Revision of the family Sphyriocephalidae Pintner, 1913 (Cestoda: Trypanorhyncha), with the description of *Heterosphyriocephalus encarnae* n. sp. and redescrptions of two species of *Sphyriocephalus*

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Abstract The family Sphyriocephalidae Pintner, 1913, which comprises the genera *Hepatoxylon* Bosc, 1811, *Sphyriocephalus* Pintner, 1913 and *Heterosphyriocephalus* Palm, 2004, is revised from newly-collected and museum material.

Heterosphyriocephalus encarnae n. sp. is described from the pelagic thresher, *Alopias pelagicus* Nakamura (Lamniformes: Alopiidae) collected from the Pacific Ocean off Boca del Alamo, Mexico. This species can be readily distinguished from the rest of sphyriocephalids by its small size, low number of proglottids and long velum with a characteristically irregular and folded border, among other features. The tentacles show a distinctive basal armature, and a heteroacanthous typical metabasal armature with heteromorphous hooks. Redescrptions are provided for *Sphyriocephalus tergestinus* Pintner, 1913 and *S. viridis* (Wagener, 1854) Pintner 1913 based on novel morphological data. A phylogenetic analysis including the available sequences of sphyriocephalid species plus new generated sequences of *S. tergestinus* has been performed, from which *S. tergestinus* is allocated into *Heterosphyriocephalus* as *H. tergestinus* n. comb. New dichotomous keys for the determination of genera of Sphyriocephalidae are provided, as well as new generic diagnoses for *Sphyriocephalus* and *Heterosphyriocephalus* and keys for the determination of species within both genera. Although the morphology of the genus *Hepatoxylon* is not addressed in the present study, the available sequence of the type-species has been incorporated in the phylogenetic analysis and its relationship to the other two genera of the family is discussed.

Keywords: parasites, cestodes, Trypanorhyncha, Sphyricephalidae, *Sphyricephalus*, *Heterosphyricephalus*

1. Introduction

The family Sphyricephalidae Pintner, 1913 was erected to accommodate two species, *Sphyricephalus viridis* (Wagener, 1854) Pintner 1913, collected as adults from the kitefin shark *Dalatias licha* (Bonnaterre) (Squaliformes: Dalatiidae) and the gulper shark *Centrophorus granulosus* (Bloch and Schneider) (Squaliformes: Centrophoridae), and *Sphyricephalus tergestinus* Pintner, 1913, collected as adults from the thresher shark *Alopias vulpinus* (Bonnaterre) (Lamniformes: Alopiidae) and as plerocercoids from the teleosts *Lepidopus caudatus* (Euphrasen) (Perciformes: Trichiuridae) and *Brama brama* (Bonnaterre) (Perciformes: Bramidae). Both trypanorhynch species were originally described from the Mediterranean Sea off Trieste, Italy. Following the re-description by Dollfus [1], this family included trypanorhynch cestodes that were characterized by (i) a large and muscular scolex, (ii) two circular bothria with fleshy rims and fused margins, (iii) tentacles emerging from the bothrial cavity, (iv) a basal swelling and characteristic basal armature, (v) transversely or obliquely oriented bulbs, (vi) a retractor muscle attached at the anterior part of the bulb, (vii) absence of a cirrus-sac (i.e. the cirrus is invaginated in the distal part of the ejaculatory duct), (viii) eggs with two prolongations on opposite ends and (ix) plerocercoid larval stages lacking a blastocyst.

Guiart [2] described a third species, *Sphyricephalus alberti* Guiart, 1935, on the basis of plerocercoid stages from the Portuguese dogfish, *Centroscymnus coelolepis* Barbosa du Bocage & de Brito Capello (Squaliformes: Somniosidae) collected from the Mediterranean Sea off Calvi, France. This species was considered a synonym of *S. viridis* by Dollfus [1]. The synonymization was later confirmed by Bussieras [3] based on a detailed re-examination of the type material. The latter author provided a very accurate description of the tentacular armature for *S. viridis*, which had initially been described as homeoacanthous, and noted that the disposition of hooks in the metabasal tentacular armature of this species clearly followed a heteroacanthous pattern. His suggestion that this feature could also be present in closely-related species was later supported by Beveridge and Campbell [4], who examined two plerocercoids of *S. tergestinus* from the blue hake *Macruronus novaezelandiae* (Hector) (Gadiformes:

Merluccidae), collected off Tasmania, Australia, and confirmed that a heteroacanthous pattern is present in the metabasal armature.

In contrast, *Sphyricephalus pelorosoma* Heinz and Dailey, 1974, described from a single adult specimen found in the bigeye thresher, *Alopias superciliosus* (Lowe) (Lamniformes: Alopiidae) from the Pacific Ocean off Bolsa Chica State Beach, California [5], and *Sphyricephalus dollfusi* Bussieras and Aldrin, 1968, described from a single plerocercoid from the stomach of the bigeye tuna *Thunnus obesus* (Lowe) (Perciformes: Scombridae) from the Atlantic Ocean off the western African coast [6], are considered to possess homeoacanthous armature patterns [7].

More recently, Palm [7] erected the new genus *Heterosphyricephalus* Palm, 2004 to accommodate *H. oheulumiae* Palm, 2004 based on plerocercoids found in the sickle pomfret, *Taractichthys steindachneri* (Döderlein) (Perciformes: Bramidae) from the Indian Ocean off Pelabuhan Ratu, Indonesia and in the yellowfin tuna *Thunnus albacares* (Bonnaterre) (Perciformes: Scombridae) from an unknown locality. Species of this genus are characterized and differ from species of *Sphyricephalus* in the possession of a typical heteroacanthous, heteromorphous armature and absence of a distinct basal swelling and basal armature.

Pintner [8] considered that the genera *Dibothriorhynchus* Blainville, 1828 (now a synonym of *Hepatoxylon* Bosc, 1811) and *Sphyricephalus* form the subfamily Sphyricephalinae. However, the former genus was later placed within the family Hepatoxylidae by Dollfus [9], who only recognized *Sphyricephalus* in the Sphyricephalidae and thus refuted the synonymy of both families. Later [10] synonymized the Hepatoxylidae with the Sphyricephalidae based on their morphological affinities regarding the tentacular armature, oncotaxy, attachment site of the retractor muscle in the bulb and features of the surface ultrastructure [7].

At present, the family Sphyricephalidae comprises the genera *Hepatoxylon*, *Heterosphyricephalus* and *Sphyricephalus*. The genus *Sphyricephalus* currently includes four valid species, namely *S. dollfusi*, *S. pelorosoma*, *S. tergestinus* and *S. viridis*. However, the necessity of a re-examination of the type material of *S. tergestinus* and *S. viridis* (to confirm their armature patterns) and of adequate re-descriptions based on museum and newly collected material (in order to provide a complete description for both species) has been stressed by several authors [3, 4, 7].

In the present study, the family Sphyricephalidae is revised and a reconfiguration of genera is performed based on molecular and morphological data. A new species of

Heterosphyriocephalus from the pelagic thresher, *Alopias pelagicus* Nakamura (Lamniformes: Alopiidae) collected from the Pacific Ocean off Boca del Alamo, Mexico, is described. Furthermore, redescrptions are provided for *Sphyriocephalus tergestinus* and *S. viridis* based on museum and newly collected material. New generic diagnoses are provided for *Sphyriocephalus* and *Heterosphyriocephalus*, as well as keys for the determination of both genera and species.

2. Materials and methods

2.1 Collection of specimens

Adult and juvenile worms and plerocercoids of all species addressed in the present study were recovered from specimens of *A. pelagicus* from the Gulf of California off Boca del Alamo (23°53'N, 109°48'W) (Mexico); *A. vulpinus* from off Bouharoun (36°40'N, 04°40'E) (Algeria); off Cap de Creus (42°20'N, 03°15'E), Sant Pol de Mar (41°35'N, 02°38'E) and Tarragona (41°02'N, 01°18'E) (Spain) and *D. licha* from off Dellys (36°55'N, 03°53'E) (Algeria) and off Nouméa (23°00'S, 167°11'E) (New Caledonia). Cestodes were placed in warm saline solution and either fixed in 10% buffered formalin and then transferred to 70% ethanol or preserved in 70% ethanol directly. In the case of the worms collected in Spain, the terminal proglottid of the strobila was preserved in pure ethanol prior to fixation. Additional plerocercoids of *S. viridis* were obtained from frozen specimens of *Galeus melastomus* Rafinesque from off Tarragona (40°35'N, 01°27'E) (Spain), *Mora moro* (Risso) from off Barcelona (40°59'N, 02°01'E) (Spain) and *Ce. coelolepis* from off Barcelona (41°04'N, 03°17'E) and Majorca (40°39'N, 03°07'E) (Spain).

2.2 Morphological study

For morphological observations, worms were stained either in celestine blue or iron acetocarmine, dehydrated in a graded series of ethanols, cleared in methyl salicylate or in clove oil and studied as permanent mounts in Canada balsam. Some of the specimens were skinned with a scalpel blade before mounting in order to reveal internal structures. Some tentacles were detached from the scolex and studied as semi-permanent mounts in pure glycerin or as permanent mounts in Canada Balsam. Pieces of strobila of each species were embedded in paraffin, sectioned at 4–5 µm and stained with hematoxylin and eosin for histology. Several samples of scolices, proglottids and eggs from all three

species addressed were prepared as described by Kuchta and Caira [11] for scanning electron microscopy (SEM).

Type and voucher material has been deposited in the South Australian Museum (SAM), Adelaide (Australia); the Instituto de Biología of the Universidad Autónoma de México (IBUNAM), Mexico City (Mexico); the Lawrence R. Penner Parasitology Collection (LRP) at the University of Connecticut, Storrs, Connecticut (USA); the Muséum National d'Histoire Naturelle (MNHN), Paris (France); the National Museum of Natural History (Smithsonian Institution, NMNH), Suitland (USA); and the Helminthological Collection of the Universitat Autònoma de Barcelona (UABhc), Barcelona (Spain).

Drawings were made with the aid of a drawing tube attached to an Olympus BH light microscope with Nomarski interference contrast. Measurements were obtained with a stage micrometer. Scolex measurements of worms preserved in ethanol were obtained from digital images taken with a Jenoptik ProgRes C3 camera attached to a Leica DM light microscope. All measurements are presented in micrometers, unless otherwise stated, as the range followed, in parentheses, by the mean $\pm$ standard deviation and the number of measurements taken (n).

The following comparative museum material was made available for examination through the courtesy of Dr. Helmut Sattmann (Naturhistorisches Museum, Vienna, Austria): five voucher specimens (VNHM 2068, 2069, 2073, 2569 and 2570) of *S. viridis* and two syntypes (VNHM 2071 and 2076), nine paratypes (VNHM 2062-2067, 2072, 2074 and 2075) and two voucher specimens (VNHM 2567 and 2568) of *S. tergestinus* (see Table 1 for details).

2.3 Molecular and phylogenetic analyses

Genomic DNA was isolated from the terminal proglottid of three adult specimens of *S. tergestinus* from *A. vulpinus* from off Cap de Creus (Spain) and used to amplify the D1-D3 regions of the nuclear large subunit ribosomal DNA (28S rDNA). Extraction was performed using QiagenTM (Valencia, California, USA) DNeasy[®] Tissue Kit following manufacturer's instructions. Polymerase chain reaction (PCR) amplifications were performed following Constenla et al. [12] using the primers and conditions described in Fyler et al. [13]. PCR amplicons were purified using QiagenTM MinElute[®] PCR Purification Kit and sequenced directly for both strands using the PCR primers, LSU5 and 1200F.

Sequences were assembled and edited using Mega v6 [14] and submitted to GenBank under accession numbers XXXX-XXXX. Sequences were aligned using Muscle as implemented in MEGA v6 together with published sequences by Palm et al. [15] and Olson et al. [16,17] for *H. oheulumiae* (GenBank FJ572941), *Hepatoxylon trichiuri* (Holten, 1802) (GenBank FJ572943), *S. viridis* (GenBank FJ572940), *Sphyricephalus* sp. (GenBank AF286974) and *Paroncomegas araya* (Woodland, 1934) (GenBank DQ642801); the latter taxon was used as an outgroup.

