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Resurrection of *Pomphorhynchus tereticollis* (Rudolphi, 1809) (Acanthocephala: Pomphorhynchidae) based on new morphological and molecular data

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Summary

Pomphorhynchus tereticollis (Rudolphi, 1809) is here redescribed on the basis of Rudolphi's material, deposited in the Museum für Naturkunde Berlin, and on acanthocephalans recently collected from the type host *Platichthys flessus* (L.) and the region embodying the type locality. Out of the paratypes of *P. tereticollis*, the lectotype and paralectotypes have been designated. Their morphology fits well with that of newly collected material of *P. tereticollis* dissected from the type fish host from the Baltic coast near Stralsund. The resurrection of *P. tereticollis*, previously considered a synonym of *Pomphorhynchus laevis* (Zoega in Müller, 1779), is supported by several morphological features distinguishing the two *Pomphorhynchus* species: 1. The basal parts of the proboscis hooks located on the posterior proboscis half possess proximal projections in *P. tereticollis* but not in *P. laevis*. This shape of the hook bases is clearly visible only in unfixed fresh worms; 2. The last hooks are situated at the anterior part of the bulbus or rarely at the posterior-most end of the proboscis in *P. tereticollis*, while they lie anterior to the end of the proboscis in *P. laevis*; 3. The proboscis hooks No. 5 or 6 are markedly stout (robust) and clearly distinct in comparison with the surrounding hooks in *P. tereticollis*, while less robust and more similar to the hooks in *P. laevis*. In addition, genetic divergence between *P. tereticollis* and *P. laevis* based on ITS1, ITS2 and COI sequencing supports the existence of two distinct species and reveals that some isolates previously identified as *P. laevis* were actually *P. tereticollis*. Previous and present morphological and genetic data show that both *Pomphorhynchus* species occur in freshwaters throughout Europe and may infect the same fish hosts, such as chub and barbel, and also several species of isopods (Gammaridae). This study also provides morphological evidence that *Pomphorhynchus intermedius* Engelbrecht, 1957 is a synonym of *P. tereticollis*, because the only discrimination character of the former species, the "existing but small proximal projections of basal parts of the proboscis hooks located on the posterior proboscis half" are present also in *P. tereticollis*.

Keywords: lectotype; paralectotype; redescription; *Pomphorhynchus laevis*; genetic differentiation

Introduction

Acanthocephalans are interesting models to study the ecology and evolution of parasites with complex life cycle, in particular to address important issues such as specificity, parasite-induced behavioural and physiological changes in their hosts, and ecotoxicology (Kennedy, 2006). A number of ecological, behavioural and biochemical studies have been carried out using the common Palaearctic species *Pomphorhynchus laevis* (Zoega in Müller, 1776) as a model, because it exhibits a broad geographical distribution and range of fish definitive hosts. However, a rather high morphological and physiological intraspecific variability has been reported in this species both in the British Islands (Kennedy *et al*., 1978; Kennedy, 1984; Brown, 1987; Guillén-Hernández & Whitfield, 2001; O'Mahony *et al*., 2004) and in continental Europe (Dudiňák & Šnábel, 2001; Chibani *et al*., 2004; Perrot-Minnot, 2004).
These data have been then supplemented by the information on genetic characters, which indicated the existence of a cryptic species within the *P. laevis* species complex, notwithstanding the final fish host and/or geographic origin (Dudiňák & Šnábel, 2001; Králová-Hromadová *et al*., 2003; Perrot-Minnot, 2004, Bombarová *et al*., 2007). Genetic studies revealed a high level of sequence divergence between two clusters of samples of acanthocephalans inferred from sequences of internal transcribed spacers 1 and 2 of nuclear rDNA and the mitochondrial cytochrome c oxidase subunit 1 gene (11.8 % and 20 %, respectively)

* Both authors contributed to the work equally.

