimková et al., 2013; Kmentová et al., 2015).

Although the identification and description of new species of monogeneans is traditionally made through morphological characterisation (Desdevises et al., 2000), the combination of molecular data with morphological analysis for the description of new species is becoming more common (e.g. Freeman & Ogawa, 2010; Bullard et al., 2015; Soo et al., 2015; Theisen et al., 2017, 2018). To date, only a few sequences are available for ancyrocephalids infecting siganids. The first gene sequence from a monogenean infecting siganids was provided by Mollaret et al. (1997). The authors analysed a LSU rDNA sequence of *Tetrancistrum* sp. obtained from *S. fuscescens* caught off Heron Island, Queensland, Australia, without providing any morphological information of this species. Later, SSU and LSU sequences of *Pseudohaliotrema sphincteroporus* Yamaguti, 1953 were obtained by Littlewood & Olson (2001) and used to evaluate the molecular phylogenetic relationships of families within the Monogenea. In addition, SSU sequences for *Tetrancistrum sigani* Goto & Kikuchi, 1917 (syn. *T. nebulosi* Young, 1967) were deposited in GenBank by Wang et al. (2010) and Ummey et al. (2015). Furthermore, a comparison of the genetic variation in populations of *G. plectocirra* was investigated by utilising the cytochrome *c* oxidase subunit 1 (*cox*1) gene, and partial SSU and the entire ITS1 region including the 5.8S gene (Stefani et al., 2012).

During a survey of the parasite fauna of *Siganus canaliculatus* (Park) from Omani waters, a new species of *Glyphidohaptor* was recovered. The objectives of the present study were to provide a morphological description of the new species and to explore

the phylogenetic relationships within the Ancyrocephalidae Bychowsky & Nagibina, 1968 (*sensu lato*) based on sequence data for the LSU, SSU and partial SSU, entire ITS1 and partial 5.8S rDNA region, focusing on *Glyphidohaptor*, *Pseudohaliotrema* Yamaguti, 1953 and *Tetrancistrum* Goto & Kikuchi, 1917.

## Materials and methods

### Sample collection and examination

A total of 245 white-spotted rabbitfish, *Siganus canaliculatus* (Perciformes: Siganidae), were purchased alive from local fish markets from seven locations along the coasts of the Sultanate of Oman (Table 1). Fish were morphologically identified by using the guidelines of Randall (1995) and FAO identification sheets (1984). Fish specimens were transported on ice to the facility of the Laboratory of Aquatic Parasitology at the Fishery Quality Control Center, Sultanate of Oman. The hosts were either immediately subjected for parasitological examination or frozen at -40°C for subsequent investigation. Upon dissection, gills were excised; arches were separated, placed in a Petri dish with filtered seawater and examined under a dissecting microscope. Parasites were detached from the gill filaments by means of fine needles and kept in filtered seawater at 4°C prior to fixation. Monogenean whole mounts were prepared from unflattened worms fixed in AFA (3 parts alcohol: 2 parts formalin: 1 part acetic acid) or 4% neutral buffered formalin, stained with Mayer's paracarmine, cleared in clove oil and mounted in Canada balsam. Measurements, all in micrometres, are given as the range, followed by the mean and the number (n) of structures measured in parentheses. Body length

includes that of the haptor; measurements of the copulatory complex, anchors and hooks and the description of the new species are according to Kritsky et al. (2007a). Additional measurements of anchors were obtained according to Gussev (1985) (see Vignon & Sasal, 2010). An analysis of the available measurements of the known congeners was carried out and statistical comparisons were carried out using Students t-test for independent samples (two-tailed; α= 95%).

Illustrations were prepared with the aid of a drawing tube attached to an Olympus BX63 motorised light microscope with Nomarski differential interference contrast optics (DIC) and digitalised using Adobe illustrator CC 2018. and the program Inkscape 0.92.2 (Scalable Vector Graphics, 2).

### Comparative morphological analysis

Voucher specimens were obtained from the British Natural History Museum (*G. phractophallus* BMNH 2006.8.8.13-14, *G. sigani* BMNH 2006.8.8.11-12), Meguro Parasitology Museum (*G. phractophallus* MPM 18828, *G. sigani* MPM 18829) and the Queensland Museum (*G. phractophallus* QMG227443-QMG 227447), *G. sigani* (QMG227453, QMG 227454, QMG 227457, QMG G227460, QMG G227461) respectively. In addition, micrographs of selected parasites were obtained for comparative purposes (*Glyphidohaptor* sp. MPM 22839 and MPM 22837 and *G. plectocirra* USNPC 98587, USNPC 98588, USNPC 98590, USNPC 98591).

### Confocal microscopy

Several specimens preserved in 95% ethanol were stained with one step Gomori's trichrome kit (Morphisto, Frankfurt, Germany), mounted in Histochoice (Amresco, Solon, OH, USA), and imaged using a Carl

**Table 1** Coordinates of sampling localities

| Locality | Coordinates | Waterbody |
| --- | --- | --- |
| Khasab fishing harbor | 26.1644°N, 56.2426°E | Persian/Arabian Gulf |
| Dibba Al-Bayya | 25.6365°N, 56.2538°E | Gulf of Oman |
| Sohar | 24.2241°N, 56.4423°E | Gulf of Oman |
| Muttrah | 23.0000°N, 58.0000°E | Gulf of Oman |
| Masirah Island | 20.4711°N, 58.8153°E | Arabian Sea |
| Al-Lakbi fish harbor | 18.113°N, 56.3255°E | Arabian Sea |
| Raysut | 16.5500°N, 54.0100°E | Arabian Sea |

Zeiss LSM780 confocal fluorescence microscope and a PL APO 63 × 1.4 oil immersion lens following the procedure of Marchiori et al. (2015). Three-dimensional (3D) stacks of the diagnostic features were acquired with a typical voxel size of 66 × 66 × 500 nm (XYZ). The samples were excited using a DP55 561 laser (AOTF 3%) and a main beam splitter 488/561. Spectral emissions (566 to 694 nm window) were detected using the internal PMT detector (grain 800). Maximum projection and three-dimensional images were obtained from a z-stack of 300 planes and the 3D rendering option in ZEN software.