SeaView v4 interface [18] was used to select blocks of evolutionarily conserved sites. Maximum likelihood (ML) and Bayesian inference (BI) algorithms were used for phylogenetic tree reconstruction after determination of the best-fit model of nucleotide substitution with jModelTest v2.1.1 [19] using the Akaike Information Criterion (AIC). The best-fitting model selected was the general time-reversible model with gamma distributed rate variation among sites (GTR + G). ML analysis was performed in PhyML v3.0 [20] with a non-parametric bootstrap of 100 replicates. BI analysis was carried out with MrBayes v3.2 [21]. Log likelihoods were estimated over 10^7 generations using Markov Chain Monte Carlo (MCMC) searches on two simultaneous runs of four chains, samplings trees every 10^3 generations. The first 25% of the sampled trees were discarded as “burn-in” and a consensus topology and nodal support estimated as posterior probability values [22] were calculated from the remaining trees. Pairwise genetic distances were calculated using the “uncorrected p-distance” model implemented in MEGA v6.

3. Results

3.1 Description of a new species and redescriptions of *S. tergestinus* and *S. viridis*

The following diagnoses are based on newly collected material and observations of the type material of *S. viridis* and *S. tergestinus* deposited in VNHM. Re-examination of the latter material revealed that many microscope slides had been labeled incorrectly. The amended identification of slides is presented in Table 1, alongside additional information.

A new species of *Heterosphyriocephalus* is described, and redescriptions are provided for *S. viridis* and *S. tergestinus* (which is herein transferred to *Heterosphyriocephalus* based on morphological and molecular results).

3.1.1 *Heterosphyriocephalus encarnae* n. sp.

Type-host: *Alopias pelagicus* Nakamura (Lamniformes: Alopiidae).

Type-locality: Gulf of California off Boca del Alamo, Mexico (23°53'N, 109°48'W).

Site of infection: Stomach.

Type material: Holotype and paratype in IBUNAM (Nos. CNHE 10182 and 10183).

Paratypes in NMNH (Nos. XXX), SAM (Nos. XXX) and LRP (Nos. XXX). Serial sections in NMNH (Nos. XXX).

Etymology: This species is dedicated to both the mother and grandmother, Encarnación Villar and Encarnación Sánchez (respectively) of the first author (SD) [= Encarna (abbreviated form of Encarnación), *encarnae*].

Description (Figs. 1–4)

[Based on 13 whole mounts of fully mature worms, 1 immature worm and 1 plerocercoid; 4 worms preserved in ethanol; 2 specimens used for SEM; 6 tentacles detached from scolices and placed in glycerine or mounted in Canada balsam; 6 serial-sectioned proglottids.]

Cestodes anapolytic, 9.5–34.0 (18.3 ± 7.0 ; $n = 12$) mm long (Fig. 1A), with 34–57 (47 ± 8 ; $n = 10$) proglottids in fully mature strobilae (Fig. 1A); maximum width at level of terminal gravid proglottids (Fig. 1A), strobila with 15–39 (26 ± 7 ; $n = 11$) immature proglottids; 3–9 (6 ± 2 ; $n = 12$) mature proglottids and 6–24 (14 ± 6 ; $n = 12$) gravid proglottids.

Scolex craspedote (Figs. 1A, C, 2A, G, E), compact, 1,507–1,938 ($1,721 \pm 149$; $n = 11$) long by 1,723–2,322 ($1,991 \pm 157$; $n = 11$) wide; maximum width at level of velum (in dorso-ventral view); pars bothrials 1,200–1,815 ($1,389 \pm 186$; $n = 11$) long by 1,714–2,139 ($1,928 \pm 189$; $n = 4$) wide (Figs. 1A, C, 2A, G); bothria two in number, oval, 1,200–1,815 ($1,389 \pm 186$; $n = 11$) long by 1,246–1,523 ($1,407 \pm 90$; $n = 8$) wide, with fused posterior margins, with thick, fleshy rims, mildly delimited (Figs. 1A, C, 2A, G); bothrial pits absent; distal bothrial surface with medial groove, covered with acicular filitriches; proximal bothrial surface covered with small cylindrical projections; projections 15 long by 4 wide, covered with acicular filitriches (Fig. 2J), decreasing in size towards scolex peduncle (Fig. 2I), disappearing towards apex of scolex and posterior margin of velum (Fig. 2E). Apex of scolex, peduncle and velum covered with acicular filitriches (Figs. 2I); pars vaginalis shorter than pars bothrials (Fig. 1C), 692–1,046 (882 ± 100 ; $n = 11$) long by 1,553–1,999 ($1,769 \pm 133$; $n = 12$) wide; tentacle

sheaths sinuous (Fig. 1C); pars bulbosa 346–584 (451 ± 69 ; $n = 13$) long by 1,692–2,230 ($1,956 \pm 151$; $n = 12$) wide (Fig. 1C); retractor muscles attach to anterior part of bulbs; prebulbar organs and gland cells inside bulbs absent (Fig. 1B); bulbs compact, oval (Figs. 1B, C), 631–923 (778 ± 95 ; $n = 13$) long by 246–354 (296 ± 31 ; $n = 13$) wide, in transverse orientation; bulb width/length ratio 1.0 : 2.3–3.0 (2.6 ± 0.2 ; $n = 13$); pars post-bulbosa absent (Fig. 1C); velum present, with irregular and folded border (Figs. 1C, 2A, G, E), 231–615 (387 ± 92 ; $n = 13$) long, scolex width at level of velum in dorso-ventral view 1,753–2,322 ($2,017 \pm 172$; $n = 12$); scolex ratio (pars bothriialis : pars vaginalis : pars bulbosa) 1.0 : 0.5–0.9 : 0.3–0.4 ($1.0 : 0.6 \pm 0.1 : 0.3 \pm 0.1$; $n = 11$). Fully everted tentacle 1,504–1,773 ($1,610 \pm 108$; $n = 5$) long, tentacle diameter without hooks 112–190 (146 ± 23 ; $n = 10$) at base (Figs. 3D-F), 158–216 (182 ± 23 ; $n = 10$) at basal swelling (Figs. 3D-F), 119–197 (144 ± 23 ; $n = 10$) in metabasal region (Figs. 3A-C), 107–137 (123 ± 12 ; $n = 10$) near tip (Figs. 3A-C). Metabasal armature typical heteroacanthous, heteromorphous (Figs. 2B-D, 3A-F); hooks hollow, in ascending half spiral rows. Hook files begin on antibothrial surface (Fig. 3A, D) and terminate on bothrial surface of tentacle (Figs. 2B, C, 3C, F); 8 hooks per principal row; hooks 1 and 1' abut (Fig. 3A, D). Hooks 1(1') falcate in early metabasal armature (Figs. 3A, B, 4B) 77–92 (87 ± 5 ; $n = 13$) long, base 45–54 (51 ± 3 ; $n = 13$) long, with transition to uncinat shape after third metabasal row (Figs. 3A, B, 4B), 62–83 (74 ± 8 ; $n = 9$) long, base 44–51 (48 ± 3 ; $n = 9$) long; hooks 2(2') falcate in early metabasal armature (Figs. 3A, B, 4B), 82–95 (90 ± 4 ; $n = 14$) long, base 45–59 (52 ± 5 ; $n = 14$) long, with transition to uncinat shape after third metabasal row (Figs. 3A, B, 4B), 62–80 (73 ± 7 ; $n = 8$) long, base 44–57 (50 ± 5 ; $n = 8$) long; hooks 3(3') falcate (Figs. 3A-C, 4B), 79–98 (90 ± 6 ; $n = 15$) long, base 48–57 (53 ± 3 ; $n = 15$) long; hooks 4(4') falcate (Figs. 3A-C, 4B), 77–109 (90 ± 10 ; $n = 15$) long, base 48–59 (54 ± 4 ; $n = 15$) long; hooks 5(5') falcate (Figs. 3B, C, 4B), 77–103 (88 ± 7 ; $n = 15$) long, base slightly narrower, 39–56 (49 ± 6 ; $n = 15$) long; hooks 6(6') falcate (Figs. 3B, C, 4B), 72–94 (84 ± 7 ; $n = 15$) long, base 41–54 (47 ± 4 ; $n = 15$) long; hooks 7(7') falcate (Figs. 3B, C, 4B), slightly smaller, 68–88 (77 ± 5 ; $n = 15$) long, base narrower, 36–50 (42 ± 4 ; $n = 15$) long; hooks 8(8') falcate (Figs. 3B, C, 4B), smaller, 57–83 (70 ± 8 ; $n = 15$) long, base narrower, 30–45 (37 ± 5 ; $n = 15$) long. Anterior extension of base longer for all hooks near base of tentacle (Figs. 3A-C).

Distinctive basal armature present (Figs. 3D-F); row 1 of basal hooks uncinat (Figs. 3D-F, 4A), 27–42 (33 ± 5 ; $n = 15$) long, base extended anteriorly, 24–35 (28 ± 3 ; $n =$

15) long. Basal swelling present (Figs. 3D-F), projected towards antiothrial surface, with 7–8 rows of hooks; rows 2–4 of basal hooks on antiothrial and bothrial surfaces falcate (Figs. 3D-F, 4A), 29–38 (31 ± 3 ; $n = 15$) long, base 15–21 (18 ± 2 ; $n = 15$) long; rows 5–7 of basal hooks on antiothrial and bothrial surfaces uncinat (Figs. 3D-F, 4A), 17–27 (21 ± 3 ; $n = 15$) long, base 13–18 (15 ± 2 ; $n = 15$) long; rows 2 and 3 of basal hooks on internal and external surfaces falcate (Figs. 3D-F, 4A), 23–35 (30 ± 4 ; $n = 12$) long, with elongate base, 12–20 (17 ± 2 ; $n = 12$) long; rows 4–9 of basal hooks on internal and external surfaces uncinat (Figs. 3D-F, 4A), densely arranged in mosaic pattern, increasing in size towards metabasal armature, 14–26 (21 ± 4 ; $n = 15$) long, with characteristically broad base 14–23 (18 ± 3 ; $n = 15$) long. First principal rows begin at level of hook row 11 (Figs. 3D-F).

Proglottids craspedote (Figs. 1A, E, 2A), covered with acicular filitriches (Fig. 2H); first immature proglottids 31–62 (42 ± 12 ; $n = 12$) long by 1,622–2,653 ($1,998 \pm 282$; $n = 12$) wide; first mature proglottids 292–461 (359 ± 62 ; $n = 11$) long by 1,922–3,737 ($2,782 \pm 573$; $n = 11$) wide; first gravid proglottids 369–784 (479 ± 110 ; $n = 12$) long by 1,992–3,868 ($2,963 \pm 613$; $n = 12$) wide; terminal gravid proglottid 385–877 (522 ± 136 ; $n = 12$) long by 2,046–3,907 ($3,065 \pm 621$; $n = 12$) wide. Genital pores pre-equatorial (Fig. 1E), covered by velum of adjacent anterior proglottid; pore inconspicuous; cirrus-sac absent (Figs. 1D, E); cirrus unarmed, invaginated in the ejaculatory duct in immature and early mature proglottids (Fig. 1E); ejaculatory duct runs parallel and close to anterior margin of proglottid until reaching to distal ovarian lobe on aporal side (Fig. 1E), tortuous in proximal and mid-regions, dilated in final portion; seminal vesicles absent (Figs. 1D, E). Testes occupy complete intervascular space (Fig. 1E), in 2–3 layers, exclusively preovarian (Fig. 1E); testes 59–98 (85 ± 12 ; $n = 15$) long by 35–50 (45 ± 4 ; $n = 15$) wide; total number of testes 264–273 (268 ± 5 ; $n = 3$); testes distribution 87–99 (92 ± 6 ; $n = 3$) postporal; 165–186 (176 ± 11 ; $n = 3$) aporal; preporal testes absent.