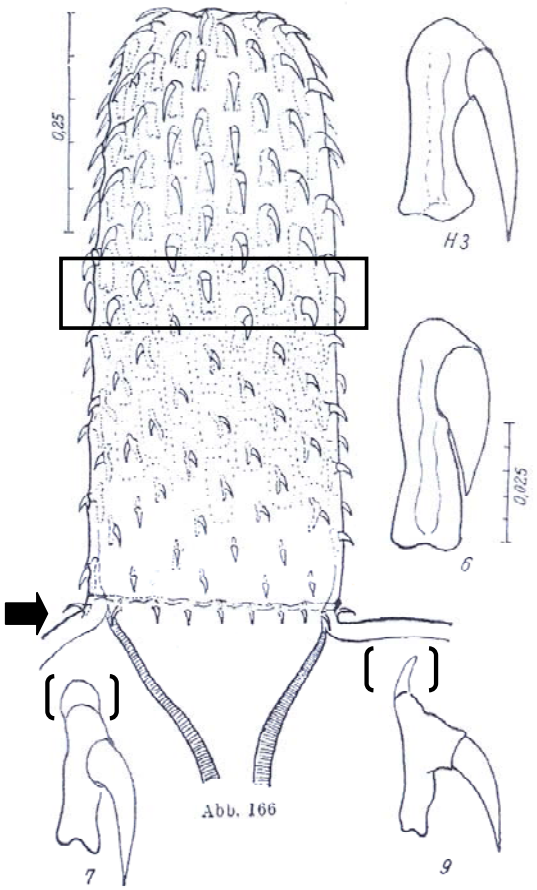

Fig. 1. Drawings of proboscis and selected hooks of *Pomphorhynchus tereticollis* (original by Meyer, 1932–1933). Rectangle indicates the proboscis area with the stoutest hooks Nos 5 and 6; arrow indicates the last hooks located on the bulbus; brackets indicate the proximal parts of the base of 7th and 9th hooks armed with a projection.

(Králová-Hromadová *et al*., 2003; Perrot-Minnot, 2004). The existence of two cryptic *Pomphorhynchus* species was further supported by a karyological study (Bombarová *et al*., 2007).

It has been suggested that one species corresponds to *P. laevis*, whereas the other may be conspecific with the previously described European species *Pomphorhynchus tereticollis* (Rudolphi, 1809) (Perrot-Minnot, 2004; Bombarová *et al*., 2007). However, these observations were awaiting a formal redescription of *P. tereticollis*, which was considered to be a synonym of *P. laevis* (Amin *et al*., 2003). In addition, *Pomphorhynchus intermedius* Engelbrecht, 1957, another synonym of *P. laevis* (Amin *et al*., 2003), can occur across the ecological and geographical range of *P. laevis*.

Pomphorhynchus tereticollis was described by Rudolphi (1809, 1819) as *Echinorhynchus tereticollis* from *Platichthys flessus* (L.) (syn. *Pleuronectes flessus*) from Baltic coast at the vicinity of Greifswald, Germany, but the author mentioned it also from other freshwater fishes. Meyer (1932 – 1933) transferred the species to *Pomphorhynchus* Monticelli, 1905 and reported that *P. tereticollis* from the Baltic *Pleuronectes platessa* L. was morphologically very close to freshwater *P. laevis,* differing in less conspicuous size difference between long anterior and short posterior proboscis hooks. The other features typical for *P. tereticollis* are apparent from the original drawings of Meyer (1932 – 1933) (Figs 1, 2). The most marked difference is the shape of the basal parts of the proboscis hooks located in the posterior proboscis half. These hook bases possess proximal projections in *P. tereticollis* (Fig. 1), which are not present in *P. laevis* (Fig. 2), as confirmed also by Petrochenko (1956). Moreover, the last hooks are localised at the anterior part of the bulbus or at the posterior-most end of the proboscis in *P. tereticollis* (Fig. 1), but more anteriorly in *P. laevis* (Fig. 2; Petrochenko, 1956). The stoutest (robust) hook in the middle of the proboscis, presented in both species, seemed to be more distinct from surrounding hooks in *P. tereticollis* than in *P. laevis.*