### DNA extraction and PCR amplification

Monogeneans infecting *S. canaliculatus* were preserved in 95% ethanol for molecular analysis. Total genomic DNA was extracted from individual worms using QIAamp mini DNA kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions with some modification. Due to the small size of the worms investigated in this study and in order to increase the DNA yield, for the final step in the DNA extraction protocol, the extracted DNA was eluted with 200 μl of elution buffer and incubated for 10 min at room temperature before final spin and collection of genomic DNA. The obtained DNA was then completely dried using a Concentrator plus/Vacufuge®–Plus (Eppendorf, Hamburg, Germany), re-suspended with 40 μl elution buffer, then stored in a freezer at −20°C. This helped increase the yield of extracted DNA. DNA concentration was quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

Polymerase chain reaction (PCR) amplification for partial SSU rDNA were performed in 20 μl reaction volume containing 10 pmol of the forward 390f (5'-AGA GGG AGC TGA GAA ACG-3') and reverse 870r (5'-GTT GAG TCA ATT AAG CCG CA-3') primers, and ∼10 ng of DNA template using illustra™ puReTaq Ready-To-Go PCR beads (0.2 ml tubes, 96 reactions). After initial denaturation at 98°C for 5 min, samples were subjected to 40 cycles of amplification (denaturation at 95°C for 30 s, primer annealing at 55°C for 45 s, and extension at 72°C for 1 min), followed by a final extension step at 72°C for of 7 min (Freeman et al., 2013).

For amplification of the D1-D2 domain of the LSU rDNA (28S) the primers C1 (5′-ACC CGC TGA ATT TAA GCA T-3′) and D2 (5′-TGG TCC GTG TTT

CAA GAC-3′) were used, following Mendlova et al. (2010) with modification of the annealing temperature to 60°C. All PCR reactions were performed in 30 μl reactions containing 5 pmol of each primer and ∼30 ng of concentrated genomic DNA using illustra™ puReTaq Ready-To-Go PCR beads. The following amplification conditions were utilised: an initial denaturation at 94°C for 2 min, followed by 40 cycles of amplification (denaturation at 94°C for 20 s, primer annealing at 60°C for 30 s, and extension at 72°C for 90 s), followed by a final extension step at 72°C for 10 min.

Furthermore, partial SSU rDNA and entire ITS1 region were amplified in one round using the primers S1 (5'-ATT CCG ATA ACG AAC GAG ACT-3') and IR8 (5'-GCT AGC TGC GTT CTT CAT CGA-3') that anneal to the SSU and 5.8S rDNA genes, respectively (Šimková et al., 2003). Each amplification reaction was performed in a final volume of 35 μl containing 10 pmol of each primer and ∼30 ng of genomic DNA using illustra™ puReTaq Ready-To-Go PCR beads (0.2 ml tubes, 96 reactions). The amplification conditions followed Šimková et al. (2013). All PCR products were viewed on a 0.8% agarose gel stained with ethidium bromide.

### Phylogenetic analyses

Contiguous sequences of the investigated worms were obtained manually using BioEdit (Hall, 1999); these were aligned using Clustal W (Thompson et al., 1994) implemented in MEGA v.7 (Kumar et al., 2015). Newly obtained sequences were subjected to a BLAST search in the GenBank database, and sequences for ancyrocephalids showing the highest similarity (88–96%) to the newly acquired data were retrieved from GenBank and used in the phylogenetic analysis (Table 2). Phylogenetic trees were reconstructed utilising three different datasets.

The first analysis used the partial LSU (D1-D2 domain) dataset containing 20 ancyrocephalid ingroup taxa and *Thaparocleidus asoti* Yamaguti, 1937 (see Wu et al., 2007) as the outgroup. The second analysis used the SSU data from 15 ancyrocephalid species, and *Lamellodiscus donatellae* Aquaro, Riva & Galli, 2009 as the outgroup. The third dataset consisted of the partial SSU, entire ITS1 and partial 5.8S rDNA sequences of *G. safiensis* n. sp. and *G. plectocirra* sequences (GenBank: HE601931-HE601933). Phylogenetic analyses were performed based on best-fit

**Table 2** List of monogenean species used for phylogenetic analysis in this study with their GenBank accession numbers

| Species | SSU | LSU | ITS1 |
| --- | --- | --- | --- |
| *Acolpenteron ureteroecetes* | | | EF650054 |
| *Bravohollisia gussevi* | KJ571007 | DQ157665 | |
| *Bravohollisia maculatus* SYSU20060429-3 | KJ571018 | KJ571008 | |
| *Bravohollisia parvianchoratus* | | KJ571009 | |
| *Bravohollisia plectorhynchus* SYSU20060502-2 | KJ571019 | KJ571010 | |
| *Bravohollisia rosetta* | | KJ571011 | |
| *Bravohollisia tecta* SYSU20060429-4 | KJ571020 | KJ571012 | |
| *Caballeria intermedius* | | KJ571013 | |
| *Euryhaliotrema johnii* | EU836214 | EU836193 | |
| *Euryhaliotrema perezponcei* | JN054405 | HQ615996 | |
| *Euryhaliotrema* sp. 3 YS-2008 | EU836215 | EU836194 | |
| *Euryhaliotrema* sp. HBDD | DQ537346 | DQ537374 | |
| *Euryhaliotrematoides annulocirrus* | EU836216 | EU836195 | |
| *Euryhaliotrematoides microphallus* | AY820606 | AY820617 | |
| *Euryhaliotrematoides* sp. 2 YS-2008 | EU836218 | EU836197 | |
| *Euryhaliotrematoides* sp. LSJ-2011 | JF938069 | HQ615997 | |
| *Glyphidohaptor plectocirra* | | | HE601932 |
| *Glyphidohaptor plectocirra* | | | HE601933 |
| *Glyphidohaptor plectocirra* | | | HE601932 |
| *Glyphidohaptor safiensis* n. sp. isolate 7 | MK192898 | MN176409 | MN213150 |
| *Glyphidohaptor safiensis* n. sp. isolate 3 | MK192832 | | |
| *Glyphidohaptor safiensis* n. sp. isolate 8 | | | MN213152 |
| *Haliotrema cromileptis* | EU523144 | EU523146. | |
| *Haliotrema platycephali* | | FJ767866 | |
| *Lamellodiscus donatellae* | FN296214 | | |
| *Parancyrocephaloides daicoci* | | | LC176447 |
| *Protogyrodactylus amacleithrium* | FM251947 | | |
| *Protogyrodactylus hainanensis* | | DQ157653 | |
| *Pseudodactylogyroides apogonis* | AB065115 | | |
| *Pseudohaliotrema sphincteroporus* | AJ287568 | AF382058 | |
| *Tetrancistrum indicum* isolate 1 | MN179328 | | |
| *Tetrancistrum indicum* isolate 5 | | MN179329 | |
| *Tetrancistrum indicum* isolate 6 | MN179330 | MN179335 | |
| *Tetrancistrum labyrinthus* isolate 6 | MN179334 | MN179332 | |
| *Tetrancistrum labyrinthus* isolate 4 | MN179331 | MN179333 | |
| *Tetrancistrum nebulosi* | HM545910 | | |
| *Tetrancistrum nebulosi* | KT267177 | | |
| *Tetrancistrum* sp. | | AF026114 | |
| *Thaparocleidus asoti* | | DQ157669 | |
| *Thylacicleidus* sp. | | | AJ490169 |