Vagina enters genital atrium posterior to ejaculatory duct (Figs. 1D, E), turns encircling a characteristic spherical dense mass at distal part (Fig. 1D), runs posterior and parallel to ejaculatory duct and anterior to uterus, turns towards and runs posterior to ovarian isthmus at level of Mehlis' gland; seminal receptacle absent (Fig. 1E). Ovary at posterior margin of proglottid (Fig. 1E), bilobed in dorsoventral view; ovarian lobes 98–183 (160 ± 42 ; $n = 4$) long by 110–189 (143 ± 37 ; $n = 4$) wide in early mature proglottids, 183–305 (242 ± 50 ; $n = 4$) long by 110–281 (191 ± 73 ; $n = 4$) wide in late

mature proglottids. Mehlis' gland large (Fig. 1E), between ovarian lobes, 79–134 (117 ± 25 ; $n = 4$) long by 92–122 (101 ± 15 ; $n = 4$) wide in early mature proglottids, 104–195 (157 ± 39 ; $n = 4$) long by 153–253 (190 ± 44 ; $n = 4$) wide in late mature proglottids. Vitelline follicles circummedullar (Fig. 1E), absent in ovarian region, 15–24 (20 ± 3 ; $n = 15$) in diameter in early mature proglottids, 21–40 (33 ± 6 ; $n = 15$) in late mature proglottids. Uterus preformed (Fig. 1E), fusiform to plicate, parallel and posterior to vagina, displaced porally. Uterine pore present, displaced porally. Eggs thin-walled, 29–37 (33 ± 2 ; $n = 20$) in diameter, with two opposite prolongations (Fig. 2F), first prolongation short and filiform, 49–77 (61 ± 7 ; $n = 20$) long, second prolongation larger and wider, 261–288 (273 ± 8 ; $n = 20$) long; egg surface rough.

Remarks

Heterosphyriocephalus encarnae n. sp. can be readily distinguished from other sphyriocephalid species by its small size, small number of proglottids, a long velum with a characteristically irregular and folded margin, presence of small cylindrical projections covering the proximal bothrial surface, scolex peduncle and anterior part of the velum and the absence of a pars post-bulbosa. The metabasal tentacular armature is typical heteroacanthous, heteromorphous with eight hooks per principal row. This species is further characterized by the low number of testes in an exclusively pre-ovarian distribution and the absence of seminal vesicles.

Heterosphyriocephalus encarnae n. sp. resembles the type-species, *H. oheulumiae*, in similar proportions of the bothria and bulbs and a typical heteroacanthous, heteromorphous metabasal armature. However, it differs from the type-species in a number of features, such as (i) a much smaller scolex (1,507–1,938 vs. 6,500–9,625, respectively), (ii) the possession of a velum (vs. absence of a velum, respectively), (iii) the pars bothrialis extending beyond the pars bulbosa (vs. pars bothrialis and pars bulbosa slightly or not overlapping, respectively), (iv) tentacles with a basal swelling and characteristic basal armature (vs. absence of both features, respectively), (v) fewer principal hooks in the metabasal armature (8 vs. 11–13, respectively), (vi) larger lengths and base lengths of basal hooks (14–38 x 12–21 vs. 10 x 5 long, respectively) and (vii) larger lengths and base lengths of metabasal hooks (57–108 x 30–59 vs. 18–60 x 18–43 long, respectively).

The examination of adult specimens and characterization of proglottids of *H. oheulumiae*, with special focus on the morphology of the eggs and the reproductive

structures (e.g., the presence/absence of seminal vesicles, the seminal receptacle, the characteristic spherical dense mass at the distal part of the vagina observed in *H. encarnae* n. sp. and *S. tergestinus*) is necessary before an accurate diagnosis of the genus and comparison among species can be made. New observations might reveal additional similarities between *H. oheulumiae* and its congeners.

The morphological resemblance between the new species and *S. tergestinus*, as well as the results of the molecular analyses presented below, resulted in the allocation of *S. tergestinus* into *Heterosphyriocephalus* (see below).

3.1.2. *Heterosphyriocephalus tergestinus* (Pintner, 1913) n. comb.

Synonyms: *Sphyriocephalus tergestinus* Pintner, 1913 (new synonym).

Type-host: *Alopias vulpinus* (Bonnaterre) [*Alopecias vulpes* (Gmelin) sensu Pintner (1913); *Vulpecula marina* Garman sensu Dollfus (1942)] (Lamniformes: Alopiidae).

Additional hosts:

Plerocerci: *Aphanopus carbo* Lowe (Perciformes: Trichiuridae), *Brama brama* (Bonnaterre) (*B. raji* Bloch and Schneider sensu [23]) (Perciformes: Bramidae), *B. dussumieri* Cuvier (Perciformes: Bramidae), *Conger conger* (L.) (Anguilliformes: Congridae), *Lepidopus caudatus* (Euphrasen) (*Le. argenteus* Bonnaterre sensu [24]) (Perciformes: Trichiuridae), *Macruronus novaezelandiae* (Hector) (Gadiformes: Merlucciidae), *Taractichthys steindachneri* (Döderlein) (Perciformes: Bramidae), *Trachurus picturatus* (Bowdich) (Perciformes: Carangidae).

Adults: *Euprotomicrus bispinatus* (Quoy and Gaimard) (Squaliformes: Dalatiidae), *Isurus oxyrinchus* Rafinesque (*Isuropsis glaucus* (Müller and Henle) sensu [31]); *I. glaucus* (Müller and Henle) sensu [1] (Lamniformes: Lamnidae).

Type-locality: Mediterranean Sea: off Trieste, Italy.

Additional localities:

Mediterranean Sea: Italy: off Naples [23], Palermo [23] and Messina [23]; Spain: off Cap de Creus (present study), Sant Pol de Mar (present study) and Tarragona (present study); Algeria: off Bouharoun (present study); Atlantic Ocean: Spain: from the Gulf of Cádiz [25]; Portugal: off Sesimbra [26] and Madeira [26, 27, 28]; North Atlantic Ocean: from the Great Meteor Seamount (GMS) [29]; Pacific Ocean: USA: from the Johnston Atoll and Hawaii (locality unknown) [30], from the eastern coastline of Japan (locality unknown) [31, 32], from the western coastline of Mexico (locality unknown) [30], from the north-central Pacific Ocean (locality unknown, see [30]); Indian Ocean: Indonesia:

off Pelabuhan Ratu [7]; Tasman Sea: Australia: off Tasmania (locality unknown, see [4]).

Site of infection: Stomach and spiral valve (plerocerci and adult worms); body cavity and gills (plerocerci).

Deposited new material: NMNH (Nos. XXX), SAM (Nos. XXX), UABpc (Nos. C1–13 and C19).

Prevalence: 100 % (3 sharks infected in Spain).

Intensity: 5–7 (in sharks from Spain).

Re-description (Figs. 5-8)

[Based on 5 whole mounts of fully mature worms; 13 fully mature worms preserved in ethanol; 3 specimens used for SEM; 4 tentacles detached from scolices and placed in glycerine or mounted in Canada balsam; 2 serial-sectioned proglottids.]

Cestodes anapolytic, 44–480 (214 ± 152 ; $n = 18$) mm long (Fig. 5A), with 126–260 (182 ± 43 ; $n = 15$) proglottids in fully mature strobilae; maximum width at level of terminal gravid proglottids; strobila with 73–130 (100 ± 16 ; $n = 14$) immature proglottids, 6–29 (14 ± 7 ; $n = 15$) mature proglottids, 8–151 (73 ± 47 ; $n = 16$) gravid proglottids.

Scolex craspedote (Figs. 5A, B, 6A), compact, 1,393–2,600 ($2,034 \pm 294$; $n = 13$) long by 1,541–2,254 ($1,974 \pm 225$; $n = 12$) wide; maximum width at level of bothria (in lateral view); pars bothrialis 1,232–1,969 ($1,487 \pm 208$; $n = 11$) long by 1,714–3,146 ($2,647 \pm 378$; $n = 13$) wide (Fig. 6A); bothria two in number, oval, 889–1,435 ($1,185 \pm 136$; $n = 15$) long by 1,143–1,523 ($1,331 \pm 114$; $n = 16$) wide, with fused posterior margins, with very thick, fleshy rims, well delimited (Figs. 5A, B, 6A, F); distal bothrial surface with medial ridge (Figs. 5B, 6F); bothrial pits absent; pars vaginalis shorter than pars bothrialis, 1,061 ($n = 1$) long by 1,676–1,861 ($n = 2$) wide; tentacle sheaths sinuous (Fig. 5B); pars bulbosa 569–692 (633 ± 62 ; $n = 3$) long by 1,676–1,846 ($n = 2$) wide; retractor muscles attach to anterior part of bulbs; prebulbar organs and gland cells inside bulbs absent (Figs. 5B, C); bulbs compact, oval (Figs. 5B, C), 689–896 (800 ± 71 ; $n = 10$) long by 238–396 (314 ± 46 ; $n = 10$) wide, in transverse orientation; bulb width/length ratio 1.0 : 2.0–3.0 (2.6 ± 0.3 ; $n = 10$); pars post-bulbosa 108–400 (246 ± 147 ; $n = 3$) long (Fig. 5B); velum present (Figs. 5A, B, 6A), 92–185 (144 ± 47 ; $n = 3$) long, covered with small projections (Fig. 6J); projections c. 5 long by c. 5 wide (Fig.

6K), decreasing in size towards scolex peduncle, disappearing towards apex of scolex; scolex width at level of velum in dorso-ventral view 1,584–1,940 ($1,797 \pm 151$; $n = 4$); scolex ratio (pars bothriialis : pars vaginalis : pars bulbosa) 1.0 : 1.9 : 2.8 ($n = 1$).

Fully everted tentacle 1,311–1,540 ($1,439 \pm 98$; $n = 5$) long, tentacle diameter without hooks 122–207 (138 ± 28 ; $n = 9$) at base (Figs. 7A-C), 168–220 (183 ± 16 ; $n = 9$) at basal swelling (Fig. 7B), 128–159 (140 ± 10 ; $n = 9$) in metabasal region (Figs. 6B-D, 7A-C), 118–151 (136 ± 10 ; $n = 9$) near tip (Figs. 6B-D, 7A-C). Metabasal armature typical heteroacanthous, heteromorphous (Figs. 6B-D, 7A-C); hooks hollow, in ascending half spiral rows. Hook files begin on antibothrial surface (Figs. 6C, 7A) and terminate on bothrial surface of tentacle (Figs. 6D, 7C); 8-9 hooks per principal row, following an irregular pattern; hooks 1 and 1' abut (Figs. 6C, 7A). Hooks 1(1') falcate in early metabasal armature (Figs. 7A, B, 8B), 76–83 (79 ± 3 ; $n = 8$) long, base 44–50 (47 ± 2 ; $n = 8$) long, with transition to uncinata shape after fifth metabasal row (Figs. 7A, B, 8B), 67–82 (75 ± 4 ; $n = 10$) long, base 47–58 (55 ± 3 ; $n = 10$) long; hooks 2(2') falcate in early metabasal armature (Figs. 7A, B, 8B), 79–85 (84 ± 2 ; $n = 8$) long, base 45–54 (51 ± 3 ; $n = 8$) long, with transition to uncinata shape after fifth metabasal row (Figs. 7A, B, 8B), 68–83 (75 ± 4 ; $n = 10$) long, base 50–59 (55 ± 3 ; $n = 10$) long; hooks 3(3') falcate (Figs. 7A, B, 8B), 70–86 (75 ± 6 ; $n = 9$) long, base slightly narrower, 50–55 (51 ± 2 ; $n = 9$) long; hooks 4(4') falcate (Figs. 7A, B, 8B), larger, 77–95 (84 ± 7 ; $n = 9$) long, base slightly wider, 51–58 (55 ± 2 ; $n = 9$) long; hooks 5(5') falcate (Figs. 7A-C, 8B), slightly larger, 77–100 (88 ± 8 ; $n = 13$) long, base 50–57 (53 ± 2 ; $n = 13$) long; hooks 6(6') falcate (Figs. 7B, C, 8B), larger, 68–98 (79 ± 10 ; $n = 15$) long, base slightly narrower, 41–56 (50 ± 5 ; $n = 15$) long; hooks 7(7') falcate (Figs. 7B, C, 8B), slightly smaller, 61–92 (75 ± 10 ; $n = 15$) long, base slightly narrower, 37–54 (47 ± 5 ; $n = 15$) long; hooks 8(8') falcate (Figs. 7B, C, 8B), slightly smaller, 56–80 (70 ± 6 ; $n = 12$) long, base narrower, 32–45 (41 ± 4 ; $n = 12$) long; hooks 9(9') falcate (Figs. 7C, 8B), smaller, 53–63 (58 ± 5 ; $n = 4$) long, base slightly narrower, 32–36 (34 ± 2 ; $n = 4$) long. All hooks decrease slightly in size towards metabasal region (Figs. 7A-C).