Later, Engelbrecht (1957) studied *Pomphorhynchus* specimens from the Baltic flounders *P. flessus* and *P. platessa* and reported a rather broad morphological variability in the shape of the hook bases. He divided flounder parasites into two forms, namely *P. laevis* f. *tereticollis* possessing hook bases "armed", with prominent proximal projections, and the new taxon *P. laevis* f. *intermedius*, having smaller projections. Golvan (1969) erected both forms to the species level as *P. tereticollis* and *P. intermedius*. Since the shape of the hook bases in *Pomphorhynchus* spp. is visible exclusively in living worms and this character disappears in fixed material, it was not considered in later studies. Overall similarity of parasites from flounders and other fish hosts throughout Europe with common *P. laevis* caused that solely this specis was considered as valid (Amin *et al*., 2003).

Formally, a thorough morphological description of *P. tereticollis* has never been published, even though the original Rudolphi's material of *P. tereticollis* exists (Hartwich *et al*., 1998). The present paper redescribes the type material

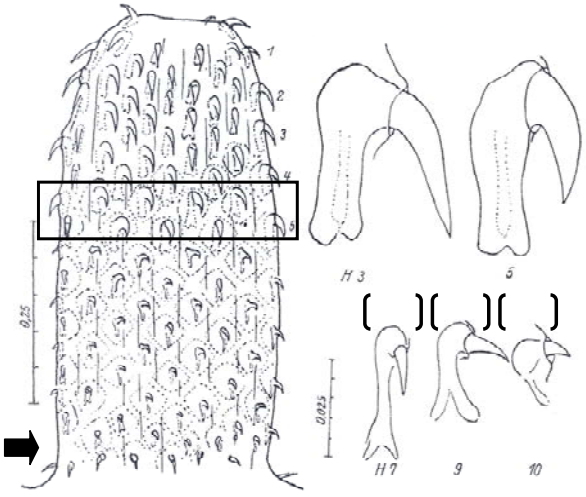

Fig. 2. Drawings of proboscis and selected hooks of *Pomphorhynchus laevis* (original by Meyer, 1932–1933). Rectangle indicates the proboscis area with less stout hooks Nos 5 and 6; arrow indicates the last hooks located on proboscis; brackets indicate the proximal parts of the base of 7th, 9th and 10th hooks devoid projections.

Table 1. ITS and COI GenBank accession numbers of *Pomphorhynchus tereticollis* and *Pomphorhynchus laevis*. Specimens of *P. tereticollis* misidentified as *P. laevis* are labelled with an asterisk. Hosts and localities of collection are also given, with abbreviations used in Fig. 6. New sequences (this study) are in bold.

	Locality	Host	ITS	COI
P. tereticollis				
Germany	Stralsund (ST), Baltic Sea	*Platichthys flessus* (Pf)	**JF06705**	**JF706706, JN695505 - JN695508**
P. laevis				
France *	Ouche River (OU)	*Gammarus pulex* (Gp)	AY424670	AY423351
	Tille (MA), Borne (BO)	*Gammarus pulex*	–	AY423352, AY423353,
	Vingeanne (VI) Rivers	*Leuciscus cephalus* (Lc)	–	**JN695504**
Slovakia *	Olšava River (OL)	*Leuciscus cephalus*	AY135413	–
	Small Vihorlat Lake (VL)	*Phoxinus phoxinus* (Pp)	AY135414	
Denmark *	Northern Øresund (NO)	*Platichthys flessus*	–	AY218096
France	Ouche River	*Leuciscus cephalus*		AY423348
	Ouche River	*Gammarus pulex*	AY424669	AY423348
	Tille River	*Gammarus roeseli* (Gr)		EF051062 – EF051065
Czech Republic	Rokytná River (RO)	*Leuciscus cephalus* *Gammarus roeseli*	AY135415	– EF051070, EF051071
Hungary	Tapolca River (TA)	*Gammarus roeseli*	–	EF051066 – EF051069 AY423349, AY423350

and morphological and molecular data on newly collected flounder acanthocephalans from the Baltic region are provided. This makes it possible to resurrect *P. tereticollis*, which occurs throughout Europe more widely than it has been supposed.

