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Molecular and morphological comparisons between *Gyrodactylus ostendicus* n. sp. (Monogenea: Gyrodactylidae) on *Pomatoschistus microps* (Krøyer) and *G. harengi* Malmberg, 1957 on *Clupea harengus membras* L.

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Abstract

Gyrodactylus ostendicus n. sp. was exclusively found on fins of the common goby *Pomatoschistus microps* (Krøyer). The haptoral hard parts are among the smallest described for species of *Gyrodactylus*. A presumed similarity between the new species and *G. harengi* Malmberg, 1957 (subgenus *Metanephrotus* Malmberg, 1964) encouraged a comparative approach. A morphological analysis showed the marginal hook sickles of *G. ostendicus* to be of quite a different type and similar to those of *G. arcuatus* Bychowsky *sensu* Bychowsky & Poljansky (1953) (subgenus *Mesonephrotus* Malmberg, 1964). The new species has a pharynx with short pharyngeal processes. Its protonephridial system has small bladders, indicating an association with the subgenera *Mesonephrotus* or *Metanephrotus*. Molecular phylogenetic analyses, including all of the species of *Mesonephrotus* and *Metanephrotus* currently available on the GenBank database, suggested that the new species belongs to *Mesonephrotus*. Combined morphological and molecular studies of the new species show that *G. ostendicus* is more closely related to *G. arcuatus* than to *G. harengi*.

Introduction

Gobiid fish are amongst the most dominant species of the Atlantic and Mediterranean coasts of Europe, playing an important role in the marine ecosystem (Miller, 1986). Hitherto, only about seven species of *Gyrodactylus* von Nordmann, 1832 are described from gobies, of which about four are referred to as '*Gyrodactylus* sp.' (see e.g. Longshaw, Pursglove & Shinn, 2003).

During a parasitological survey of the gyrodactylid fauna of various species of goby occurring in the North Sea, several undescribed *Gyrodactylus* species were found, one of them on the fins of *Pomatoschistus microps* (Krøyer). None of the other gobies collected were infected with this species. The haptoral hard parts of the species are clearly of a different type to those of other *Gyrodactylus* species found on *P. microps*, i.e. *G. rugiensoides* Huyse & Volckaert, 2002 and *G. rugiensis* Gläser, 1974, which belong to the subgenus *Paranephrothus* Malmberg, 1964 (protonephridial system with large bladders; see Gläser, 1974; Malmberg, 1970). Although a designation to subgenus, based on specific features of the excretory system (Malmberg, 1970), was not included in our study, the presence of small excretory bladders was established. This fact points to an association with either the subgenus *Mesonephrotus* Malmberg, 1964 or the subgenus *Metanephrotus* Malmberg, 1964. When our molecular investigations started, the specimens under study were referred to under the 'working name' *G.* cf. *harengi* due to their superficial similarity with *G. harengi* Malmberg, 1957. However, the following morphological and molecular analyses clearly differentiate this species from *G. harengi*, a member of the subgenus *Metanephrotus* (see Malmberg, 1957). In order to elucidate the position of the species within

*TH and GM take responsibility for the molecular and morphological aspects of this work, respectively.

Gyrodactylus on a molecular basis (see e.g. Ziętara et al., 2002), all species of the subgenera *Mesonephrotus* and *Metanephrotus* presently available on GenBank were included. The interrelationship between the present material, *G. harengi* and certain other species belonging to these two subgenera will be discussed below.

Materials and methods

Hosts and parasites

Gobies were collected in the Spuikom at Ostend (Belgium), at Ambleteuse (France) and at Yerseke and Texel (The Netherlands). Fish were transported alive in local water to the laboratory and killed by pithing before investigation. Using a stereomicroscope, *Gyrodactylus* specimens were individually removed from the fish by means of preparation needles. After morphological identification in local water, the parasites were removed from the slide and transferred to a 0.5 ml microcentrifuge tube containing 5 μl of milli-Q water and stored at $-20\,^{\circ}$C. For the examination of the haptoral hard parts using phase contrast microscopy, *Gyrodactylus* specimens were fixed and mounted between slide and coverslip in ammonium picro-glycerine (Malmberg, 1970).