Distinctive basal armature present (Figs. 6B, D, E, H, 7A-C); row 1 of basal hooks uncinata (Figs. 7A-C, 8A), 32–46 (39 ± 3 ; $n = 12$) long, base extended anteriorly, 24–35 (31 ± 3 ; $n = 12$) long. Basal swelling present (Figs. 6B, D, E, H, 7A-C), projected towards antibothrial surface, with 9–10 rows of hooks; rows 2–4 of basal hooks on antibothrial and bothrial surfaces falcate (Figs. 7A-C, 8A), 23–33 (28 ± 4 ; $n = 11$) long, base 9–23 (17 ± 4 ; $n = 11$) long; rows 5–7 of basal hooks on antibothrial and bothrial

surfaces uncinata (Figs. 7A-C, 8A), 14–27 (20 ± 4 ; $n = 11$) long, base 9–18 (14 ± 2 ; $n = 11$) long; rows 2 and 3 of basal hooks on internal and external surfaces falcate (Figs. 7A-C, 8A), 38–44 (40 ± 2 ; $n = 10$) long, with elongate base, 21–27 (24 ± 2 ; $n = 10$) long; rows 4–9 of basal hooks on internal and external surfaces uncinata (Figs. 7A-C, 8A), densely arranged in mosaic pattern, increasing in size towards metabasal armature, 18–38 (25 ± 6 ; $n = 10$) long, with characteristically broad base, 15–24 (19 ± 3 ; $n = 10$) long. First principal rows begin at level of hook row 11 (Figs. 7A-C).

Proglottids craspedote (Figs. 5A, E, 6I); first immature proglottids 8–12 (10 ± 2 ; $n = 4$) long by 1,492–1,830 ($1,687 \pm 175$; $n = 3$) wide; first mature proglottids 369–584 (492 ± 109 ; $n = 4$) long by 2,999–3,860 ($3,364 \pm 398$; $n = 4$) wide; first gravid proglottids 1,061–1,338 ($1,188 \pm 140$; $n = 4$) long by 4,245–4,783 ($4,587 \pm 241$; $n = 4$) wide; terminal gravid proglottid 1,323–2,169 ($1,712 \pm 427$; $n = 4$) long by 2,476–3,799 ($3,004 \pm 700$; $n = 4$) wide. Genital pores equatorial (Fig. 5E); pore conspicuous; cirrus-sac absent (Figs. 5D, E); cirrus unarmed, with unarmed sheath, invaginated in the ejaculatory duct in immature and early mature proglottids; ejaculatory duct runs parallel and close to anterior margin of proglottid until reaching to Mehlis' gland in early mature proglottids and overpassing distal ovarian lobe on aporal side in late mature proglottids (Fig. 5E), dilated in final portion, where it connects with large pyriform seminal vesicle (Fig. 5E). Testes occupy complete intervacular space (Fig. 5E), in 2–3 layers, mainly preovarian, some posterior to ovary and Mehlis' gland; testes 90–127 (107 ± 12 ; $n = 15$) long by 55–72 (63 ± 6 ; $n = 15$) wide; total number of testes 570–715 (658 ± 62 ; $n = 4$); testes distribution 70–105 (93 ± 17 ; $n = 4$) preporal, 185–268 (233 ± 36 ; $n = 4$) postporal, 313–358 (333 ± 22 ; $n = 4$) aporal.

Vagina enters genital atrium posterior to ejaculatory duct (Figs. 5D, E), turns encircling a characteristic spherical dense mass at distal part (Figs. 5D, E), runs posterior and parallel to ejaculatory duct and anterior to uterus, turns towards and runs posterior to ovarian isthmus at level of Mehlis' gland; small and elongate seminal receptacle present (Fig. 5E), becoming larger with maturity. Ovary in posterior region of proglottid (Fig. 5E), not reaching to posterior margin, bilobed in dorsoventral view; ovarian lobes 162–229 (198 ± 28 ; $n = 4$) long by 122–223 (188 ± 46 ; $n = 4$) wide in early mature proglottids, 274–451 (367 ± 73 ; $n = 4$) long by 226–335 (288 ± 53 ; $n = 4$) wide in late mature proglottids. Mehlis' gland between ovarian lobes (Fig. 5E), reaching to posterior margin of proglottid, 113–162 (145 ± 22 ; $n = 4$) long by 107–204 (171 ± 44 ; $n = 4$) wide in early mature proglottids, 220–274 (241 ± 27 ; $n = 4$) long by 232–311 (281 ± 34 ;

n = 4) wide in late mature proglottids. Vitelline follicles circummedullar (Fig. 5E), absent in ovarian region, 11–18 (15 ± 3 ; n = 15) in diameter in early mature proglottids, 34–61 (45 ± 9 ; n = 15) in late mature proglottids. Uterus preformed (Fig. 5E), fusiform, parallel and posterior to vagina, displaced porally, sometimes inducing a large round swelling at side of late gravid proglottids (Fig. 5A). Uterine pore present, displaced porally.

Eggs thin-walled, 34–40 (37 ± 1 ; n = 20) in diameter, with two opposite prolongations (Fig. 6G), first prolongation short and filiform, 57–76 (67 ± 6 ; n = 20) long, second prolongation larger and wider, 303–376 (335 ± 21 ; n = 20) long; egg surface rough.

Remarks

This species is characterized by a typical heteroacanthous, heteromorphous metabasal armature with eight to nine hooks per principal row following an irregular pattern.

Other features characteristic for this species are the presence of small projections covering the velum and scolex peduncle, genital pores in an equatorial position, the presence of a large pyriform seminal vesicle entering the proximal part of the ejaculatory duct and the presence of a small seminal receptacle.

Phylogenetic analysis based on 28S rDNA (Fig. 9) has shown that this species is a congener to *H. oheulumiae*. Consequently, *S. tergestinus* is hereby transferred to the genus *Heterosphyriocephalus* as *Heterosphyriocephalus tergestinus* n. comb.

Furthermore, the relocation of this species is supported by the presence of shared morphological features with *H. encarnae* (the only species of the genus for which complete morphological data exist), which differ from morphological characteristics of members of the genus *Sphyriocephalus*.

Heterosphyriocephalus tergestinus n. comb. resembles the type-species of the genus, *H. oheulumiae*, in similar bothria and bulb proportions and in the presence of a typical heteroacanthous, heteromorphous armature pattern. However, it differs from *H. oheulumiae* in (i) a smaller plerocercoid larval stage (3–4 vs. 6 mm, respectively), (ii) a much smaller scolex (1,393–2,600 vs. 6,500–9,625, respectively), (iii) the possession of a velum (vs. absence of a velum, respectively), (iv) a pars bothrialis reaching posteriorly beyond the pars bulbosa (vs. pars bothrialis not reaching to beyond the pars bulbosa, respectively), (v) a larger tentacle diameter (128–159 vs. 95–135, respectively), (vi) the presence of a basal swelling and characteristic basal armature (vs. absence of both features, respectively), (vii) a metabasal armature with fewer hooks per principal row

(8–9 vs. 11–13, respectively), (viii) larger lengths and base lengths of basal hooks (13–44 x 9–27 vs. 10 x 5 long, respectively) and (ix) larger lengths and base lengths of metabasal hooks (53–100 x 32–59 vs. 18–60 x 18–43 long, respectively).

Heterosphyriocephalus tergestinus n. comb. is morphologically very similar to *H. encarnae*. Both species share many morphological features, such as the presence of a velum, similar scolex proportions, a pars bothriialis extending beyond the pars bulbosa and the presence of small projections covering different parts of the scolex. The tentacle diameters of both species are also very similar, as are the basal and metabasal tentacular armature patterns and the shape and sizes of basal and metabasal hooks. Both species possess a characteristic, spherical, dense mass at the distal part of the vagina, which seems to be an exclusive feature of the genus. The eggs are almost identical in shape and size but show a slightly longer prolongation in *H. tergestinus* n. comb.

However, *H. tergestinus* n. comb. can be readily differentiated from *H. encarnae* in (i) different body sizes (200–300 mm vs. 9.5–34 mm, respectively), (ii) the number of proglottids (more than 150 vs. 34–57, respectively), (iii) a shorter velum (92–185 vs. 231–615, respectively), (iv) the absence of small cylindrical projections covering the proximal bothrial surface (vs. the presence of such projections in this area, respectively), (v) the presence of a pars post-bulbosa (vs. absence of a pars post-bulbosa, respectively), (vi) an irregular pattern of 8–9 hooks per principal row in the metabasal tentacular armature (vs. consistently 8 hooks per principal row, respectively), (vii) the number of testes (570–715 vs. 264–273, respectively), (viii) the presence of a large, pyriform seminal vesicle in the proximal part of the ejaculatory duct (vs. absence of this structure, respectively), and (ix) the presence of a small seminal receptacle (vs. absence of a seminal receptacle, respectively).

The spherical dense mass observed at the proximal part of the vagina was already noted and described by Pintner [8] and Yamaguti [31]. The latter author interpreted this structure as a distal vaginal sphincter, which was rejected later by Dollfus [1].

Following Pintner [8], Dollfus [1] described it as a muscular mass bearing elongate spines with rounded bases in a regular pattern. Although we also consider this structure as a large and nearly spherical muscular mass as observed in histological sections and whole mounts, we have not been able to observe the spines described by former authors. We believe that Pintner [8] misinterpreted either the muscular fibers forming this mass, which are thick and abundant and very easily observed in sections and whole mounts, or the cellular nuclei that are interspersed with such fibers and that in histological sections

often appear elongate and triangular in shape as elongate spines. As mentioned by Dollfus [1], this structure seems to be unique within the Eucestoda.

The detailed morphological description of two species of this genus (i.e. *H. encarnae* and *H. tergestinus*), provided in the present study, as well as the confirmation of their phylogenetic relationships based on molecular results (presented in section 3.3), enables us to provide a more accurate generic diagnosis. However, as it was stated above, an examination of adult specimens of the type-species becomes necessary in order to identify some unknown morphological characteristics and to complete the generic diagnosis.

3.1.3. *Sphyriocephalus viridis* (Wagener, 1854) Pintner, 1913

Synonyms: *Tetrarhynchus viridis* Wagener, 1854; *Rhynchobothrium viride* Wagener, 1854 (sensu Parona, 1894); *Sphyriocephala viridis* (Wagener, 1854) Pintner, 1913 [sic]; *Sphyriocephala richardi* Guiart, 1935 [sic]; *Sphyriocephalus richardi* Guiart, 1935; *Sphyriocephala alberti* Guiart, 1935 [sic]; *Sphyriocephalus alberti* Guiart, 1935.

Type-host: *Dalatias licha* (Bonnaterre) (Squaliformes: Dalatiidae).

Additional hosts:

Plerocerci: *Alepocephalus rostratus* Risso (Osmeriformes: Alepocephalidae), *Centroscymnus coelolepis* Barbosa du Bocage & de Brito Capello (Squaliformes: Somniosidae), *Dalatias licha* (Bonnaterre) (Squaliformes: Dalatiidae), *Galeus melastomus* Rafinesque (*Pristiurus melanostomus* (Rafinesque) sensu [8]) (Carcharhiniformes: Pentachidae), *Mora moro* (Risso) (Gadiformes: Moridae) (new host record), *Pseudotriakis microdon* de Brito Capello (*Pseudotriakis microdon* Capello sensu [2]) (Carcharhiniformes: Pseudotriakidae), *Squalus acanthias* L. (Squaliformes: Squalidae), *Synaphobranchus brevidorsalis* Lloyd (*Sy. pinnatus* (Gronovius) sensu [2]) (Anguilliformes: Synaphobranchidae), *Trachyrincus scabrus* (Rafinesque) (*Lepidoleprus trachyrhynchus* Risso sensu [8]) (Gadiformes: Macrouridae), *Xiphias gladius* L. (Perciformes: Xiphiidae).

Adults: *Alopias superciliosus* Lowe (Lamniformes: Alopiidae), *Centrophorus granulosus* (Bloch and Schneider) (Squaliformes: Centrophoridae).