model in MEGA version 7 (Kumar et al., 2015). For the LSU dataset, maximum likelihood method based on the General Time Reversible model was the best model. For the SSU dataset, maximum likelihood method based on the Kimura 2-parameter model was used. The methods chosen for the third dataset was

Neighbor-Joining method based on Kimura 2-parameter model. The robustness of the inferred phylogeny was assessed using a bootstrap procedure with 1,000 replications (Wu et al., 2005).

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

### Genus *Glyphidohaptor* Kritsky, Galli & Yang, 2007

#### *Glyphidohaptor safiensis* n. sp.

*Type-host*: *Siganus canaliculatus* (Park) (Perciformes: Siganidae), white-spotted rabbitfish.
*Type-locality*: Off Muttrah (23.6249°N, 58.5624°E), Sea of Oman, Sultanate of Oman.
*Other localities*: Off Khasab fishing harbour (26.1644°N, 56.2426°E), Sea of Oman, Sultanate of Oman; off Dibba Al-Bayya (25.6365°N, 56.2538°E), Sea of Oman, Sultanate of Oman; off Masirah Island (20.4711°N, 58.8153°E), Arabian Sea, Sultanate of Oman; off Al-Lakbi fishing harbour (18.113°N, 56.3255°E), Arabian Sea, Sultanate of Oman; and off Raysut (16.5500°N, 54.0100°E), Arabian Sea, Sultanate of Oman (November and December 2012).
*Type-material*: The holotype (ZMB Monogenea 7434) and 5 paratypes (ZMB Monogenea 7435) were deposited in the Berlin Natural History Museum, Berlin, Germany; 9 paratypes (MPM. Coll. No. 20959) were deposited in the Meguro Parasitology Museum, Tokyo, Japan.
*Site in host:* Gills.
*Prevalence*: Off Khasab fishing harbour: 83% (29 out of 35 fish); off Dibba Al-Bayya: 94% (33 out of 35 fish); off Muttrah: 97% (34 out of 35 fish); off Masirah Island: 97% (34 out of 35 fish); off Al-Lakbi fishing harbour: 97% (34 out of 35 fish); off Raysut: 100% (35 out of 35 fish).
*Representative DNA sequences*: GenBank MK192832 and MK192898.
*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 *Glyphidohaptor safiensis* n. sp. is urn:lsid: zoobank.org:act:D6911FB7-3E39-4B56-ABF6-4B7B7E0FECAF.
*Etymology*: The specific name (*safiensis*) is after the Arabic local name of the host (safi).

## Description

[Based on 25 adult specimens; Figs. 1, 2.] Body fusiform, slightly flattened dorso-ventrally, 926–1,326 (1,113; n = 25) in total length (Fig. 1A); greatest width 217–313 (266; n = 25) at level of gonads; tegument smooth. Cephalic lobes well developed; each head organ consisting of groupings of terminations of cephalic-gland ducts posteriolateral to pharynx. Pharynx a muscular, glandular bulb, spherical to subovate, 43–67 (54; n = 25) long, 37–49 (43; n = 21) wide. Eye-spots absent; chromatic granules scattered throughout cephalic region and posterior trunk. Mouth subterminal, midventral at level of head organs, opening into buccal tube; buccal tube extending posteriorly along
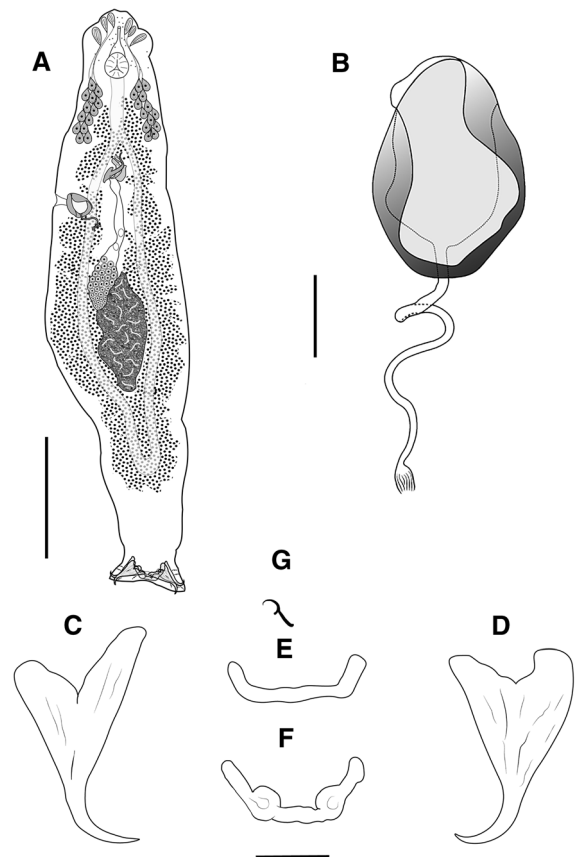