Materials and methods

Materials and morphology

Original Rudolphi's material of *P. tereticollis* from *P. flessus* from the Baltic coast near Greifswald, deposited in the helminthological collection of the Museum für Naturkunde Berlin (acronym ZMB), was studied during a stay of M. Š. in ZMB under the project SYNTHESYS (DE-TAF-2337). In a vial No. ZMB Entozoa 1226, 5 males and 3 females out of 56 syntypes were freely available, whereas the remaining 48 acanthocephalans were destroyed or burrowed into the fish gut wall by their proximal body part. Eight specimens were cleared in glycerin-ethanol for a short time, drawn and measured with the help of the light microscope Zeiss Axioskop 50 equipped with a drawing tube.

Fresh *Pomphorhynchus* material was obtained from 15 flounders *P. flessus*, which were purchased alive from a fisherman in Barhöft port at the Baltic coast, Germany, in October 2006. Fish were caught in the Baltic Sea area called Plantagenet-Ground, which is connected with the type locality in Greifswalder Bodden via Strela Sund near Stralsund, Germany (distance about 50 km). The fish were 335 – 490 mm long and were all infected with *Pomphorhynchus* parasites (below mentioned as Stralsund samples), with the intensity of infection 1 – 30 (mean = 11.7). Parasites were located in the posterior part of the fish gut.

Out of 175 acanthocephalans obtained, 30 unfixed females and 30 males were used for drawings of entire proboscis hooks. Afterwards, the material was divided into two parts and fixed either with 4 % formalin for morphology and with 99 % ethanol for molecular analysis, respectively. A total of 17 males (1 – 5 worms from 9 flounder hosts) and 14 females (1 – 2 worms from 9 fish) were used for measurements and drawings after clearing in glycerin-ethanol. Measurements of proboscis hooks represent the length and width of the outer visible hook part as used by Wayland (2010).

Molecular characterization

Pomphorhynchus tereticollis individual samples from the type host (*P. flessus*) and the type region (Stralsund locality) were compared with previously published sequences of freshwater *P. laevis* (Králová-Hromadová *et al*., 2003; Giribet *et al*., 2004; Perrot-Minnot, 2004; Moret *et al*., 2007) (Table 1). DNA extraction, amplification and sequencing of the ITS1 + 5.8S + ITS2 rDNA region and the COI gene were done following Perrot-Minnot (2004). Sequences were aligned using ClustalW procedure in BioEdit editor (Hall, 1999), and the rate of genetic divergence between Stralsund isolates and sequences of *P. laevis* available in GenBank were calculated. Two types of phylogenetic analyses were performed using MEGA v.5.04 with 1000 bootstrap replications: a distance-based Neighbor-Joining (NJ) analysis and Maximum Likelihood (ML) analysis.

Results

Pomphorhynchus tereticollis (Rudolphi, 1809)

Redescription

Based on measurements of 5 males and 3 females, all juvenile, from ZMB Entozoa 1226 (Fig. 3A – C) and freshly collected material of 17 males and 14 females, all adult (Fig. 4A – E). Measurements are in μm unless otherwise stated.