Type-locality: Mediterranean Sea off Nice, France.

Additional localities: Mediterranean Sea: France: off Calvi [2]; Italy: off Naples [23] and Trieste [23]; Algeria: off Bou Ismaïl (Castiglione sensu [3]) and Dellys (present study); Spain: off Barcelona (present study) and Majorca (present study); Atlantic

Ocean: Cape Verde (locality unknown, see [1,2]), Portugal: Azores (locality unknown, see [1,2]), from the Gulf of Guinea [33]; north-eastern Atlantic Ocean: locality unknown [34]; tropical, equatorial and central South Atlantic Ocean: localities unknown [35]; Pacific Ocean: USA: off Bolsa Chica State Beach, California [5], New Caledonia: off Nouméa (present study).

Site of infection: stomach (plerocerci and adult worms), body cavity (plerocerci).

Deposited new material: NMNH (Nos. XXX), SAM (Nos. XXX), MNHN (No. JNC395), UABpc (Nos. C14–18).

Re-description (Figs. 9–12)

[Based on 6 whole mounts of fully mature worms and 3 scolices; 8 specimens used for SEM; 6 detached tentacles in glycerin or Canada balsam; 2 serial-sectioned proglottids; several plerocerci and fragments of adult worms preserved in ethanol.]

Cestodes anapolytic, with more than 200 proglottids in fully mature strobilae (Fig. 10A); maximum length more than 20 cm (Fig. 10A); maximum width at level of terminal gravid proglottids (Fig. 10A); strobila with 141 ($n = 1$) immature proglottids, 27–99 ($n = 2$) mature proglottids, more than 99 ($n = 1$) gravid proglottids.

Scolex acraspedote (Figs. 10A, B, 11A), compact, 4,952–6,183 ($5,632 \pm 405$; $n = 6$) long by 3,614 ($n = 1$) wide, bearing a small apical protuberance (Figs. 10A, 11B); maximum width at level of bothria (in lateral view); pars bothrialis 2,415–3,737 ($3,111 \pm 405$; $n = 7$) long by 3,876–4,322 ($4,086 \pm 224$; $n = 3$) wide (Figs. 10A, 11A); bothria two in number, oval, well delimited, 1,779–3,076 ($2,546 \pm 412$; $n = 12$) long by 2,207–2,907 ($2,599 \pm 288$; $n = 7$) wide, with fused posterior margins, with very thick, fleshy rims (Figs. 10A, B, 11A, B); distal bothrial surface markedly concave, with medial ridge (Fig. 11B); bothrial pits absent; pars vaginalis longer than pars bothrialis, 2,830–3,783 ($3,402 \pm 451$; $n = 5$) long by 3,399 ($n = 1$) wide (Fig. 10B); tentacle sheaths sinuous (Fig. 10B); pars bulbosa 1,230–1,846 ($1,630 \pm 277$; $n = 4$) long by 2,907–3,614 ($n = 2$) wide (Fig. 10B); retractor muscles attach at anterior part of bulbs (Fig. 10C); prebulbar organs and gland cells inside bulbs absent (Figs. 10B, C); bulbs compact, oval (Figs. 10B, C), 1,200–1,707 ($1,454 \pm 197$; $n = 10$) long by 615–723 (672 ± 34 ; $n = 10$) wide, in transverse orientation; bulb width/length ratio 1.0 : 1.8–2.6 (2.2 ± 0.3 ; $n = 10$); pars post-bulbosa prominent, 477–923 (665 ± 187 ; $n = 34$) long (Fig. 10B); scolex ratio (pars bothrialis : pars vaginalis : pars bulbosa) 1.0 : 1.0–1.2 : 0.4–0.8 ($1.0 : 1.1 \pm 0.1$:

0.5 ± 0.2; n = 5). Scolex peduncle covered with acicular filitriches. Scolex delimited from strobila by a narrow constriction (Figs. 10A, B).

Fully everted tentacle 2,876–4,353 (3,511 ± 760; n = 3) long; tentacle diameter without hooks 223–246 (234 ± 12; n = 3) at base (Figs. 11G, 12A-C), 246–288 (265 ± 21; n = 3) at basal swelling (Figs. 11C, G, 12A-C), 238–261 (251 ± 11; n = 3) in transition area towards metabasal region (Figs. 11C, F, G, 12A-C), 215–269 (243 ± 25; n = 4) in metabasal region (Figs. 11F, G, 12A-C), 223–261 (243 ± 19; n = 4) near tip (Figs. 11F, G, 12A-C). Metabasal armature typical heteroacanthous, heteromorphous (Figs. 11C, F, G, 12A-C); hooks hollow, in ascending half spiral rows. Hook files begin on antibothrial surface (Figs. 11G, 12A) and terminate on bothrial surface of tentacle (Fig. 12C); 10–13 hooks per principal row, decreasing in number towards metabasal region; hooks 1 and 1' abut (Figs. 11G, 12A). Metabasal hooks of identical shape, differ in size on different tentacular surfaces (Figs. 11F, G, 12A-C, 13B). Hooks on antibothrial surface of transition area towards metabasal region uncinata (Figs. 12A, 13B), 86–92 (90 ± 2; n = 8) long, base 60–70 (65 ± 4; n = 8) long; hooks on antibothrial surface of metabasal region uncinata (Figs. 12B, 13B), slightly larger, 83–107 (95 ± 9; n = 10) long, base 52–69 (62 ± 5; n = 10) long; hooks on external and internal surfaces of transition area towards metabasal region uncinata (Figs. 11C, 12A, C, 13B), 80–96 (89 ± 6; n = 10) long, base 52–70 (64 ± 5; n = 10) long; hooks on external and internal surfaces of metabasal region uncinata (Figs. 12A, C, 13B), larger, 92–107 (101 ± 6; n = 10) long, base wider, 64–75 (71 ± 4; n = 10) long; hooks on bothrial surface of transition area towards metabasal region uncinata (Figs. 12B, 13B), 73–95 (90 ± 7; n = 10) long, base 52–70 (62 ± 6; n = 10) long; hooks on bothrial surface of metabasal region uncinata (Figs. 12B, 13B), larger, 98–119 (110 ± 8; n = 10) long, base wider, 67–86 (78 ± 8; n = 10) long.

Distinctive basal armature and basal swelling present (Figs. 11C, F, G, 12A-C); basal swelling with 8–9 rows of hooks on antibothrial surface; rows 1–5 of basal hooks on antibothrial and bothrial surfaces falcate (Figs. 12B, 13A), 41–56 (50 ± 5; n = 10) long, base 26–32 (29 ± 2; n = 10) long; rows 6–13 of basal hooks on antibothrial and bothrial surfaces uncinata (Figs. 12B, 13A), 37–53 (44 ± 6; n = 10) long, base 26–39 (33 ± 5; n = 10) long. Rows 1–3 of basal hooks on internal and external surfaces falcate (Figs. 12A, C, 12A), 42–62 (53 ± 5; n = 10) long, base 21–32 (26 ± 3; n = 10) long; rows 4–13 of basal hooks on internal surface uncinata (Figs. 11C, 12A, C, 13A), 29–44 (36 ± 5; n = 10) long, base 27–33 (30 ± 2; n = 10) long; rows 4–13 of basal hooks on external

surface uncinata (Figs. 11C, 12A, C, 13A), larger, 44–58 (51 ± 4 ; $n = 10$) long, base longer, 36–47 (42 ± 4 ; $n = 10$) long. All basal hooks increase in size as progressing further into transition area towards metabasal region (Figs. 11C, 12A-C). First principal rows begin at level of hook row 13 (Figs. 12A-C). Boundary between transition area and metabasal armature around hook row 19 (Figs. 12A-C).

Proglottids craspedote (Figs. 10A, 11E), covered by acicular filitriches; first immature proglottids 15–21 ($n = 2$) long by 1,578–3,322 ($n = 2$) wide; first mature proglottids 523–761 (625 ± 121 ; $n = 4$) long by 2,476–7,000 ($4,348 \pm 1,952$; $n = 4$) wide; first gravid proglottids 646–1,261 ($1,038 \pm 270$; $n = 4$) long by 3,676–6,500 ($4,901 \pm 1,196$; $n = 4$) wide; terminal gravid proglottid 1,546 ($n = 1$) long by 3,783 ($n = 1$) wide. Genital pores pre-equatorial (Fig. 10E); pore inconspicuous; cirrus-sac absent (Figs. 10D, E); cirrus unarmed, invaginated in ejaculatory duct in immature and early mature proglottids (Figs. 10D, E); accessory seminal vesicle round (Figs. 10D, E), 150–162 (156 ± 5 ; $n = 5$) in diameter, followed by seminal vesicle, oval to pyriform (Figs. 10D, E), 217–272 (241 ± 20 ; $n = 5$) long by 110–150 (126 ± 15 ; $n = 5$) wide; accessory seminal vesicle and seminal vesicle located posterior to cirrus (Figs. 10D, E); ejaculatory duct strongly coiled, runs parallel and close to anterior margin of proglottid until reaching to ovarian isthmus (Fig. 10E). Testes occupy complete intervacular space (Fig. 10E), in 2–3 layers, mainly preovarian, some posterior to ovary and Mehlis' gland (Fig. 10E); testes oval (Figs. 10D, E), 81–101 (91 ± 5 ; $n = 15$) long by 55–73 (61 ± 5 ; $n = 15$) wide; total number of testes 965–1,100 ($1,038 \pm 68$; $n = 3$); testes distribution 100–135 (119 ± 18 ; $n = 3$) preporal, 280–370 (335 ± 48 ; $n = 3$) postporal, 563–595 (583 ± 18 ; $n = 3$) aporal.

Vagina joins posteriorly the ejaculatory duct to form an hermaphroditic duct (Figs. 10D, E), runs posterior and parallel to ejaculatory duct, turns towards and runs posterior to ovarian isthmus at level of Mehlis' gland (Fig. 10E); seminal receptacle absent. Ovary in posterior region of proglottid (Fig. 10E), not reaching to posterior margin, bilobed in dorsoventral view; ovarian lobes 239–349 (305 ± 54 ; $n = 4$) long by 166–331 (245 ± 77 ; $n = 4$) wide in early mature proglottids, 349–509 (425 ± 68 ; $n = 5$) long by 184–319 (255 ± 48 ; $n = 5$) wide in late mature proglottids. Mehlis' gland large (Fig. 10E), round, positioned between ovarian lobes, almost reaching to posterior margin of proglottid, 162–288 (234 ± 54 ; $n = 4$) long by 172–264 (221 ± 42 ; $n = 4$) wide in early mature proglottids, 202–368 (314 ± 68 ; $n = 5$) long by 239–380 (311 ± 63 ; $n = 5$) wide in late mature proglottids. Vitelline follicles circummedullar (Fig. 10E), absent in ovarian

region, 18–31 (24 ± 3 ; $n = 15$) in diameter in early mature proglottids, 34–46 (40 ± 3 ; $n = 15$) in late mature proglottids. Uterus preformed (Fig. 10E), fusiform, highly folded, parallel and overlapping vagina and ejaculatory duct, displaced porally. Uterine pore present (Fig. 10A), displaced porally.

Eggs thin-walled, variable in shape, oval to pyriform (Fig. 11D), normally pointed on both sides and with mucron at one pole, side opposite to mucron occasionally completely blunt; with no prolongations present; 82–121 (95 ± 10 ; $n = 20$) long by 55–73 (61 ± 5 ; $n = 20$) wide; egg surface smooth.

Remarks

This species was originally described by Wagener [36] as *Tetrarhynchus viridis*. Pintner [23] redescribed the species, allocated it within the genus *Sphyriocephalus* and erected the family Sphyriocephalidae. Although later authors reviewed the work of Wagener [36] and Pintner [23] and addressed different features of the morphology of this species [1,3,7], the description remained incomplete and several morphological characteristics were still unknown. The present work provides the most advanced redescription of this species and accurately addresses the features that until now have remained unknown or dubious.