Molecular analysis

DNA extraction, ITS amplification and sequencing of individual parasites were performed as described by Ziętara et al. (2002). The forward primer ITS-5′-TTTCCGTAGGTGAACCT-3′ was used in combination with ITS2R5′-GGTAATCACGCTTGAATC-3′; two additional internal primers were used for sequencing: ITS1R 5′-ATTTGCGTTCGAGAGACCG-3′, and ITS2F 5′-TGGTGGATCACTCGGCTCA-3′. Sequences were aligned with the Clustal X multiple sequence alignment program (version 1.81, Thompson et al., 1997). The sequences have been submitted to the GenBank database under the accession numbers AJ576064 and AJ576065. Regions with an ambiguous alignment were excluded from further analyses. With respect to the discussion on subgeneric status, the following species (available at September, 2003 from the GenBank database) of the subgenera *Mesonephrotus* and *Metanephrotus* were included: *G. arcuatus* Bychowsky, 1933 (AY338442), *G. branchicus* Malmberg, 1964 (AF156669), *G. rarus* Wegener, 1910 (AY338445), *G. bullatarudis* Turnbull, 1956 (AJ011410), *G. turnbulli* Harris, 198 (AJ001846) and *Gyrodactylus* sp. 1 of Ziętara et a (2002) (AF328866). *G. nipponensis* Ogawa & Egusa 1978 (AB063295) was included because molecula analyses of the species indicate a close relationshi with species of *Mesonephrotus* (see Huyse et al 2003). *G. rugiensis* Gläser, 1974 (AY338446) wa used as the outgroup, since it belongs to another sub genus, i.e. *Paranephrotus* (see Gläser, 1974; Huys et al., 2003).

To infer phylogenetic relationships, maximur likelihood (ML), distance-based methods and max imum parsimony (MP) were applied using PAUP* 4.01b (Swofford, 2001). ModelTest 3.06 selected th GTR + Γ model (gamma shape parameter = 0.5) c DNA evolution based on log likelihood scores (Posad & Crandall, 1998). The ML analysis was performe using the parameters estimated under the best-f model. With the minimum-evolution distance metho the distance matrix was calculated using the ML para meters. MP trees were inferred with the branch an bound algorithm (1,000 replicates). In these analyse gaps were treated both as fifth character and as missin data; all sites were equally weighted. Nodal suppor was assessed by running 1,000 bootstrap samples.

Morphological analysis

The microscopical analyses of *Gyrodactylus* speci mens were carried out in the Department of Zoology Stockholm University. A Leitz Dialux microscope with a Heine phase contrast condenser, a 90× oi immersion objective and a Leitz drawing attachmer with a 16× eye-piece, was used. This equipmer (Malmberg, 1970) was linked to a Leica DC 30 Digital Camera and Archiving System, and digital im ages of the adult haptoral hard parts of 20 specimen and the marginal hook sickle of large embryos in th uterus, when present, were analysed. Drawings of th new species were compared with drawings of *G. har engi* in the 'Malmbergs collection' at the Departmen of Invertebrate Zoology, Swedish Museum of Nat ural History, Stockholm. Measurements were mad using an image analysis system (Leica Q500/W witl a Hamamatsu 3 CCD camera, C5810); those of th marginal hook handle, sickle filament loop and sickl area by detection and the other measurements by in teractive measuring on the computer screen. In total 21 features of the anchors, ventral bars and margina hook sickles were measured (Figure 1; Table 1).

Table 1. Measurements in micrometres (mean, with range in parentheses) of body, pharynx and haptoral hard parts of *G. ostendicus* n. sp. The numbers to the left of the hard parts relate to Figure 1.

	N	Adult 1	Adult 2	Embryo
Body				
Total length	19	524.6 (440.0-659.5)		
Haptor, length × width	18	73.9 (61-96.5) × 95.5 (81.5-116)		
Pharynx				
Length × width	11	54.2 (49-61) × 53.1 (49-62.5)		
Marginal hooks				
1. Total length of marginal hook	19	19.2 (17.5-21)		
2. Length of marginal hook filament loop	21	7.9 (7-9)		
3. Length of marginal hook handle	17/17/3	15.7 14-17)	16.3 (15.5-17)	15.8 (15.5-16)
4. Length of marginal hook sickle	20/17/11	3.7 (3-4)	3.8 (3.5-4)	3.7 (3.5-4)
5. Proximal width of marginal hook sickle	20/17/11	2.7 (2.5-3)	2.8 (2.5-3.5)	2.7 (2.5-3)
6. Distal width of marginal hook sickle	20/17/11	3.1 (2.5-3.5)	3.2 (3-3.5)	3.2 (3-3.5)
7. Marginal hook toe length	20/17/11	1.0 (0.5-1)	0.9 (0.5-1)	1.0 (0.5-1)
8. Marginal hook heel length	20/17/11	1.9 (1.5-2)	1.9 (1.5-2.5)	1.8 (1.5-2.0)
9. Marginal hook sickle aperture distance	20/17/11	2.1 (1.9-2.3)	2.2 (2-2.5)	2.2 (2-2.5)
10. Marginal hook sickle shaft length	20/17/11	2.5 (2-2.5)	2.6 (2.5-3)	2.5 (2-3)
11. Length of marginal hook sickle point	20/17/11	1.6 (1.5- 2)	1.7 (1.5-2)	1.6 (1.5-2)
Area of marginal hook	20/17/11	4.9 (4.1-5.8)	5.3 (4-6.5)	5.0 (4-5.5)
Ventral bar				
12. Length of ventral bar	18	11.2 (9.5-14)		
13. Basal width of ventral bar	35	5.1 (3.5-7)		
14. Median width of ventral bar	13	5.2 (4-6.5)		
15. Length of ventral bar membrane	7	8.2 (6-10)		
Median width of ventral bar + ventral bar membrane	8	13.4 (12-15)		
Anchors				
16. Total length of anchor	19	28.7 (26.5-31.5)		
17. Length of anchor point	20	15.6 (13.5-17.5)		
18. Length of anchor shaft	19	24.8 (23-26.5)		
19. Length of anchor root	20	7.7 (6-9)		
Dorsal bar				
20. Length	18	11.2 (10-13)		