**Fig. 1** *Glyphidohaptor safiensis* n. sp. ex *Siganus canaliculatus*. A, Holotype, ventral view; B, Vaginal vestibule, ventral view; C, Dorsal anchor; D, Ventral anchor; E, Ventral bar; F, Dorsal bar; G, Hook. *Scale-bars*: A, 200 μm; B–G, 20 μm
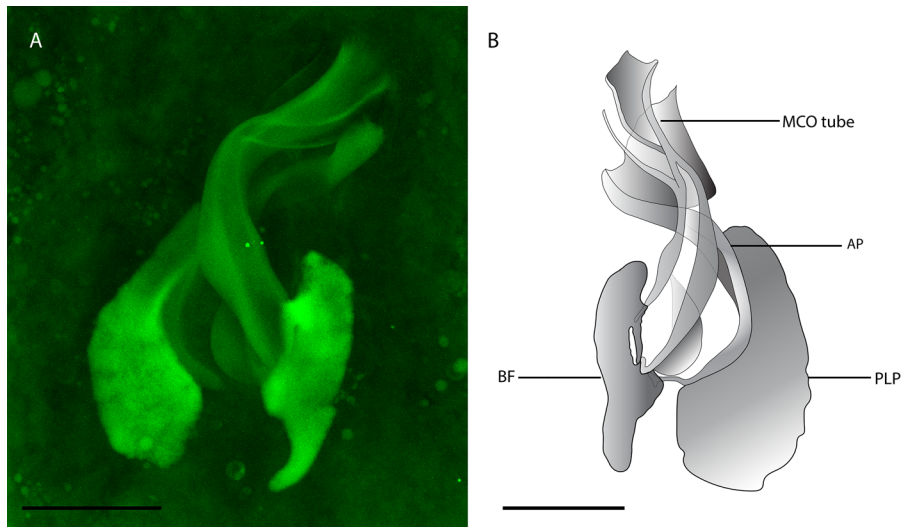
**Fig. 2** *Glyphidohaptor safiensis* n. sp. ex *Siganus canaliculatus*. Male copulatory complex. A, Confocal microscope maximum projection image showing the composition of the MCO and the semi-circular sub-basal flange positioned distally to the fan-shaped basal flange. B, Drawing of the male copulatory organ, dorsal view. *Abbreviations*: BF, basal flange; PLP, plate-like projection; AP, accessory piece. *Scale-bars*: 20 μm

body midline to pharynx; intestinal caeca bifurcating posterior to pharynx, confluent, lacking diverticula.

Testis fusiform, 173–258 (217; n = 21) long, 35–78 (61; n = 21) wide. Germarium pyriform, 64–132 (96; n = 19) long, 21–43 (32; n = 19) wide. Vaginal vestibule (Fig. 1B) slightly sclerotised, sub-oval; vaginal opening 39–52 (45; n = 16) long, 26–33 (30; n = 6) wide; vaginal tube sinuous or coiled, with frayed opening at the end, 44–70 (54; n = 6) long. Male copulatory organ (MCO), tubular, curved, enclosed in heavy sheath, with semi-circular sub-basal flange (Fig. 2A), 58–76 (64; n = 24) long. Accessory piece rod-shaped, distally flattened, with reniform plate-like projection.

Haptor 41–58 (51; n = 24) long, 84–131 (118; n = 24) wide. Ventral anchor 48–57 (53; n = 17) long, base 32–49 (40; n = 21) long, 29–36 (32; n =17) wide (Fig. 1D), inner roots 12–14 (17; n = 20) long, outer roots 11–25 (17; n= 20) long, length of point 6–10 (8; n = 20). Dorsal anchor 54–63 (58; n = 16) long, base 30–44 (38; n = 22) long, 24–33 (28; n = 16) wide (Fig. 1C), inner roots 13–23 (16; n = 20) long; outer roots 21–32 (25; n = 20) long, length of point 6–10 (8; n = 20). Ventral bar 32–46 (39; n = 16) long (Fig. 1E); dorsal bar 37–48 (41; n = 12) long (Fig. 1F); hooks 11–12 (11; n = 18) long (Fig. 1G).

Remarks

Comparative statistical analysis (see Supplementary Table S1) of the morphometric characteristics of *Glyphidohaptor* species revealed that *Glyphidohaptor safiensis* n. sp. can be distinguished from other members of the genus by its significantly larger body length and width. The length of the haptor is significantly shorter in comparison to those of *G. phractophallus*, *G. plectocirra* (*S. luridus*) and *G. sigani* (*S. doliatus* and *S. lineatus*) (Table 3). However, the haptor is significantly wider in comparison to *G. phractophallus*, *G. plectocirra* and *G. sigani* (*S. corallinus*, *S. doliatus* and *S. lineatus*). The size of the pharynx and the male copulatory organ of the new species is significantly larger than all of its congeners. The length of ventral anchor of the new species is larger than that of *G. plectocirra* and *G. sigani* (*S. doliatus* and *S. corallinus*). The base of the ventral sucker of *G. safiensis* n. sp. is smaller than those of *G. phractophallus* and *G. sigani* (*S. doliatus*, *S. corallinus* and *S. lineatus*), but is significantly wider than that of *G. plectocirra* (*S. luridus*). The dorsal anchor length and width are larger in the new species in comparison to its congeners. Also, the ventral and dorsal bars of the new species are significantly larger in comparison to other members of the genus.