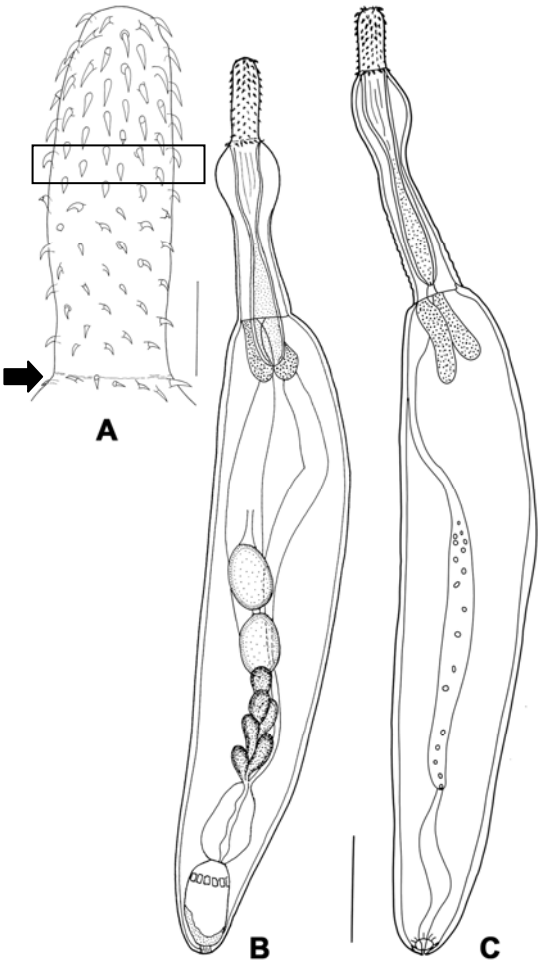

Fig. 3. *Pomphorhynchus tereticollis*. Male lectotype (A, B; ZMB Vermes Entozoa 1226) and female paralectotype (C; ZMB Vermes Entozoa 7500a) from Rudolphi's original material. Bar = 200 µm in (A) and 1 mm in (B, C). Rectangle indicates the proboscis area with the stoutest hooks Nos 5 and 6; arrow indicates the last hooks located on the bulbus (A).

General. Pomphorhynchidae, with characters of genus. Trunk elongated (Figs 3B, C, 4C, D). Neck of moderate length, somewhat tapered, broader at base, with prominent distal bulb. Proboscis cylindrical, with 13–18 longitudinal rows of 8 – 12 hooks each (Figs 3A, 4A, B, 5A). Hooks directed posteriorly; last hooks situated on proximal end of bulb or posterior-most margin of proboscis, directed oblique (Figs 3A, 5A). First four to five hooks (from anterior end) longest; fifth or sixth hooks stoutest. Hooks posterior to fifth or sixth hook remarkably shortest (Figs 3A, 4A, B, 5A). Hook roots, visible only in fresh unfixed material, without proximal protrusions (Fig. 4A) or exceptionally with small projections (Fig. 4B) in anterior hooks including the stoutest one. Hook bases at posterior half of proboscis with marked proximal projections (Figs 4A, B, 5C). Proboscis receptacle swollen posteriorly, in some specimens extending into body cavity (Fig. 3B). Muscular wall of receptacle incomplete posteriorly. Lemnisci subequal, slightly broader posteriorly, not extending to anterior testis (Figs 3B, 4C).

Male: based on 5 juvenile specimens - lectotype and 4 paralectotypes (Fig. 3A, B) and 17 adult specimens (Fig. 4A, C, data in parentheses).