N, Number of specimens measured.
Adult 1, Marginal hook No. 1/2, one of the two most anterior hooks.
Adult 2, Marginal hook No. 7/8, one of the most posterior hooks.
Embryo, Marginal hook sickle of large embryo.

For Scanning Electron Microscopy (SEM), live specimens were fixed in glutaraldehyde (2% solution in sodium cacodylate buffer), rinsed in sodium cacodylate buffer, dehydrated in acetone and dried in a Balzers Union Critical Point Dryer. The specimens were subsequently sputter coated with gold in a Balzers Union Sputter Coater Device and scanned in a Philips-515 scanning electron microscope.

Results

Molecular identification

About 950 bp of the rDNA complex spanning the 3′ end of the 18S subunit, ITS1, 5.8S subunit, ITS2, and the 5′ end of the 28S subunit were obtained. The ITS1 sequence of *Gyrodactylus harengi* was 362 bp long, 5.8S rDNA 157 bp and ITS2 388 bp; the total segment was 907 bp long. Sequences of *G. ostendicus* n. sp. (see below) were obtained in a previous study (Huyse

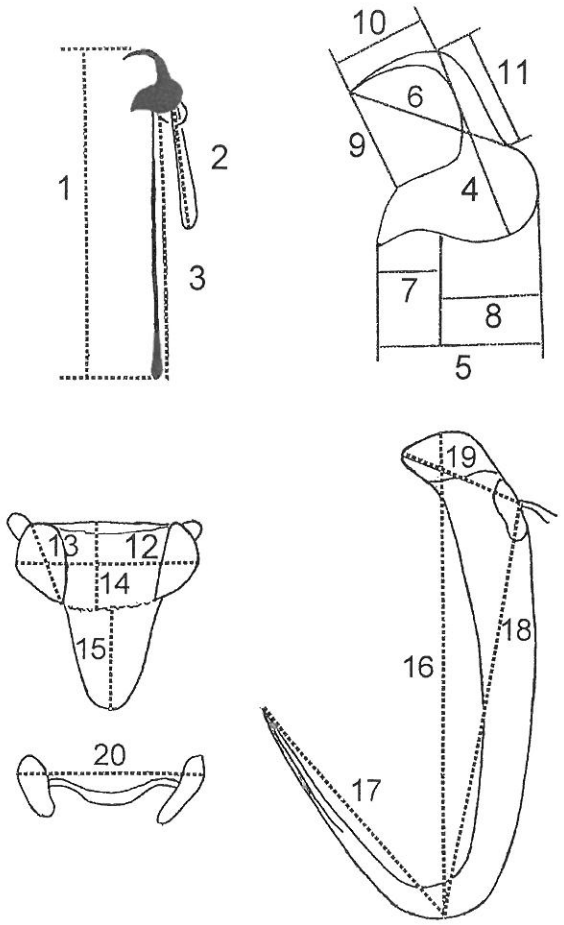

Figure 1. Method of measuring haptoral hard parts of *Gyrodactylus ostendicus* n. sp. 1-3. **Marginal hook**: 1. Total length. 2. Length of filament loop, 3. Length of handle. 4-11. **Marginal hook sickle:** 4. Length. 5. Proximal width. 6. Distal width. 7. Toe length. 8. Heel length. 9. Aperture distance. 10. Point length. 11. Shaft length. Area of sickle (measured by detection at the image analysis). 12-15. **Ventral bar**: 12. Length. 13. Basal width. 14. Median width. 15. Membrane, length. 14 + 15. Median width + membrane. 16-19. **Anchor**: 16. Total length. 17. Length of point. 18. Length of shaft. 19. Length of root. 20. **Dorsal bar**: Length.

et al., 2003); the DNA sequence of ITS1 was 367 bp, 5.8S rDNA 157 bp and ITS2 394 bp; the total segment was 918 bp long. All of the *G. ostendicus* specimens (they were only found on the fins), collected at Ostend, Ambleteuse, Yerseke and Texel, had an identical ITS rDNA sequence (Huyse et al., 2003).