**Table 3** Comparative metrical data for *Glyphidohaptor* spp.

| Species | *G. phractophallus* | *G. plectocirra* | *G. plectocirra* | *G. sigani* | *G. sigani* | *G. sigani* | *G. sigani* |
|---|---|---|---|---|---|---|---|
| Host | *S. fuscescens*[a] | *S. luridus*[a] | *S. rivulatus*[a] | *S. doliatus*[a] | *S. punctatus*[a] | *S. corallinus*[a] | *S. lineatus*[a] |
| **Body** | | | | | | | |
| Length | 608–876 (731; n = 26) | 432–614 (522; n = 24) | 662–1064 (870; n = 9) | 325–500 (407; n = 29) | 527–644 (576; n = 5) | 435–539 (470; n = 10) | 372–648 (557; n = 17) |
| Width | 141–254 (186; n = 27) | 106–179 (138; n = 29) | 130–314 (186; n= 17) | 134–213 (171; n = 27) | 159–228 (186; n = 5) | 129–199 (160; n = 10) | 122–213 (164; n = 18) |
| **Haptor** | | | | | | | |
| Length | 58–80 (65; n = 21) | 50–77 (65; n = 22) | 57–81 (68; n = 8) | 51–74 (64; n = 25) | 58–79 (68; n = 6) | 47–69 (61; n = 10) | 58–82 (68; n = 18) |
| Width | 90–112 (99; n = 20) | 60–89 (79; n = 19) | 85–106 (94; n = 9) | 84–118 (97; n = 28) | 83–110 (99; n = 6) | 84–114 (99; n = 10) | 87–118 (102; n = 15) |
| Pharynx | 29–45 (39; n = 27) | 26–41 (34; n = 27) | 36–47 (41; n = 10) | 25–36 (30; n = 30) | 31–36 (35; n = 7) | 29–33 (31; n = 10) | 31–41 (37; n = 16) |
| Copulatory complex | 44–60 (52; n = 12) | 34–50 (41; n = 11) | 42–54 (48; n = 5) | 40–51 (47; n = 8) | 45–52 (49; n = 11) | 42–50 (47; n = 8) | 40–51 (46; n = 8) |
| **Ventral anchor** | | | | | | | |
| Length | 47–57 (52; n = 10) | 42–47 (45; n = 5) | 46–52 (48; n = 6) | 44–52 (48; n = 7) | 52–57 (54; n = 7) | 45–53 (49; n = 6) | 51–58 (54; n = 8) |
| Base width | 31–38 (35; n = 10) | 24–33 (29; n = 6) | 28–34 (31; n = 5) | 30–36 (33; n = 7)[d] | 34–38 (37; n = 6) | 32–38 (35; n = 5) | 29–38 (34; n = 6) |
| Length of base[e] | 32–39 (36; n = 5) | | | 34–41 (38; n = 4)[d] | | | |
| Length of inner root[e] | 12–15 (14; n = 6)[c] | | | 11–16 (13; n = 2)[d] | | | |
| Length of outer root[e] | 16–21 (19; n = 6)[c] | | | 17–21 (19; n = 2)[d] | | | |
| Length of point[e] | 8–10 (9; n = 6)[c] | | | 7–9 (8; n = 2)[d] | | | |
| **Dorsal anchor** | | | | | | | |
| Length | 50–56 (53; n = 9) | 45–49 (47; n = 5) | 43–51 (47; n = 6) | 49–56 (53; n = 7) | 57–61 (59; n = 9) | 50–55 (53; n = 8) | 56–62 (58; n = 8) |
| Base width | 32–36 (34; n = 9) | 27–32 (30; n = 5) | 28–34 (32; n = 5) | 28–38 (32; n = 7) | 33–39 (37; n = 7) | 29–37 (34; n = 7) | 32–37 (34; n = 6) |
| Length of base[e] | 28–35 (31; n = 6)[c] | | | 34–38 (37; n = 4)[d] | | | |
| Length of inner root[e] | 16–20 (17; n = 6)[c] | | | 12–17 (15; n = 4)[d] | | | |
| Length of outer root[e] | 21–27 (23; n = 6)[c] | | | 17–27 (21; n = 4)[d] | | | |
| Length of point[e] | 6–9 (8; n = 5)[c] | | | 8(8; n = 1)[d] | | | |
| Ventral bar | 30–39 (36; n = 9) | 24–28 (26; n = 4) | 24–33 (29; n = 6) | 28–34 (31; n = 7) | 30–35 (32; n = 5) | 27–33 (30; n = 5) | 27–35 (30; n = 10) |
| Dorsal bar | 33–40 (36; n = 5) | 32 (30–34; n = 2) | 28–34 (31; n = 4) | 36–37 (n = 1) | 32–39 (35; n = 4) | 31–38 (33; n = 3) | 29–45 (35; n = 7) |
| Hooks | 11–13 (12; n = 9) | 11–12 (n = 12) | 11–13 (12; n = 16) | 11–13 (12; n = 7) | 11–12 (n = 13) | 10–12 (11; n = 13) | 11–12 (n = 10) |

**Table 3** continued

| Species | G. phractophallus | G. plectocirra | G. plectocirra | G. sigani | G. sigani | G. sigani | G. sigani |
|---|---|---|---|---|---|---|---|
| Host | S. fuscescens[a] | S. luridus[a] | S. rivulatus[a] | S. doliatus[a] | S. punctatus[a] | S. corallinus[a] | S. lineatus[a] |
| Germarium width | 26–39 (31; n = 25) | 24–33 (28; n = 14) | 28–34 (32; n = 3) | 18–30 (24; n = 27) | 28–35 (31; n = 6) | 23–30 (27; n = 10) | 21–32 (27; n = 17) |
| Testis | | | | | | | |
| Length | 119–193 (155; n = 24) | 84–170 (128; n = 24) | 158–241 (211; n = 8) | 51–96 (74; n = 25) | 112–151 (132; n = 7) | 81–124 (95; n = 9) | 67–142 (105; n = 18) |
| Width | 37–70 (53; n = 24) | 48–87 (59; n = 23) | 62–106 (84; n = 8) | 40–76 (53; n = 25) | 60–74 (68; n = 7) | 43–68 (54; n = 9) | 35–80 (54; n = 18) |

[a]Kritsky et al. (2007a); [b]Paperna (1972); [c]Measurements obtained from G. phractophallus museum slides QMG 227444, QMG 227443 and MPM 1882; [d]Measurements obtained from G. sigani (ex Siganus doliatus) museum slides GBR-G227454 and GBR-G227457; [e]According to Gussev (1985)

Morphologically, the male copulatory complex of all members of *Glyphidohaptor* is composed of a basally articulated MCO that is equipped with a basal flange and an accessory piece with a plate-like projection (Kritsky et al., 2007a). The shape of the MCO is variable among species of the genus. The MCO tube of the new species is curved, while that of *G. phractophallus* is arched. In comparison to the new species the MCO tube of *G. sigani* is recurved and that of *G. plectocirra* is straight. The accessory piece of *Glyphidohaptor* spp. appears to be the most suitable structure to differentiate between species of the genus as it is unique to each *Glyphidohaptor* species. The accessory piece of *G. safiensis* n. sp. is rod-shaped and distally expanded and flattened. In *G. phractophallus* the accessory piece is flat and blade-like. *Glyphidohaptor sigani* displays a rod-shaped accessory piece with a bifid proximal end, while the accessory piece of *G. plectocirra* is distally pointed. The basal flange of all *Glyphidohaptor* spp. is fan-shaped, with some species-specific variations among members of the genus. Finally, all *Glyphidohaptor* spp. possess a plate-like projection that is positioned along the proximal half of the accessory piece whereas the plate-like projection of *G. safiensis* n. sp. is large and reniform.