Body length 7.4 – 9.4 (10.7 – 15.9) mm. Trunk 5.3 – 6.4 (7.4 – 11.4) mm long, 1.1 – 1.6 (1.4 – 2.1) mm wide. Neck 0.9 – 1.4 (1.2 – 1.9) mm long, 300 – 400 (250 – 500) wide just posterior to bulb and 420 – 530 (450 – 800) wide close to trunk. Bulb ovoid, round or ellipsoid, 600 – 950 (520 – 1470) long and 520 – 650 (640 – 1540) wide. Proboscis 440 – 790 (590 – 880) × 210 – 300 (270 – 360) wide in middle, with 13 – 14 (14 – 17) longitudinal rows of 10 – 11 (8 – 12) hooks each. Proboscis hook length and width from anterior: [First hook] 50 – 53/13 – 15 (30 – 55/8 – 15); [3 – 4 subsequent hooks] 40 – 55/13 – 17 (40 – 62/9 – 17); [stoutest fifth or sixth hook] 35 – 45/17 – 22 (36 – 52/15 – 22); [3 – 5 hooks posterior to stoutest hook] 26 – 31/7 – 10 (21 – 48/7 – 13); [last hook directed oblique] 22 – 31/7 – 9 (20 – 40/6 – 12). Proboscis receptacle 1.9 – 2.2 (1.5 – 3.1) mm × 210 – 290 (200 – 360) wide at posterior end. Two lemnisci 570 – 650 (500/555 – 1270/1325) × 150 – 250 (90/140 – 540/570). Testes two or exceptionally single (in one paralectotype from five and three adult males from 17), pre-postequatorial, subequal, ovoid, usually contiguous, occasionally detached up to 440. Anterior testis 540 – 635 (540 – 985) × 330 – 390 (380 – 750), posterior testis 515 – 620 (570 – 1020) × 330 – 370 (360 – 600), in case of single testis 960 × 500 (640 – 1520 × 570 – 650). Six elongate-pyriform cement glands with cylindrical core, 240 – 380 (180 – 750) × 130 – 170 (125 – 540). In 2 of 17 adult males (other than males with a single testis), two larger glands (680 – 1160 × 460 – 540) and two smaller glands (360 – 480 × 360 – 400) (Fig. 4C). Saefftigen's pouch just posterior to cement glands, 800 – 925 (800 – 1430) × 350 – 410 (320 – 640). Copulatory bursa (625 – 1130 × 320 – 570) when retracted and (412 – 805 × 570 – 720) when protruded (Fig. 4C). Fully everted bursa in single male (90 × 50). Gonopore terminal.

Female: based on 3 juvenile female paralectotypes (Fig. 3C) and 14 egg-bearing females (Fig. 4B, D, E, data in parentheses).

Body length 7.8 – 9.3 (15.4 – 21.0) mm. Trunk 4.5 – 6.3 (7.6 – 16.5) mm long, 1.2 – 1.7 (1.2 – 2.4) mm wide. Neck 1.3 – 1.9 (1.4 – 2.4) mm long, 270 – 290 (300 – 380) wide just posterior to bulb and 450 – 540 (450 – 540) wide close to trunk. Bulb ovoid, round or ellipsoid, 840 (700 – 1570) long and 530 – 750 (930 – 1630) wide. Proboscis 530 – 630 (630 – 930) × 215 – 330 (250 – 380) wide in middle, with 15 – 17 (15 – 18) longitudinal rows of 11 – 12 (9 – 12) hooks each. Proboscis hook length/width from anterior: [First hook] 45 – 50/12 – 13 (30 – 56/6 – 14); [2 – 4 subsequent hooks] 45 – 50/12 – 13 (40 – 65/12 – 19); [stoutest fifth or sixth hook] 30 – 40/17 – 21 (36 – 50/18 – 22); [3 – 5 hooks posterior to stoutest hook] 27 – 30/7 – 9 (23 – 40/9 – 15); [last hook directed oblique] 26 – 28/8 – 9 (22 – 38/10 – 15). Proboscis receptacle 2.3 – 2.7 (2.5 – 3.6) mm × 230 – 300 (250 – 380) wide at posterior end.