The *G. harengi* specimens from the fins and gills of *Clupea harengus membras* L. differed in three transitions in the ITS1 region, and four transitions and three transversions in the ITS2 region, resulting in a distance of 1.1% (uncorrected p-distances). The genetic divergence between *G. ostendicus* and *G. harengi* was very

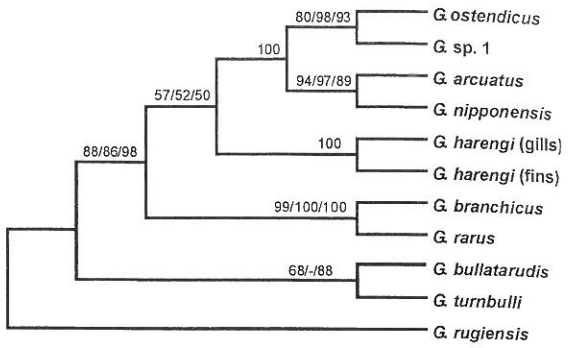

Figure 2. Phylogenetic relationships between *Gyrodactylus osten dicus* n. sp. and the available species on GenBank of the sub genera *Mesonephrotus* and *Metanephrotus* as expressed by th minimum-evolution tree. *G.* (*Paranephrotus*) *rugiensis* is take as the outgroup. Bootstrap values are shown for the maximur likelihood/maximum parsimony/minimum-evolution analyses.

high, differing in 92 transitions, 87 transversions an 23 indels in the complete ITS region, resulting in (uncorrected) genetic distance of 20% (Table 2; 30% gamma corrected distances). The lowest distance were found between *G. ostendicus* and *Gyrodactylu* sp. 1 of Ziętara et al. (2002) from the gills and fin of *Pomatoschistus minutus* (Pallas) and *P. lozanoi* (d Buen) (Table 2).

The phylogenetic relationships are presented i Figure 2, the cladogram being rooted by *G. rugien sis*. There were 236 parsimony informative sites. Th MP tree (tree length = 486; C.I. = 0.73; R.I. = 0.76 was identical with the ML tree (− *Ln* likelihood = 3930.79) and the minimum-evolution tree; the boot strap values were fairly high (Figure 2). Only th clustering of *G. bullatarudus* and *G. turnbulli* was nc supported by the MP analyses and the grouping of th two *G. harengi* genotypes appeared unresolved. *G. os tendicus* clustered strongly with *Gyrodactylus* sp. 1 subsequently grouping with *G. arcuatus* and *G. nip ponensis*. *G. bullatarudus* and *G. turnbulli* branche off most basally.

Microscopical identification

A special digital analysis of the 20 specimens of *G. os tendicus* n. sp. revealed a small variation in size but nc in shape between the marginal hook sickles (Figure 4 This is valid not only for the sickles of adult spec mens but also for the sickles of an adult and its large or fully-grown embryo. Thus a marginal hook sickl of an adult could either be slightly larger or slightl smaller than a sickle of its embryo.

Table 2. Uncorrected pairwise genetic distances between species of the subgenera *Metanephrotus* (nos 1-4) and *Mesonephrotus* (nos 7-10). *Gyrodactylus rugiensis* belongs to the subgenus *Paranephrotus*.

Gyrodactylus spp.	1	2	3	4	5	6	7	8	9	10	11
1. *G. branchicus*											
2. *G. rarus*	0.01										
3. *G. harengi gills*	0.13	0.13									
4. *G. harengi fins*	0.13	0.13	0.01								
5. *G. bullatarudus*	0.18	0.18	0.21	0.20							
6. *G. turnbulli*	0.17	0.17	0.19	0.19	0.17						
7 *G. ostendicus* n. sp.	0.18	0.18	0.20	0.20	0.25	0.24					
8. *Gyrodactylus* sp. 1	0.18	0.18	0.19	0.19	0.24	0.23	0.03				
9. *G. arcuatus*	0.19	0.19	0.19	0.20	0.25	0.24	0.08	0.07			
10. *G. nipponensis*	0.18	0.18	0.19	0.19	0.24	0.23	0.05	0.05	0.04		
11. *G. rugiensis*	0.26	0.27	0.29	0.29	0.26	0.26	0.29	0.28	0.30	0.28	