**Phylogenetic position of *Glyphidohaptor safiensis* n. sp. using the SSU dataset**

The length of the partial SSU sequences of the monogeneans sequenced in the present study was 890 bp for *Glyphidohaptor safiensis* n. sp. and *c.*900 bp for both *Tetrancistrum labyrinthus* Al-Jufaili &

Palm, 2017 (GenBank: MN179331 and MN179334) and *T. indicum* Paperna, 1972 (GenBank: MN179330 and MN179328). The BLAST results showed that *G. safiensis* n. sp. is 96% similar to *P. sphincteroporus*, and both sequences of *Tetrancistrum* spp. investigated in this study showed 98% and 99% similarity to *T. sigani* (as *T. nebulosi*; GenBank: KT267177) and 98% similarity to *T. sigani* (as *T. nebulosi*; GenBank: HM545910).

The maximum-likelihood tree based on Kimura 2-parameter distance, gamma distributed with invariant sites constructed using the SSU data set revealed that *G. safiensis* n. sp. and *P. sphincteroporus* formed a separate clade clustering as sister taxa with a bootstrap value of 69% (Fig. 3). The newly generated SSU rDNA sequences of the two *Tetrancistrum* spp. and the available SSU rDNA sequence of *T. sigani* (syn. *T. nebulosi*) (only one sequence was used, HM545910) formed a well-supported monophyletic clade within the marine ancyrocephalids. The clade containing *Glyphidohaptor* and *Pseudohaliotrema* was sister to the clade comprising members of *Bravohollisia* Bychowsky & Nagibina, 1970 but with low bootstrap support (43%). In the *Tetrancistrum* clade, *T. labyrinthus* clustered with *T. indicum* (Fig. 3).

**Phylogenetic position of *Glyphidohaptor safiensis* n. sp. using the LSU dataset**

For the LSU rDNA gene, sequences reaching *c.*851 bp were successfully generated from *G. safiensis* n. sp. (accession number: MN176409) and 856 bp and 863 bp sequences were generated for *T. indicum* (accession numbers: MN179335 and MN179329) and *T.*
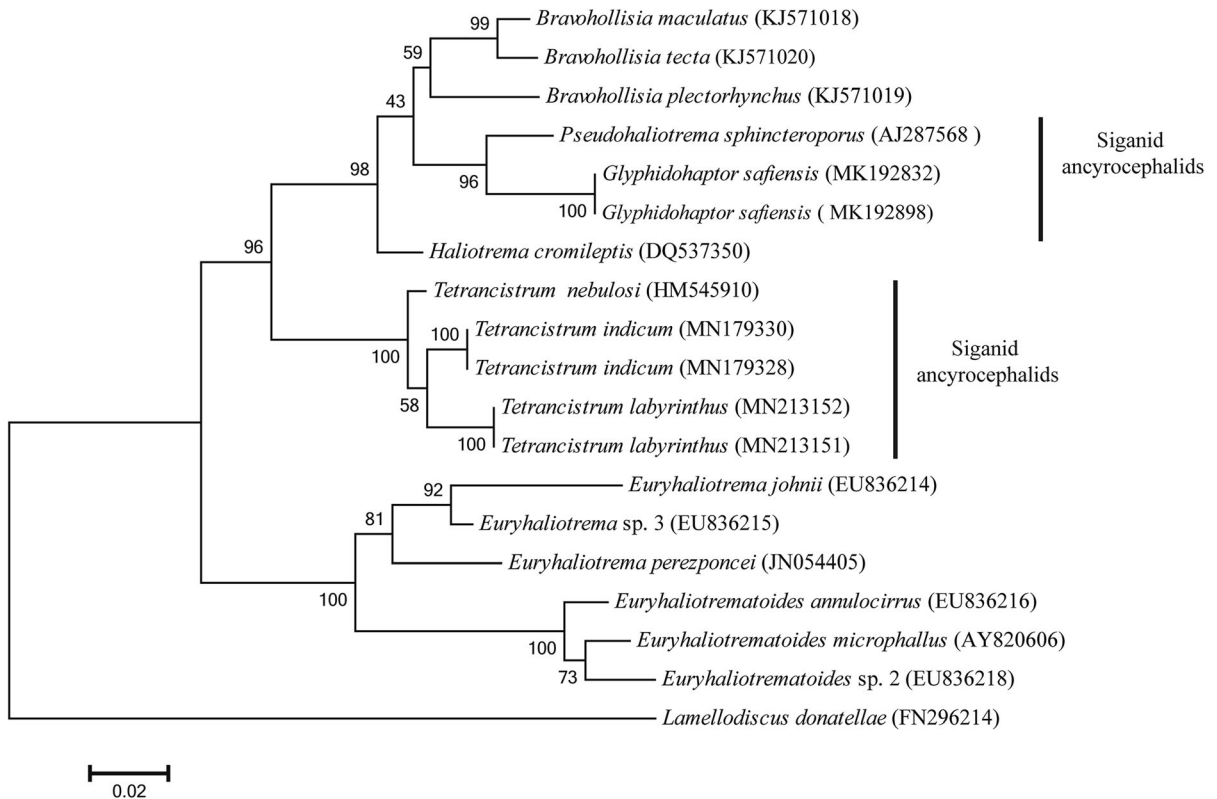
**Fig. 3** Maximum-likelihood tree based on Kimura 2-parameter model, gamma distributed with invariant sites inferred from analysis of SSU rDNA sequences of 15 species of ancyrocephalid monogeneans

*labyrinthus* (accession numbers: MN179333 and MN179332), respectively. The highest similarity to *G. safiensis* n. sp. was with the LSU rDNA sequence of *Tetrancistrum* sp. (GenBank: AF026114) collected from *S. fuscescens* from Australia (97% similarity), which was deposited by Mollaret et al. (1997). As for the *Tetrancistrum* spp. investigated in this study, the closest match generated by a BLAST search was with species of *Euryhaliotrema* Kritsky & Boeger, 2002, especially with *Euryhaliotrema perezponcei* Garcia-Vargas, Fajer-Ávila & Lamothe-Argumedo, 2008. The resulting neighbor-joining tree that was constructed using the LSU gene data (Fig. 4) showed that *G. safiensis* n. sp. formed a well-supported sister species relationship with *Tetrancistrum* sp. (100% bootstrap value), and the two species showed a close phylogenetic relationship clustering with *P. sphincteroporus*, (bootstrap value of 89%). The species of *Tetrancistrum* from the Gulf of Oman formed a well-supported monophylytic clade within the

Ancyrocephalidae (Fig. 4). The genetic divergence value was the lowest between *G. safiensis* n. sp. and *Tetrancistrum* sp. (p-distance of 0.017%) and the highest between *G. safiensis* n. sp. and *Thaparocleidus asoti* (p-distance of 0.341%) (see Supplementary Table S2 for details).