Sphyriocephalus viridis can be characterized by (i) the presence of a small protuberance on the apical part of the scolex, (ii) a different tentacle diameter (i.e. smaller than in *Sphyriocephalus pelorosoma* and *Sphyriocephalus dollfusi* but larger than in members of the genus *Heterosphyriocephalus*), (iii) a different size of the uncinata hooks arranged in mosaic pattern on internal (i.e. smaller hooks) and external (i.e. larger hooks) surfaces of the basal tentacular armature, (iv) the presence of two seminal vesicles in the distal part of the ejaculatory duct, (v) the possession of a large number of testes (i.e. around 1,000) and (vi) a different size and shape of eggs (i.e. oval to pyriform, without any prolongations and with mucron on one pole).

Sphyriocephalus viridis resembles its congeners *S. pelorosoma* and *S. dollfusi* in similar proportions of the scolex and bothria (Table 2) and in a similar number of principal hooks in the metabasal armature (i.e. 20–23 hooks). Furthermore, it also resembles *S. dollfusi* in its possession of an acraspedote scolex.

However, *S. viridis* can be differentiated from both *S. pelorosoma* and *S. dollfusi* in a number of features, such as (i) a larger body size (i.e. total length up to 300 mm vs. up to 118 and 71 mm, respectively), (ii) a different orientation of bulbs (i.e. transversely vs.

longitudinally oriented, respectively), (iii) differently shaped bulbs (i.e. compact vs. elongate in the other two species, respectively), (iv) longer tentacles (i.e. 2,876–4,353 vs. 2,000–2,125 and 2,020–2,530 in length, respectively), (v) a smaller tentacle diameter (i.e. basal armature: 223–246 vs. 475–500 and 460–600, respectively; metabasal armature: 215–269 vs. 375–450 and 375–475, respectively), (vi) the presence of a basal swelling and a characteristic basal armature (vs. absence of both features, respectively), (vii) a typical heteroacanthous, heteromorphous metabasal armature (vs. a homeoacanthous, homeomorphous metabasal armature, respectively) and (viii) smaller metabasal hooks with narrower base ($95\text{--}110 \times 62\text{--}78$ vs. $150\text{--}160 \times 135\text{--}140$ and $126\text{--}145 \times 110\text{--}126$, respectively). *Sphyriocephalus viridis* can be further distinguished from *S. pelorosoma* by (i) the absence of a velum (vs. presence of a velum, respectively) and (ii) a different shape of eggs (i.e. absence vs. presence of a long prolongation, respectively).

As it has been stated above for the type species of *Heterosphyriocephalus*, detailed observations on the morphology of *S. pelorosoma* and *S. dollfusi* with special focus on characteristics of the reproductive system are necessary in order to differentiate between *S. viridis* and its congeners.

As Dollfus [1] noted, the apical protuberance of the scolex was described and clearly illustrated by Wagener [36] but ignored by Pintner [23]. Histological sections revealed a small concavity in the centre, with radially departing muscular fibres.

Palm [7] described the scolex of *S. viridis* as slightly craspedote with the presence of a short velum. However, Dollfus [1] stated that the posterior limitation of the scolex is marked by an irregular constriction instead of a velum. According to this author, this line corresponds to the posterior margin of a velum, which is not free, and he considered the scolex as “cryptocraspedote” (i.e. apparently acraspedote due to the reduction and fusion with the strobila of the real velum). A velum has not been observed in any of the examined specimens (i.e. museum specimens and new material). Therefore, the scolex of *S. viridis* is considered as acraspedote.

Although this species was originally thought to have an homeoacanthous armature pattern, Bussieras [3] re-examined the type specimens of *S. alberti* (now *S. viridis*) deposited in the Oceanographic Museum in Monaco and noted that the metabasal armature follows a typical heteroacanthous pattern. According to this author, this feature could only be detected in the distal part of the tentacle, since the half-spiral rows were not distinguishable in the early metabasal armature. Herein we confirm this pattern

observed by Bussieras [3] and determine that *S. viridis* possesses a typical heteroacanthous armature, with half-spiral rows starting on the antibothrial surface and terminating on the bothrial surface. This characteristic is only visible in vastly extended tentacles, since in the early metabasal armature hooks are fairly small and close to one another which makes it hard to observe this pattern. This fact has also been stated by Bussieras [3]. However, Bussieras [3] misidentified the tentacular surfaces on which the patches of small, uncinat hooks forming a mosaic pattern on the basal armature are located. He stated that these hooks are located on bothrial and antibothrial surfaces, whereas these structures are in fact located on the internal and external tentacular surfaces.

Former authors noted the presence of a “globular-pyriform seminal vesicle” [1] in the distal part of the ejaculatory duct. Although this suggests only a single structure, the present study revealed the presence of two distinct seminal vesicles in a tandem position. Both vesicles are clearly separated, connected only by a short and narrow duct. At present, this feature is unique within the family Sphyricephalidae and none of the species in this group possesses seminal vesicles in the distal part of the ejaculatory duct. However, examinations of adult specimens of *S. pelorosoma* and *S. dollfusi* are necessary in order to determine whether this characteristic resembles a generic feature. The presence of one, two or even three seminal vesicles in the distal part of the ejaculatory duct is a common feature in several groups of trypanorhynch cestodes (e.g. all members of the order Gymnorhynchoidea, some members of Eutetrarhynchidae or Otobothriidae) and seems to be highly variable, even among genera of the same family [7].

The eggs of *S. viridis* were described by Wagener [36] and Pintner [23] as bearing a “spiniform process” on either side. However, examination of eggs from the new material reveals that, although most eggs are pointed at both sides, only one side bears a true, spiniform process or mucron. In fact, egg shapes can vary significantly from semi-circular or oval to even pyriform, with one mucronated pole and a completely blunt opposite pole. Eggs described in the original description by Wagener [36] were also smaller than the ones observed in the present study (i.e. 75×45 vs. 95×61 , respectively). Based on these differences, we can only speculate that he could have examined eggs that belong to a different species or which had been observed *in utero*.

3.2 Keys and generic diagnoses

3.2.1 Key to the genera of Sphyricephalidae

Features of the segment morphology presented in the key only apply for species for which adult specimens are well-known (i.e. *S. viridis*, *H. encarnae* and *H. tergestinus*).

- 1 Tentacles short, bulbous or conical in shape, emerging from anterior bothrial margins; bothria elongate; two sets of genitalia per proglottid.....
***Hepatoxylon* Bosc, 1811**
- Tentacles long and cylindrical in shape, emerging from inside the bothria; bothria round or oval; one set of genitalia per proglottid..... 2
- 2 Bulbs more than 1,200 in length and 600 in width; two seminal vesicles present in distal part of ejaculatory duct; absence of spherical dense mass at the distal part of the vagina; tentacles more than 2,000 in length; absence of small projections covering different parts of the scolex..... ***Sphyricephalus* Pintner, 1913**
- Bulbs less than 1,000 in length and 500 in width; seminal vesicles absent or only a single seminal vesicle present in proximal part of ejaculatory duct; presence of spherical dense mass located at the distal part of the vagina; tentacles less than 1,800 in length; presence of small projections covering different parts of the scolex..... ***Heterosphyricephalus* Palm, 2004**

3.2.2. Genus *Sphyricephalus* Pintner, 1913

Diagnosis: Scolex large, compact, craspedote or acraspedote. Bothria two in number, oval, well delimited, with fused posterior margins, with thick, fleshy rims; bothrial pits absent; tentacle sheaths sinuous or straight; retractor muscles attach at anterior part of bulbs; prebulbar organs and gland cells inside bulbs absent; bulbs compact, oval, in transverse or longitudinal orientation. Four elongate tentacles; metabasal armature homeoacanthous, homeomorphous or typical heteroacanthous, heteromorphous; hooks hollow. Distinctive basal armature and basal swelling present or absent. Proglottids (unknown for *S. pelorosoma* and *S. dollfusi*) craspedote. Genital pores marginal, irregularly alternating; cirrus-sac absent; cirrus unarmed, invaginated in ejaculatory duct; two seminal vesicles present in distal part of ejaculatory duct, posterior to cirrus. Testes oval, occupy complete intervascular space, in 2–3 layers, mainly preovarian.

Vagina joins posteriorly the ejaculatory duct to form an hermaphroditic duct; seminal receptacle absent. Ovary in posterior region of proglottid, bilobed in dorsoventral view; Mehlis' gland posterior, between ovarian lobes. Vitelline follicles circummedullar, absent in ovarian region. Uterus preformed, displaced porally. Uterine pore present, displaced porally. Eggs thin-walled, variable in shape.

Type-species: *S. viridis* (Wagener, 1854) Pintner, 1913

Key to the species of *Sphyricephalus*

- 1 Metabasal tentacular armature typical heteroacanthous, heteromorphous; basal swelling and characteristic basal armature present; tentacles more than 2,800 in length and less than 300 in diameter; bulbs in transverse orientation..... ***S. viridis* (Wagener, 1854) Pintner, 1913**
- Metabasal tentacular armature homeoacanthous, homeomorphous; basal swelling and characteristic basal armature absent; tentacles less than 2,600 in length and more than 350 in diameter; bulbs in longitudinal orientation..... **2**
- 2 Scolex craspedote, more than 6.0 mm in length; pars vaginalis of similar length or shorter than pars bothriialis; metabasal hooks more than 150 in length..... ***S. pelorosoma* Heinz & Dailey, 1974**
- Scolex acraspedote, less than 5.4 mm in length; pars vaginalis longer than pars bothriialis; metabasal hooks less than 145 in length..... ***S. dollfusi* Bussieras & Aldrin, 1968**

3.2.3. Genus *Heterosphyriocephalus* Palm, 2004

Diagnosis: Scolex large, compact, craspedote or acraspedote. Bothria two in number, oval, well delimited, with fused posterior margins, with thick, fleshy rims; bothrial pits absent; small projections covering different parts of the scolex (i.e. scolex peduncle, velum, proximal bothrial surface) (latter character unknown for *H. oheulumiae*); tentacle sheaths sinuous; retractor muscles attach at anterior part of bulbs; prebulbar organs and gland cells inside bulbs absent; bulbs compact, oval, in transverse orientation. Four elongate tentacles; metabasal armature typical heteroacanthous, heteromorphous or homeomorphous; hooks hollow. Distinctive basal armature and

basal swelling present or absent. Proglottids (unknown for *H. oheulumiae*) craspedote. Genital pores marginal, irregularly alternating; cirrus-sac absent; cirrus unarmed, invaginated in ejaculatory duct; external seminal vesicle present or absent. Testes oval, occupy complete intervacular space, in 2–3 layers, mainly preovarian. Vagina enters genital atrium posterior to ejaculatory duct; seminal receptacle present or absent. Ovary in posterior region of proglottid, bilobed in dorsoventral view; Mehlis' gland between ovarian lobes. Vitelline follicles circummedullar, absent in ovarian region. Uterus preformed, displaced porally. Uterine pore present, displaced porally. Eggs thin-walled, with two opposite prolongations; first prolongation short and filiform, second prolongation elongate and wide.

Type-species: *H. oheulumiae* Palm, 2004

Key to the species of *Heterosphyriocephalus*

- 1 Scolex acraspedote, more than 6 mm in length; metabasal tentacular armature with 11–13 hooks per principal row; basal swelling and characteristic basal armature absent..... ***H. oheulumiae* Palm, 2004**
- Scolex craspedote, less than 5 mm in length; metabasal tentacular armature with less than 10 hooks per principal row; basal swelling and characteristic basal armature present..... **2**
- 2 Worms between 200 and 300 mm in length and with more than 150 proglottids; bothria well delimited; proximal bothrial surface lacking small projections; pars post-bulbosa present; scolex velum with regular border, not folded; large, pyriform seminal vesicle in proximal part of ejaculatory duct; seminal receptacle present..... ***H. tergestinus* n. comb (Pintner, 1913)**
- Worms between 10 and 34 mm in length and with fewer than 100 proglottids; bothria less delimited; proximal bothrial surface covered with small projections; pars post-bulbosa absent; scolex velum with irregular and folded border; seminal vesicles absent; seminal receptacle absent..... ***H. encarnae* n. sp.**

3.3 Relationships between species of sphyriocephalids based on molecular data

Three identical partial 28S rDNA (domains D1-D3, 1,250 nt) sequences were generated from three adult specimens of *H. tergestinus*.