Family Gyrodactylidae Cobbold, 1864
Genus *Gyrodactylus* Nordmann, 1832
Subgenus *G. (Mesonephrotus)* Malmberg, 1964

Gyrodactylus ostendicus n. sp.*

Type-host: *Pomatoschistus microps* (Krøyer) [Gobiidae].
Site: Fins.
Type-locality: Spuikom, Ostend, Belgium (51° 14′ N; 2° 57′ E).
Water temperature, salinity and date of collection: 18 °C; 31.1 ppm; 18.8.1999.
Other localities: Ambleteuse, France; Yerseke and Texel, The Netherlands.
Specimens studied for molecular analysis: Four specimens from Ostend, 2 from Ambleteuse, 2 from Yerseke and 2 from Texel. DNA sequences have been submitted to the GenBank database under accession number AY338439 - AY338441 (Huyse et al., 2003).
Specimens studied for morphological analysis: The holotype specimen and 17 other specimens from Ostend and 2 from Ambleteuse were digitised and the haptoral hard parts of 7 specimens (2 from Ambleteuse) were drawn by means of a drawing apparatus (Figures 3, 4). The holotype under (Reg. No. 5918) and 19 paratypes (Reg. Nos 5919-5937) are deposited at the Department of Invertebrate Zoology, Swedish Museum of Natural History, Stockholm, Sweden.
Etymology: The species name refers to the locality of the holotype.

* Previously recorded as *G.* cf. *harengi* in a thesis (Huyse, 2002) and as *Gyrodactylus* sp. 4 in Huyse et al. (2003).

Molecular diagnosis

The DNA sequence of ITS1 was 367 bp, 5.8S rDNA 157 bp and ITS2 394 bp; the total segment was 918 bp long. All 10 sequences studied were identical (Huyse et al., 2003). Sequences were compared with those of *G. harengi* Malmberg, 1957. In total, 92 transitions, 87 transversions and 23 indels were found.

Microscopical diagnosis (Figures 3, 4, 5; Table 1)

Coverslip-flattened specimens in ammonium picroglycerine 525 (440-660) μm long. Other measurements in Table 1. Haptor delineated from body (Figure 5B). Pharynx with short processes (Figure 5A); anterior and posterior parts of similar length and width. Cirrus situated posteriorly to pharynx, with single large spine and 5-6 small spines in single arched row. Protonephridial system with small bladders. Marginal hook sickle of different type to, and both shorter and broader than, that of *G. harengi* Malmberg, 1957, but similar to that of *G. arcuatus* Bychowsky *sensu* Bychowsky & Poljansky (1953). Marginal hook handle shorter than in *G. harengi*. Ventral bar and anchors small, resembling those of *G. harengi* but even smaller. Ventral bar usually with small processes; membrane thin and often difficult to observe. Anchor with long, slender point; anchor shaft slightly curved, lacking fold for ventral bar process; root short, shorter than in *G. harengi*; attachment points for dorsal bar with posteriorly directed extension (Figure 3c).