## Phylogenetic position of *Glyphidohaptor safiensis* n. sp. using a partial SSU-entire ITS1 region dataset

Approximately 994 bp were generated for the partial SSU, entire ITS1 and partial 5.8S region of rDNA of *Glyphidohaptor safiensis* n. sp. (accession numbers: MN213150 and MN213152). The two newly obtained sequences were identical. The new sequence was 98.83–98.97% similar to three sequences for another member of *Glyphidohaptor* available on GenBank, *G. plectocirra* (GenBank: HE601931-HE601933). The uncorrected p-distance was 0.01% ± 0.004 between *G. safiensis* n. sp. and *G. plectocirra* (see
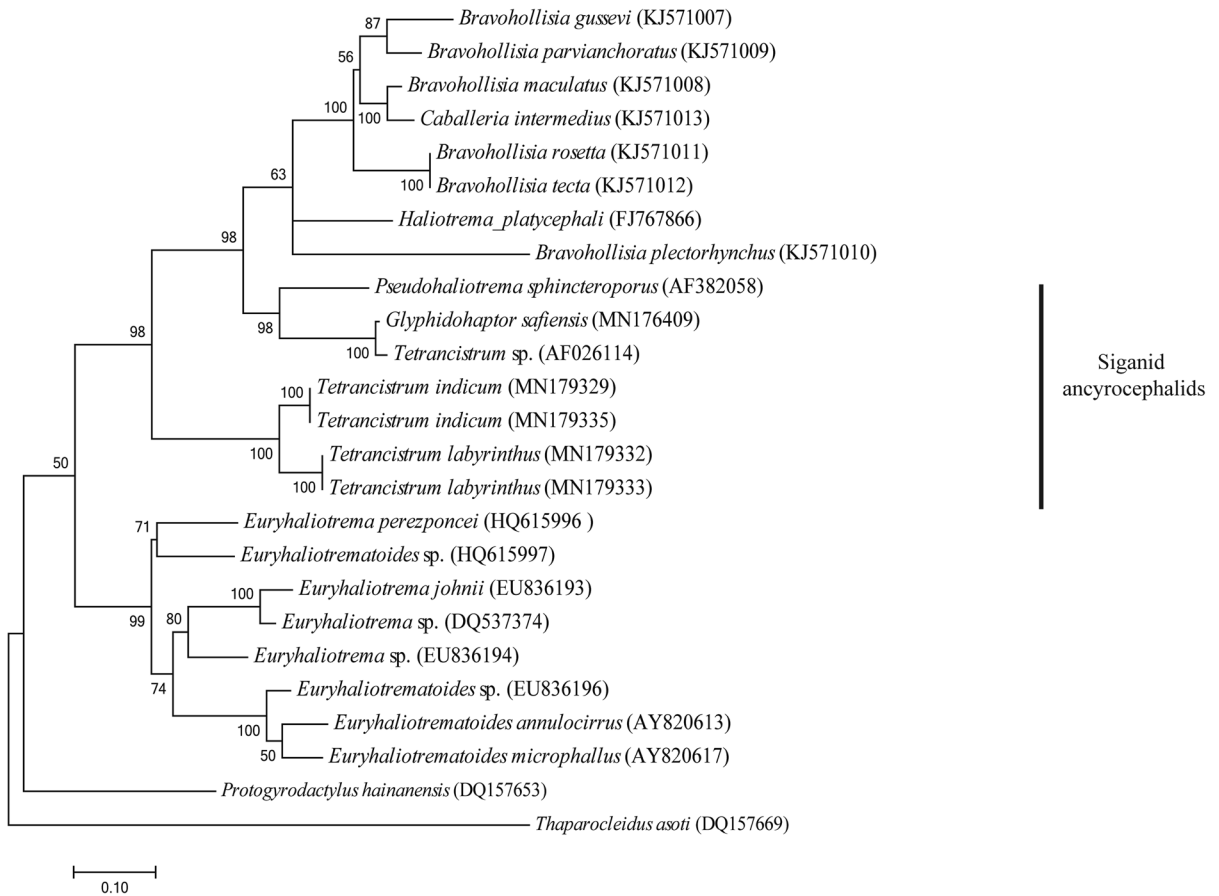
**Fig. 4** Phylogenetic tree inferred from LSU rDNA based on general time reversible model using 20 species of ancyrocephalid monogeneans
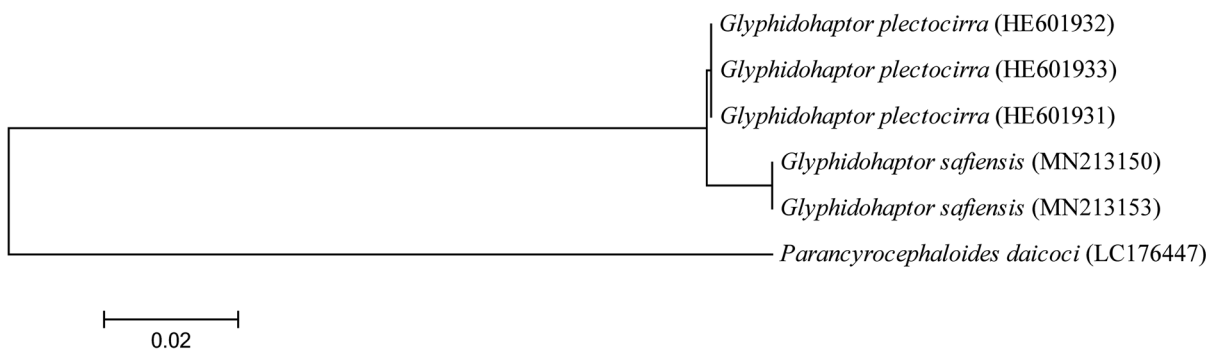