Both ML and BI algorithms produced trees with identical topology, which are illustrated in Figure 9. The molecular analysis has shown that the newly-generated sequences of *H. tergestinus* grouped with that of *H. oheulumiae* ex. *T. steindachneri* collected in Indonesia [15] forming a strongly-supported monophyletic clade. The sequences for both species differed by 1.0–1.1% (13–14 nt). *Hepatoxylon trichiuri* ex. *Taractes rubescens* (Jordan & Evermann) (Perciformes: Bramidae) from Indonesia [15] formed a clade with members of *Heterosphyriocephalus*, though not strongly-supported, and differed by 5.5–6.0% (69–75 nt) from the newly-generated sequences. The next clade combined the sequences of *S. viridis* ex *D. licha* from Indonesia [15] and *Sphyriocephalus* sp. ex. *D. licha* from northeastern Atlantic Ocean (off the southwestern part of Ireland) [16], thus confirming the identity of the sequence obtained by Olson et al. [16]. The sequences of the latter species were identical and differed by 7.9–8.2% (99–103 nt) from our newly-generated sequences.

4. Discussion

4.1 Contributions to the knowledge of sphyriocephalid tapeworms

This is the first comprehensive study on the family Sphyriocephalidae including morphological and molecular data.

With the description of *H. encarnae* and the allocation of *S. tergestinus* in the genus *Heterosphyriocephalus* this genus is no longer monotypic and now includes three valid species (i.e. the two mentioned species and the type-species *H. oheulumiae*). Therefore, it now has an equal number of species as the genus *Sphyriocephalus*, but might become the most speciose genus within the family given that *S. pelorosoma* and *S. dollfusi* might be transferred to it, either as a single species or separately, as it has been suggested by Palm [7] (see below).

The genus *Sphyriocephalus* now consists of three valid species, out of which only the type-species, *S. viridis*, is adequately characterized. *Sphyriocephalus pelorosoma* was originally described on the basis of a single, adult specimen [5] and *S. dollfusi* only on the basis of a single plerocercoid [6]. Despite the recovery of adults for both species (several for *S. pelorosoma* and only one for *S. dollfusi*) in Indonesia off Pelabuhan Ratu by Palm [7,37], information and measurements regarding the genital organs and eggs are almost entirely absent. Although Palm [7] considered both species valid, he suggested that they might be conspecific, due to their high morphological similarity,

with the exception of slight differences in some scolex measurements (see Table 2) and hook sizes. Furthermore, the eggs of *S. pelorosoma* possess a long filament [7], for which the length has not been provided. According to this description, such eggs clearly differ from those found in *S. viridis*. These eggs could resemble those of *H. encarnae* and *H. tergestinus*, which bear a short and filiform prolongation and a larger and wider one (present study, see sections 3.1.1. and 3.1.2.). This trait might indicate a possible allocation of *S. pelorosoma* and *S. dollfusi* to the genus *Heterosphyriocephalus*, for which the egg shape is highly characteristic. However, further studies completing the morphological description for both species and including molecular data will be required to confirm the synonymy of *S. pelorosoma* and *S. dollfusi*, which will furthermore clarify their phylogenetic position within the Sphyriocephalidae. The genus *Hepatoxylon* remains unchanged. Although the morphology of its members has not been addressed in the present study, the available sequence of the type-species *He. trichiuri* has been incorporated in the phylogenetic analyses and its relation to other members of the family are discussed.

4.2 Phylogenetic relationships

As suggested by morphology (see remarks section for *H. tergestinus* n. comb. in section 3.1.2.) and confirmed by molecular data (Fig. 9), *H. tergestinus* n. comb. and *H. oheulumiae* are congeners and therefore have to be placed within the same genus. One of the questions that arises from the phylogenetic analysis is the position of the genus *Hepatoxylon* within the Sphyriocephalidae and in relation to the remaining genera. According to present results, *He. trichiuri* groups with members of *Heterosphyriocephalus*, while species of *Sphyriocephalus* remain as a separate clade. This configuration, although weakly supported, agrees with the results published by Palm et al. [15] based on 18S rDNA and 28S rDNA. However, according to morphological data, *Sphyriocephalus* and *Heterosphyriocephalus* are the most similar genera within the family and one might expect that their members group together while representatives of *Hepatoxylon* form a separate clade. Palm [15] carried out a molecular study on the phylogeny of trypanorhynch cestodes, where the same topology as the one presented herein was observed: *H. oheulumiae* and *He. trichiuri* grouped together, while *S. viridis* appeared in a more derived position. As highlighted by Bazsalovicsová et al. [38], this conflict between morphology and genetic data is of high relevance in taxonomy and systematics. Scholz et al. [39] encountered the same situation with

species of the genus *Khawia* Hsü, 1935 (Cestoda: Caryophyllidea), which was regarded as a possible consequence of morphological divergence (without the corresponding genetic differentiation) which might have occurred after the switch of one of two related parasite species to a different host group. Following this argument, *Hepatoxylon* is the only genus of the family which parasitizes carcharhiniform sharks, while the other two genera seem restricted to lamniform and squaliform selachians. Within these, the family Alopiidae seems to be of special importance, since all valid species within *Sphyrrocephalus* and *Heterosphyrrocephalus* (with the exception of *H. oheulumiae*, for which the final host remains unknown) have been recovered from this host family (see below). This possible host-related phenotypic plasticity, despite frequently encountered in multiple other parasite groups [39,40,41,42], is not yet well understood, and furthermore highlights the importance of combining morphological with genetic data in systematic studies.

4.3 Host-specificity

Adult specimens of trypanorhynchs vary greatly in their host specificity, from species with a euryxenous to oioxenous host specificity [43,44]. Larval stages, on the other hand, are known to show a very low host specificity [45].

Overall, plerocerci of *S. viridis* and *H. tergestinus* have been reported from a large number of teleosts acting as intermediate hosts (see sections 3.1.2. and 3.1.3.). The same has been observed for members of *Hepatoxylon* [1]. When addressing the adult forms of sphyrrocephalids, it can be stated that the most well-known species are able to parasitize a diverse array of mostly pelagic selachians as their final hosts. Adults of *S. viridis* and *H. tergestinus* have each been reported from three hosts belonging to two different orders and three different families [i.e. *S. viridis* from *A. superciliosus* (Lamniformes: Alopiidae), *D. licha* (Squaliformes: Dalatiidae) and *C. granulatus* (Squaliformes: Centrophoridae); *H. tergestinus* from *A. vulpinus* (Lamniformes: Alopiidae), *E. bispinatus* (Squaliformes: Dalatiidae) and *I. oxyrinchus* (Lamniformes: Lamnidae)] [3,5,8,23,25,30,31,32,34,36,present study]. Similarly, the adults of *He. trichiuri* have been recovered from four hosts belonging to three different orders and four different families, namely *Carcharias* sp. (Lamniformes: Odontaspidae), *Lamna nasus* (Bonnaterre) (Lamniformes: Lamnidae), *Prionace glauca* (L.) (Carcharhiniformes: Carcharhinidae) and *Somniosus microcephalus* (Bloch & Schneider) (Squaliformes: Somniosidae)) (citations listed in Dollfus [1]).

In contrast, adult specimens of *S. pelorosoma* and *S. dollfusi* are solely known from *A. superciliosus* (Lamniformes: Alopiidae) [7,37], *H. encarnae* from *A. pelagicus* (Lamniformes: Alopiidae) (present study), and *He. megacephalum* from *Carcharodon carcharias* (L.) (Carcharhiniformes, Carcharhinidae) [1]. Until now, *H. oheulumiae* has not been found in its adult form [7].

Taking into account the low host-specificity shown by adults of the most well-known sphyriocephalids, we hypothesize that more definitive hosts are likely to emerge and will add to the list of infected host species for the least-known or recently described species of this group.

4.4 Different patterns of host-infection among genera

Despite an existing overlap in host preference among the three genera in terms of orders and families of selachians serving as definitive hosts, some patterns can be revealed. The genera *Sphyriocephalus* and *Heterosphyriocephalus* seem restricted to the orders Lamniformes and Squaliformes. Within these two orders, the family Alopiidae seems to be of a special importance, since all the existing species within both genera (with the exception of *H. oheulumiae*, for which the definitive host has not yet been described) have been reported from alopiids. In contrast, *Hepatoxylon* represents the only genus that has been reported from sharks of the order Carcharhiniformes. Members of Lamniformes and Squaliformes also serve as definitive hosts, but in these cases the families differ as follows: Alopiidae and Dalatiidae represent typical definitive hosts for members of *Sphyriocephalus* and *Heterosphyriocephalus*, while members of *Hepatoxylon* have been recorded from Somniosidae and Odontaspidae. The family Lamnidae seems to be infected by members of both groups (namely by *H. tergestinus* and *He. trichiuri*).

4.5 Patterns in the geographical distribution

Sphyriocephalus viridis and *H. tergestinus* both possess an interoceanic distribution (molecularly confirmed for the former species by Palm [15]). Both species have been reported from the Atlantic (including the Mediterranean Sea) and Pacific Oceans and *H. tergestinus* also occurs in the Indian Ocean (see sections 3.1.2. and 3.1.3. for a list of localities). This is not surprising given the cosmopolitan distribution patterns and migratory habits of the typical final hosts of both species. *Alopias vulpinus*, which acts as the final host for *H. tergestinus*, is a cosmopolitan species of tropical and temperate

waters [46] with highly migratory habits (sensu [47]), while *D. licha*, typically hosting the adults of *S. viridis*, is found at the continental shelves and slopes of tropical and temperate latitudes worldwide [46].

In the case of *H. encarnae* we assume a presence in the Indian and Pacific Oceans since its final host, *A. pelagicus*, seems restricted to the tropical areas in the Indo-Pacific [46]. However, there is a partial lack of reliable records due to its confusion with *A. vulpinus* (sensu [47]).

Alopias superciliosus, same as *A. vulpinus*, occurs in tropical and temperate waters and displays a cosmopolitan distribution [46]. Therefore, *S. dollfusi* and *S. pelorosoma* are expected to follow the same distributional pattern. At present, *S. dollfusi* has been recorded from the Atlantic and Indian Oceans [6,7] and *S. pelorosoma* from the Indian and Pacific Oceans [5,7].

Although three species of *Alopias* are officially recognized, Eitner [48] suggested the presence of a fourth unrecognized species based on fixed allelic differences among individuals in a group of specimens identified as *A. superciliosus*. Since each species of *Alopias* seems to host its corresponding sphyriocephalid parasite (*S. dollfusi* and *S. pelorosoma* would make an exception if they are confirmed as congeners) we could speculate the possible existence of an additional sphyriocephalid species parasitizing this (at present) undescribed alopiid shark.

In the case of *Hepatoxylon*, the type species *He. trichiuri* also has a cosmopolitan distribution, as it was recorded from the Atlantic and Pacific Oceans as well as from the Mediterranean Sea (see Dollfus [1] for a list of references; [4]). *Hepatoxylon megacephalum* has only been recorded from the Pacific Ocean [4] and the Mediterranean Sea (see Dollfus [1] for references). Although only very few records exist for the latter species, its final host, *Ca. carcharias* is found worldwide mostly in temperate waters and undergoes interoceanic migrations (sensu [47]). Therefore, we expect further geographical records for *H. megacephalum* in the future.

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Tables

Table 1. Summary data for the type-material of *Sphyricephalus tergestinus* and *S. viridis* from the Naturhistorisches Museum Wien (VNHM) collected by Pintner and re-examined in the present study.