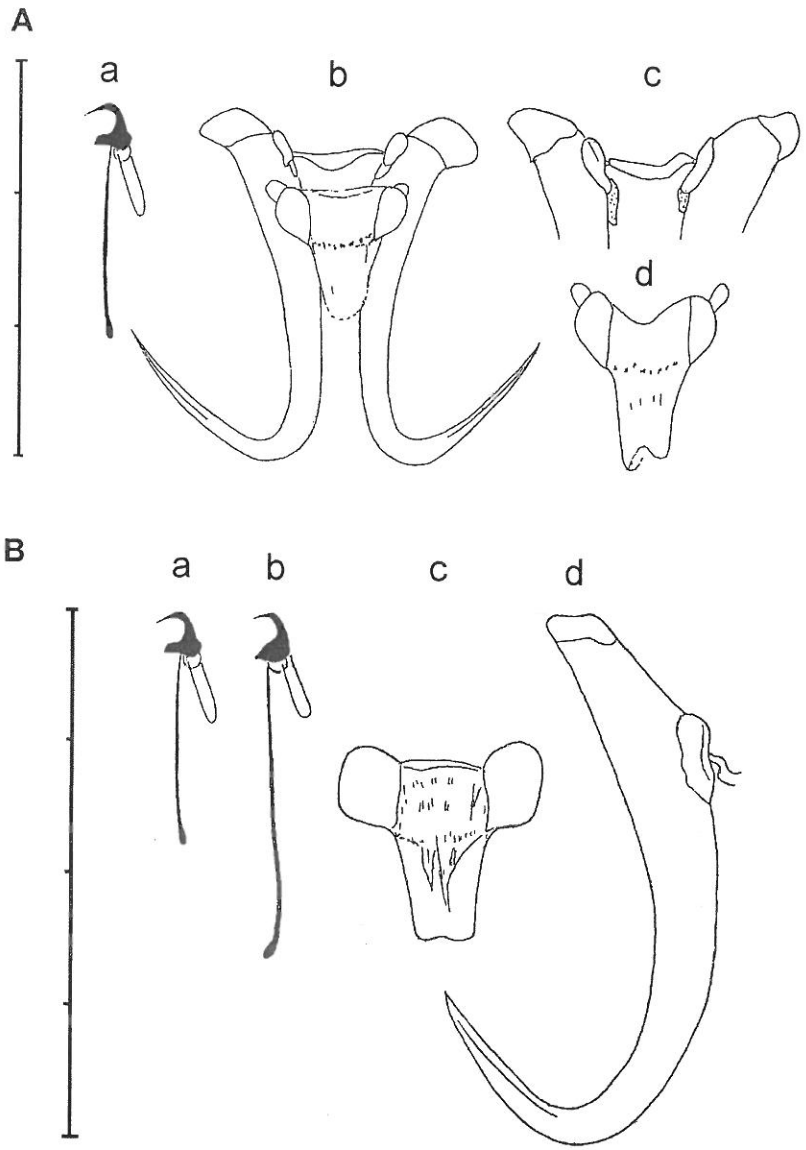

Figure 3. **A.** Haptoral hard parts of *Gyrodactylus ostendicus* n. sp. from the fins of *Pomatoschistus microps*, Ostend, Belgium: **a,b,** of holoty **c,d,** paratype specimen; **c,** dorsal bar; note the attachment points with posteriorly pointing extensions, **d**, ventral bar with small processes an weakly-developed median part and membrane. **B.** Haptoral hard parts of *G. ostendicus* n. sp. and *G. harengi*: **a,** marginal hook of *G. ostendi* n. sp. (holotype); **b-d**, marginal hook, ventral bar and anchor of *G. harengi* from a fin of *Clupea harengus membras* from off Tvärmin Finland (Baltic Sea); **a,b,** note the difference in shape between the marginal hook sickle of *G. ostendicus* n. sp. (**a**) and *G. harengi* (**b**); **c-d**, *harengi*: **c**, ventral bar; note the absences of processes; **d**, anchor; as in *G. ostendicus* n. sp., there are no anchor folds. *Scale-bars*: A, 30 μ B, 40 μm.

Discussion

Molecular analyses

The differences at the molecular level between *G. ostendicus* n. sp. and *G. harengi* were very distinct. The uncorrected p-distances exceeded 20% (Table 2). Especially in the ITS1 region, many insertion and deletion events were found and the 5.8S gene, kno to be very conservative between members of the sa subgenus (Ziętara et al., 2002; Huyse et al., 200 differed in one transversion and two transitions. T suggests that the two species belong to different s genera. In the phylogenetic analyses, *G. ostendic* clustered strongly with *Gyrodactylus* sp. 1 of Zięt et al. (2002), which is found on the gills and fi