**Fig. 5** The evolutionary history of *Glyphidohaptor* spp. using the partial SSU, entire ITS-1 and partial 5.8S rDNA region inferred using the Neighbor-Joining method using the Kimura 2-parameter model. All positions containing gaps and missing data were eliminated. There were a total of 678 positions in the final dataset

Supplementary Table S3) with a difference of seven nucleotides (Supplementary Table S4). The phylogenetic tree shows that *G. safiensis* n. sp. and *G. plectocirra* formed separate clades (Fig. 5).

## Discussion

### Diversity of *Glyphidohaptor* spp.

Prior to this study *Glyphidohaptor* spp. were only reported from the Great Barrier Reef and the Red Sea. Several micrographs of *Glyphidohaptor* sp. from an unknown *Siganus* sp. from Macassar, Celebes, deposited by Yamaguti (1953) were obtained for comparative analysis. It was noted that this species is unlike any of the previously described members of *Glyphidohaptor* and does not resemble the new species described herein. Thus, it is possible that this undescribed species is another new species of *Glyphidohaptor*, thereby extending the geographical distribution of these worms.

Geets et al. (1997) reported an unidentified species of *Pseudohaliotrema* from the gills of *Siganus sutor* (Valenciennes) off East Africa. It is noteworthy that the species of two of the three ancyrocephalid genera recorded from siganids, *Glyphidohaptor* and *Pseudohaliotrema*, are restricted to hosts of the Siganidae (see Kritsky et al., 2007a). However, *Glyphidohaptor* exhibits a wider geographical range and has been registered from both, reef-associated and colored siganids (e.g. *S. corallinus*, *S. doliatus*, *S. lineatus* and *S. punctatus*) and drab-coloured off-reef siganids (e.g. *S. luridus*, *S. fuscescens* and *S. rivulatus*). On the other hand, members of *Pseudohaliotrema* are so far geographically restricted to the Eastern Indian Ocean and Western Pacific and seem to be limited to the deep-bodied reef-associated siganids (Lim, 2002; Kritsky et al., 2007a). Given that the misidentification of *Glyphidohaptor* and *Tetrancistrum* species as members of *Pseudohaliotrema* was a common misconception among researchers (Kritsky et al., 2007b) and based on the geographical restriction of members of *Pseudohaliotrema*, we believe that the registration of *Pseudohaliotrema* sp. from *S. sutor* by Geets et al. (1997) from off East Africa is probably another misidentification of a *Glyphidohaptor* species. Collection of new material from off East Africa is deemed necessary to confirm this statement.

### Molecular characterisation

To the best of our knowledge, the present study provides the first phylogenetic analyses using data for three genera of marine ancyrocephalids known to infect siganids. These three genera show affinities to each other and affinities to other monogeneans within the marine Ancyrocephalidae. The analysis of the SSU rDNA dataset revealed that *Glyphidohaptor* and *Pseudohaliotrema* formed a separate clade within the marine Ancyrocephalidae and clustered as sister taxa. Kritsky et al. (2007a) stated that within the order Dactylogyridea, only *Glyphidohaptor* spp. and *Pseudohaliotrema* spp. displayed a germarium lying to the right of the anterior portion of the testis and suggested that this feature is apomorphic and that they are phylogenetically related. Future investigations including molecular analysis of all known members of the two genera, in addition to their relationships with their representative hosts, could provide some fascinating insights into the host-parasite phylogenetic relationships and co-evolution.

Species of *Tetrancistrum* which are found on both siganids and members of the acanthurid genus *Naso* (Lacépède), formed a distinct monophyletic group within the Ancyrocephalidae (Figs. 4 and 5). All of the *Tetrancistrum* spp. included in this analysis are collected from siganids; they were divided into two groups, one composed of *T. sigani* (syn. *T. nebulosi*) and *T. labyrinthus* and a separate group containing *T. indicum*. At the current state of knowledge, it is not clear what main characters are responsible for this phylogenetic division within the members of *Tetrancistrum*. Obtaining additional molecular data from other species of *Tetrancistrum* from siganid and nasoid hosts will help clarify the factors influencing the formation of these molecular divisions. The findings of the present study suggest that the SSU rDNA is a reliable marker to understand phylogenetic relationships within the Ancyrocephalidae.

The data obtained from the LSU rDNA region of the worms investigated in the present study revealed that *G. safiensis* n. sp. disclosed highest similarity to the sequence of *Tetrancistrum* sp. deposited by Mollaret et al. (1997). Since molecular divergence among species representing different genera is usually higher than that among congeneric species within the family (Wu et al., 2007), it can be suggested that the *Tetrancistrum* sp. analysed by Mollaret et al. (1997) is

actually a species belonging to *Glyphidohaptor* rather than *Tetrancistrum*. However, re-evaluation of the species analysed by Mollaret (1997) will require confirmation before drawing any conclusions. Subsequently, the newly generated sequences of *Tetrancistrum* spp. were always clustering as a separate monophyletic group regardless of the type of dataset used in the molecular analyses. This observation supports the notion that *Tetrancistrum* sp. analysed by Mollaret et al. (1997) is a member of *Glyphidohaptor*, and most probably represents *G. phractophallus* since it is the only species reported from *S. fuscescens* to date. This suggestion requires further investigation and confirmation by morphological and molecular analysis of *Glyphidohaptor* and *Tetrancistrum*-like specimens from *S. fuscescens* off Australia.

In their study Stefani et al. (2012) observed no intraspecific variability of the partial SSU-ITS1 rDNA marker among individuals of *G. plectocirra*. Thus, the fact that the p-distance among individuals of *G. plectocirra* was negligible, supports that *G. safiensis* n. sp. with a p-distance of 0.010% and a difference of seven nucleotides, is genetically distant from *G. plectocirra*. The low p-distance value between these two species could be attributed the close geographical localities of these species.

**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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