Accession No.	Species label	Host species	Locality	Material	Medium	Amended identification
VNHM2062	<i>S. viridis</i> ^a	<i>L. caudatus</i>	Neapel, 1890	Cross-sections of plerocercoid	Permanent mount	<i>H. tergestinus</i>
VNHM2063	<i>S. viridis</i> ^a	<i>L. caudatus</i>	Neapel, 1890	Cross-sections of plerocercoid	Permanent mount	<i>H. tergestinus</i>
VNHM2064	<i>S. viridis</i> ^a	<i>L. caudatus</i>	Neapel, 1890	Longitudinal sections of plerocercoid	Permanent mount	<i>H. tergestinus</i>
VNHM2065	<i>S. viridis</i>	<i>L. caudatus</i>	Neapel, 1890	Tentacle fragments	Permanent mount	<i>H. tergestinus</i>
VNHM2066	<i>S. viridis</i>	<i>L. caudatus</i>	Neapel, 1890	Cross-sections of plerocercoid	Permanent mount	<i>H. tergestinus</i>
VNHM2067	<i>S. viridis</i>	<i>A. vulpinus</i> ^b	–	Sections of gravid segments	Permanent mount	<i>H. tergestinus</i>
VNHM2068	<i>S. viridis</i>	<i>D. licha</i> ^c	Neapel, 1890	Tentacle fragments	Permanent mount	
VNHM2069	<i>S. viridis</i>	<i>C. granulatus</i>	Neapel, 1890	Single tentacle	Permanent mount	
VNHM2071	<i>S. tergestinus</i>	<i>A. vulpinus</i> ^b	Trieste, 1898	Tentacles	Permanent mount	
VNHM2072	<i>S. viridis</i>	<i>A. vulpinus</i> ^b	Trieste, 1898	Longitudinal sections of scolex & cross-sections of gravid segment	Permanent mount	<i>H. tergestinus</i>
VNHM2073	<i>S. viridis</i>	<i>D. licha</i> ^c	Neapel, 1890	Longitudinal sections of scolex	Permanent mount	

VNHM2074	<i>S. viridis</i>	<i>A. vulpinus</i> ^b	Trieste	Tentacles	Permanent mount	<i>H. tergestinus</i>
VNHM2075	<i>S. viridis</i>	<i>A. vulpinus</i> ^b	Trieste	Strobila fragments	Permanent mount	<i>H. tergestinus</i>
VNHM2076	<i>S. tergestinus</i>	<i>A. vulpinus</i> ^b	Trieste, 1923	Strobila fragments	Permanent mount	
VNHM2567	<i>S. tergestinus</i>	<i>A. vulpinus</i> ^b	Trieste, 1898	Entire specimen, fragmented	Ethanol	
VNHM2568	<i>S. tergestinus</i>	<i>A. vulpinus</i> ^b	Trieste, 1898	Specimen fragments	Ethanol	
VNHM2569	<i>S. viridis</i>	<i>C. granulatus</i>	Neapel, 1890	Entire specimen	Ethanol	
VNHM2570	<i>S. viridis</i>	<i>C. granulatus</i>	Neapel, 1890	Specimen fragments	Ethanol	

^a, labelled as *Tertrarrhynchus viridis*; ^b, labelled as *Alopecias vulpes*; ^c, labelled as *Scymnorhinus lichia*

Table 2. Metrical data of species of *Sphyriocephalus* and *Heterosphyriocephalus*.

Abbreviations: TL, total length; NS, number of segments; SL, scolex length; SW, scolex width; PboL, pars bothrials length; PboW, pars bothrials width; BoL, bothrium length; BoW, bothrium width; PvaL, pars vaginalis length; PvaW, pars vaginalis width; PbuL, pars bulbosa length; PbuW, pars bulbosa width; BL, bulb length; BW, bulb width; PpbuL, pars postbulbosa length.

Species	<i>S. viridis</i>	<i>S. dollfusi</i>	<i>S. pelorosoma</i>	<i>H. oheulumiae</i>	<i>H. tergestinus</i> n. comb.	<i>H. encarnae</i> n. sp.
<i>Measurements</i>						
TL (mm)	>200	71	69–118	-	44–480	9.5–34.0
NS	>200	-	>300	-	126–260	34–57
SL	4,952–6,183	5,000–5,375	6,000–6,500	6,5–9,625	1,393–2,600	1,507–1,938
SW	3614	3,000–5,800	6,550–6,675	2,475–4,000	1,541–2,254	1,723–2,322
PboL	2,415–3,737	2,020–3,000	2,625–2,750	1,950–2,000	1,232–1,969	1,200–1,815
PboW	3,876–4,322	3,829 ^a	-	2,545 ^b	1,714–3,146	1,714–2,139

BoL	1,779–3,076	2,020	-	1,450–1,750	889–1,435	1,200–1,815
BoW	2,207–2,907	2,140–2,500	-	1,225–1,350	1,143–1,523	1,246–1,523
PvaL	2,830–3,783	2,020–3,375	2,940–4,000	1,800–2,800	1061	692–1,046
PvaW	3,399	3,286 ^a	-	-	1,676–1,861	1,553–1,999
PbuL	1,230–1,846	2,000–2,500	2,390–2,750	925	569–692	346–584
PbuW	2,907–3,614	3,371 ^a	-	-	1,676–1,846	1,692–2,230
BL	1,200–1,707	2,025–2,330	2,200–2,390	750–925	689–896	631–923
BW	615–723	620–1,000	750–1,000	275–425	238–396	246–354
PpbuL	477–923	-	9,640	-	108–400	0
<i>Ratios</i>						
BL : BW	1.8–2.6	2.3–3.5	2.3–3.0	1.9–3.3	2.0–3.0	2.3–3.0
PvaL : PboL	1.0–1.2	1.2–1.4	1.0–1.2	2.1–2.2	1,9	0.5–0.9
PbuL : PboL	0.4–0.8	1.1–1.7	1.2–1.6	2.0–3.0	2,8	0.3–0.4

^a, inferred from line-drawing of Bussieras & Aldrin (1968, fig. 1); ^b, inferred from line-drawing of Palm (2004, fig. 69a)

Figure captions

Fig. 1. Line drawings of *Heterosphyriocephalus encarnae* n. sp. from *Alopias pelagicus* Nakamura from the Pacific Ocean off Boca del Álamo, Mexico. A, outline of entire specimen, dorsoventral view; B, bulb; C, scolex, dorsoventral view; D, terminal genitalia; E, mature segment. *Abbreviations:* c, cirrus; ed, ejaculatory duct; mg, Mehlis' gland; ov, ovary; sdm, spherical dense mass; t, testis; ut, uterus; va, vagina; vt, vitelline follicles.

Fig. 2. Scanning electron micrographs of *Heterosphyriocephalus encarnae* n. sp. from *Alopias pelagicus* Nakamura from the Pacific Ocean off Boca del Álamo, Mexico. A, complete specimen, dorsoventral view; B, metabasal tentacular armature, bothrial surface; C, basal and metabasal tentacular armature, bothrial surface; D, metabasal tentacular armature, internal surface; E, velum, detailed view; F, egg; G, scolex, dorsoventral view, arrowhead shows medial groove in distal bothrial surface; H, acicular filitriches covering strobila; I, small projection with acicular filitriches covering scolex peduncle; J, small cylindrical projections with acicular filitriches covering proximal bothrial surface.

Fig. 3. Line drawings of tentacular armature of *Heterosphyriocephalus encarnae* n. sp. from *Alopias pelagicus* Nakamura from the Pacific Ocean off Boca del Álamo, Mexico. A, metabasal tentacular armature, antibothrial surface; B, metabasal tentacular armature, internal surface; C, metabasal tentacular armature, bothrial surface; D, basal tentacular armature, antibothrial surface; E, basal tentacular armature, external surface; F, basal tentacular armature, bothrial surface.

Fig. 4. Line drawings of individual tentacular hooks of *Heterosphyriocephalus encarnae* n. sp. from *Alopias pelagicus* Nakamura from the Pacific Ocean off Boca del Álamo, Mexico. A, basal individual hooks. Upper figures show hooks on internal and external surfaces, while lower figures show hooks on antibothrial and bothrial surfaces; B, individual hooks on metabasal tentacular armature. *Change to uncinata shape as progressing further into metabase.

Fig. 5. Line drawings of *Heterosphyriocephalus tergestinus* n. comb from *Alopias vulpinus* (Bonnaterre) from the Mediterranean Sea off Cap de Creus, Spain (A) and off Bou Haroun, Algeria (B-E). A, outline of entire cestode, dorsoventral view; B, scolex,

dorsoventral view; C, bulb; D, terminal genitalia; E, mature segment. *Abbreviations:* c, cirrus; ed, ejaculatory duct; mg, Mehlis' gland; ov, ovary; sdm, spherical dense mass; sr, seminal receptacle; sv, seminal vesicle; t, testis; ut, uterus; va, vagina; vt, vitelline follicles.

Fig. 6. Scanning electron micrographs of *Heterosphyriocephalus tergestinus* n. comb from *Alopias vulpinus* (Bonnaterre) from the Mediterranean Sea off Cap de Creus, Spain. A, scolex, lateral view; B, full tentacles, external/internal to antibothrial surface; C, metabasal tentacular armature, antibothrial surface; D, entire tentacle, bothrial surface; E, basal tentacular armature, bothrial surface; F, bothria, detailed view; G, egg; H, basal tentacular armature, external to antibothrial surface; I, segments of mature strobila; J, small projections covering the velum; K, detail view on small projection on velum.

Fig. 7. Line drawings of tentacular armature of *Heterosphyriocephalus tergestinus* n. comb from *Alopias vulpinus* (Bonnaterre) from the Mediterranean Sea off Cap de Creus, Spain. A, tentacular armature, antibothrial surface; B, tentacular armature, internal surface; C, tentacular armature, bothrial surface.

Fig. 8. Line drawings of individual tentacular hooks of *Heterosphyriocephalus tergestinus* n. comb from *Alopias vulpinus* (Bonnaterre) from the Mediterranean Sea off Cap de Creus, Spain. A, basal individual hooks. Upper figures show hooks on internal and external surfaces, while lower figures show hooks on antibothrial and bothrial surfaces; B, individual hooks on metabasal tentacular armature. *Change to uncinata shape as progressing further into metabase.

Fig. 9. Maximum likelihood (ML) phylogram reconstructed using the newly-generated and retrieved from GenBank 28S rDNA sequences for *Heterosphyriocephalus tergestinus* n. comb, *Heterosphyriocephalus oheulumiae*, *Hepatoxylon trichiuri*, *Sphyriocephalus viridis* and *Sphyriocephalus* sp. with nodal support from ML and Bayesian inference (BI) analyses indicated as ML/BI. Outgroup: *Paroncomegas araya*. The scale-bar indicates the expected number of substitutions per site.

Fig. 10. Line drawings of *Sphyriocephalus viridis* from *Dalatias licha* (Bonnaterre) from the Mediterranean Sea off Dellys, Algeria (A, C-E) and the Pacific Ocean off Nouméa, New Caledonia (B). A, outline of entire specimen, scolex in lateral view,

strobila in dorsoventral view; B, scolex, dorsoventral view; C, bulb; D, terminal genitalia; E, mature segment. *Abbreviations:* asv, accessory seminal vesicle; c, cirrus; ed, ejaculatory duct; mg, Mehlis' gland; ov, ovary; sv, seminal vesicle; t, testis; ut, uterus; va, vagina; vt, vitelline follicles.

Fig. 11. Scanning electron micrographs of *Sphyricephalus viridis* from *Dalatias licha* (Bonnaterre) from the Mediterranean Sea off Dellys, Algeria. A, scolex, lateral view; B, pars bothrialis, detailed view; C, basal to metabasal tentacular armature, antiothrial surface; D, egg; E, segments of mature strobila; F, proximal to distal metabasal tentacular armature, external surface; G, basal and metabasal tentacular armature, antiothrial surface.

Fig. 12. Line drawings of tentacular armature of *Sphyricephalus viridis* from *Centroscymnus coelolepis* Barbosa du Bocage & de Brito Capello from the Mediterranean Sea off Majorca, Spain. A, tentacular armature, antiothrial surface; B, tentacular armature, internal surface; C, tentacular armature, bothrial surface.

Fig. 13. Line drawings of individual tentacular hooks of *Sphyricephalus viridis* from *Centroscymnus coelolepis* Barbosa du Bocage & de Brito Capello from the Mediterranean Sea off Majorca and Barcelona, Spain. A, basal individual hooks. Upper figures show hooks on antiothrial and bothrial surfaces, while lower figures show hooks on internal and external surfaces (hooks of internal surface are shown on upper level; hooks of external surface are shown on lower level); B, individual hooks on metabasal tentacular armature. Left figures on each tentacular surface show hooks on transition area from basal to metabasal region, while right figures on each tentacular surface show hooks on metabasal region.

A

B

A

B

A **B** **C** **D** **E**

A

B

Heterosphyriocephalus encarnae n. sp.

Heterosphyriocephalus tergestinus n. comb.

Sphyriocephalus tergestinus

Heterosphyriocephalus tergestinus n. comb.

Sphyriocephalus viridis