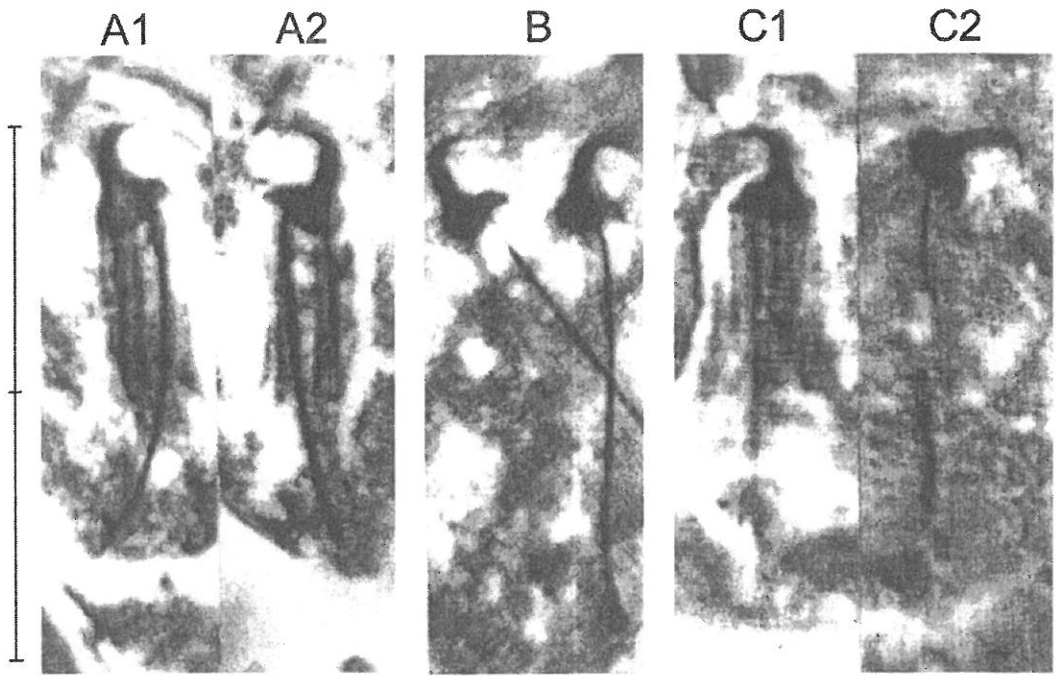

Figure 4. Comparative micrographs (phase contrast) of marginal hooks from three specimens (**A**, **B**, **C**) of *Gyrodactylus ostendicus* n. sp. **A1.** Hook no. 1 left side; **A2.** Hook no. 1 right side of the holotype (adult specimen); **B** Hook nos 1 and 2 left side in an embryo; **C1.** Hook no. 1 right side of an adult; **C2.** Hook from an embryo of C1. *Scale-bar*: 20 μm.

of *Pomatoschistus minutus* and *P. lozanoi*. These two species grouped together with *G. arcuatus* Bychowsky, 1933 and *G. nipponensis* Ogawa & Egusa, 1978. As such, from a molecular point of view, *G. ostendicus* appears more closely related to species of *Mesonephrotus* than to species of *Metanephrotus*, although both subgenera cluster together. This fits well with the morphological diagnosis (see below). The clustering of *G. arcuatus* and *G. nipponensis* was recently described by Huyse et al. (2003) and suggests that the latter species migth belong to *Mesonephrotus*.

The *G. harengi* specimens consisted of two genotypes, one found on the gills and one found on the fins. They clustered together but their position remained unresolved (Figure 2). Based on the genetic distances (Table 2), they were most closely related to *G. branchicus* Malmberg, 1964 and *G. rarus* Wegener, 1910, two species belonging to the subgenus *Metanephrotus* which are morphologically as well as genetically very similar (Ziętara & Lumme, 2003). Although *G. bullatarudis* Turnbull, 1956 is supposed to belong to *Mesonephrotus* (see Harris, 1986), it branched off basally and appeared very divergent to the *Mesonephrotus* species included in this study. Except in the MP analyses, this species clustered with *G. turnbulli* Harris, 1986, which belongs to *Metanephrotus*, although the genetic distance between both species was relatively high.

Morphological analyses

The anchors, ventral bar and marginal hook sickles of *G. ostendicus* are among the smallest described for species of *Gyrodactylus*. The form of its marginal hook sickles is similar to that of *G. arcuatus*, a member of *Mesonephrotus*. Specimens of *G. ostendicus* from all localities, Ostend, Ambleteuse, Yerseke and Texel, had an identical ITS rDNA sequence, indicating the presence of one and the same species in all localities. On this basis, the degree of morphological differences between the marginal hook sickles of different specimens was assessed. To the best of our knowledge, there have been no such previous studies based on both morphological and DNA evidence. Without molecular data, differences in the haptoral hard parts of two morphologically similar species could be interpreted as intraspecific variation. For example, the two types of marginal hook sickles of *G. macronychus* Malmberg, 1957 were originally presumed to represent one and the same species, but complementary molecular analyses revealed a genetic distance of 21.8% (ITS region)

between the two forms (Ziętara & Lumme, 2003). These results motivated the splitting of *G. macronychus* into two species, *G. macronychus* and *G. jussi* Ziętara & Lumme, 2003.

For the study of morphological differences between the marginal hook sickles of *G. ostendicus*, 18 specimens from Ostend and two from Ambleteuse were included. Very small differences in size were found. This is valid not only for the sickles of different specimens but also for those of the adult and the embryo in its uterus. A marginal hook sickle of an adult can be either slightly larger or slightly smaller than a sickle of its embryo (Figure 4). The shape of the sickles, however, was always the same, strongly indicating the presence of a single species. The combined morphological and molecular results presented here are of relevance to further studies on intraspecific morphological variations within species of *Gyrodactylus*.

The protonephridial system of *G. ostendicus* has small bladders, which indicates membership of either *Mesonephrotus* or *Metanephrotus* (see Malmberg, 1964). The molecular phylogenetic analyses of *G. ostendicus* point to an association with *Mesonephrotus* (Figure 2). The small haptoral hard parts appear to be more similar to species of *Mesonephrotus* than to species of other subgenera, e.g. to *G. arcuatus*, a skin and fin parasite which is sometimes also found inside the mouth of its host, *Gasterosteus aculeatus*. So far, *G. ostendicus* has only been found on the fins of its host, *Pomatoschistus microps*. Based on studies of *c.*85 *Gyrodactylus* species, Malmberg (1970) found that *Gyrodactylus* species with small haptoral hard parts often correlate with a host species of small size and/or a parasitic mode of existence inside the mouth of the host (pharynx, gill-arches, gill-filaments). Furthermore, members of *Mesonephrotus* were found to be parasites of gasterosteids and gadids, teleosts that are phylogenetically less advanced than gobiids, which are members of the Gobiesociformes. The presence of small haptoral hard parts may favour the secondary adaptation of *Gyrodactylus* species to small fish hosts, such as gobies. It is, therefore, possible that *G. ostendicus* is the result of a host-switch from a host at the gasterosteid/gadid phylogenetic level to a more advanced gobiid host.

G. (*Metanephrotus*) *emembranatus* Malmberg, 1970 is a good example of a buccal species which lives inside the mouth of its host and has small, reduced haptoral hard parts, i.e. with small anchors, diverging anchor roots, anchors lacking an anchor fold

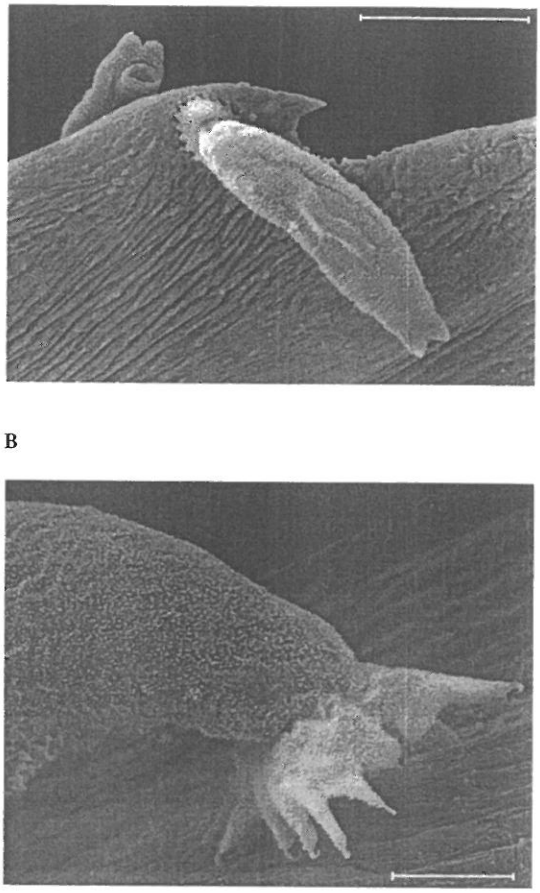

Figure 5. Scanning electron micrographs of *Gyrodacylus os dicus* n. sp. **A.** Two specimens, one on each side of a fir *Pomatoschistus microps* (Ostend, Belgium); in the uppermost s cimen, the protruded prepharynx with the mouth is seen; the lo specimen has its bi-lobed anterior end downwards in the figure haptor is clearly delineated from the posterior end; **B.** Posterior of a specimen, postero-dorsal aspect: the haptor, clearly delinea from the body. The two 'fingers' of No. 8 are seen to the left; t more 'fingers' of right side and the marginal hook sickle of e 'finger' are seen. Scale-bars: A, 100 μm; B, 20 μm.

for a ventral bar process, no ventral bar proces and no ventral bar membrane. The small anchors *G. ostendicus* also lack an anchor fold, the ventral processes are small and not always present, and ventral bar membrane is very thin and often diffic to observe, giving a rudimentary impression of the b Judging by the anchors and ventral bar, *G. ostendi* could originally have been a buccal species and nc fin parasite.

Gyrodactylus harengi was originally descrit from Baltic herring *Clupea harengus membras*

Nämdö in the Stockholm Archipelago (Malmberg, 1957). Its small diverging anchors without folds and ventral bar without processes point to it being a buccal species. However, most specimens were found on the fins and only a few were inside the mouth (Malmberg, 1957). The *G. harengi* specimens in the present investigation were also collected from the Baltic herring (June, 2002; off Edesö Stockholm Archipelago). The infection intensity was higher on this occasion and most specimens were found on the gills. Fin and gill specimens were dropped separately into 96% ethanol, and some of these specimens were removed and determined as *G. harengi*. The subsequent DNA analysis showed that the presumed *G. harengi* specimens consisted of gill and fin genotypes, differing in 1.1% of the complete ITS rDNA region. Further investigations are needed to assess whether or not morphological differences between specimens from the fins and gills can be detected.

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