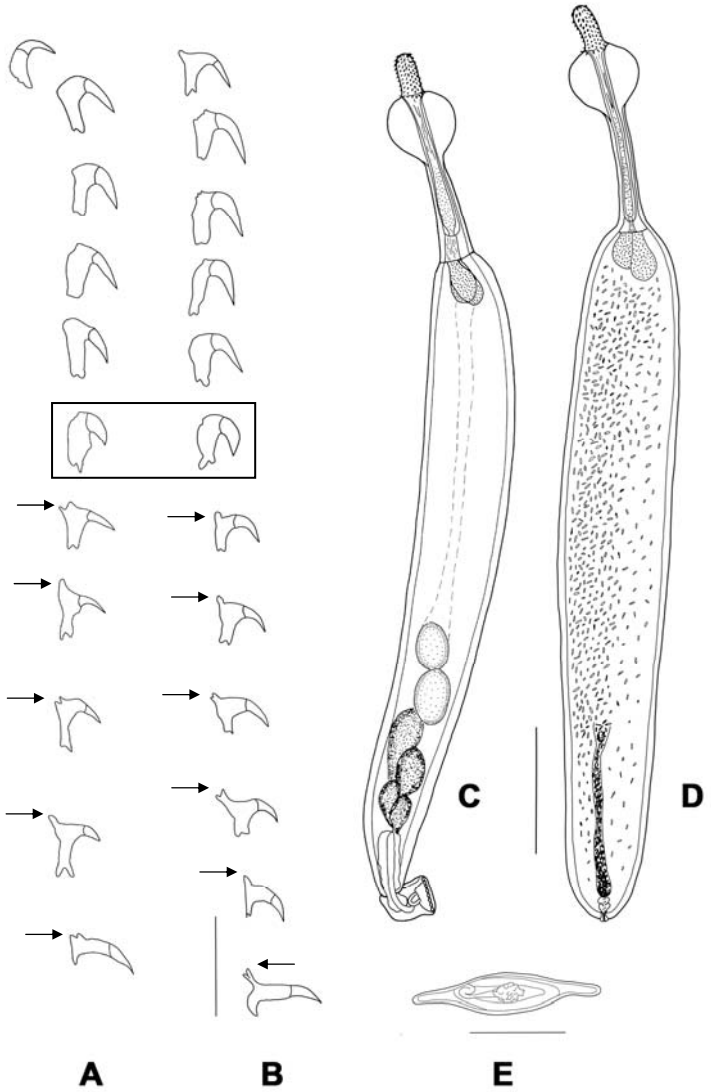

Fig. 4. *Pomphorhynchus tereticollis*. New material (ZMB Vermes Entozoa 7502) of adult males (A, C) and females (B, D, E). A, B – longitudinal row of proboscis hooks as visible in live specimens. Rectangle indicates the stoutest hooks No. 6, arrows indicate the proximal projections of bases of hooks Nos 7–11 (A) or 7–12 (B). Bar = 100 µm. C – male with atypical number of 4 cement glands and partially protruded copulatory bursa. Bar = 1 mm. D – mature female, filled with eggs indicated schematically. Bar = 1 mm. E – Egg. Bar = 50 µm.

Two lemnisci 90 – 710 (540/900 – 1340/1430) × 185 – 190 (110/160 – 480/540). Gonopore terminal (Fig. 4D). Numerous eggs and ovarian balls within trunk, eggs (95 – 126 × 12 – 23) (Fig. 4E)

Taxonomic summary

Type material

Type-host: Platichthys flessus (L.) (Pleuronectiformes, Pleuronectidae).

Type-locality: Baltic Sea near Greifswald (Gryphiae), probably Greifswalder Bodden (54°13.22'N; 13°32.48'E).

Date of collection: 25 October of unspecified year (before 1809).

Site of infection: intestine.

Helminth collection of Museum für Naturkunde Berlin (ZMB), originally catalogued under ZMB Vermes Entozoa 1226. Out of 56 syntypes, the male lectotype (ZMB Vermes Entozoa 1226) and one female paralectotype (ZMB Vermes Entozoa 7500a) were designated, figured and measured here. Measurements of six other paralectotypes are supplemented (ZMB Vermes Entozoa 7500b-g). The remaining 48 specimens are now catalogued under ZMB Vermes Entozoa 7501.

New material

Host: Platichthys flessus (L.) (Pleuronectiformes, Pleuronectidae).

Locality: Baltic Sea near Barhőft port (54°26.10'N; 13°02.00'E)

Date of collection: 13 October 2006.

Site of infection: posterior intestine

Deposition of material: Helminth collection of the Museum für Naturkunde Berlin (ZMB), ZMB Vermes Entozoa 7502. 17 males and 14 females, all adult.

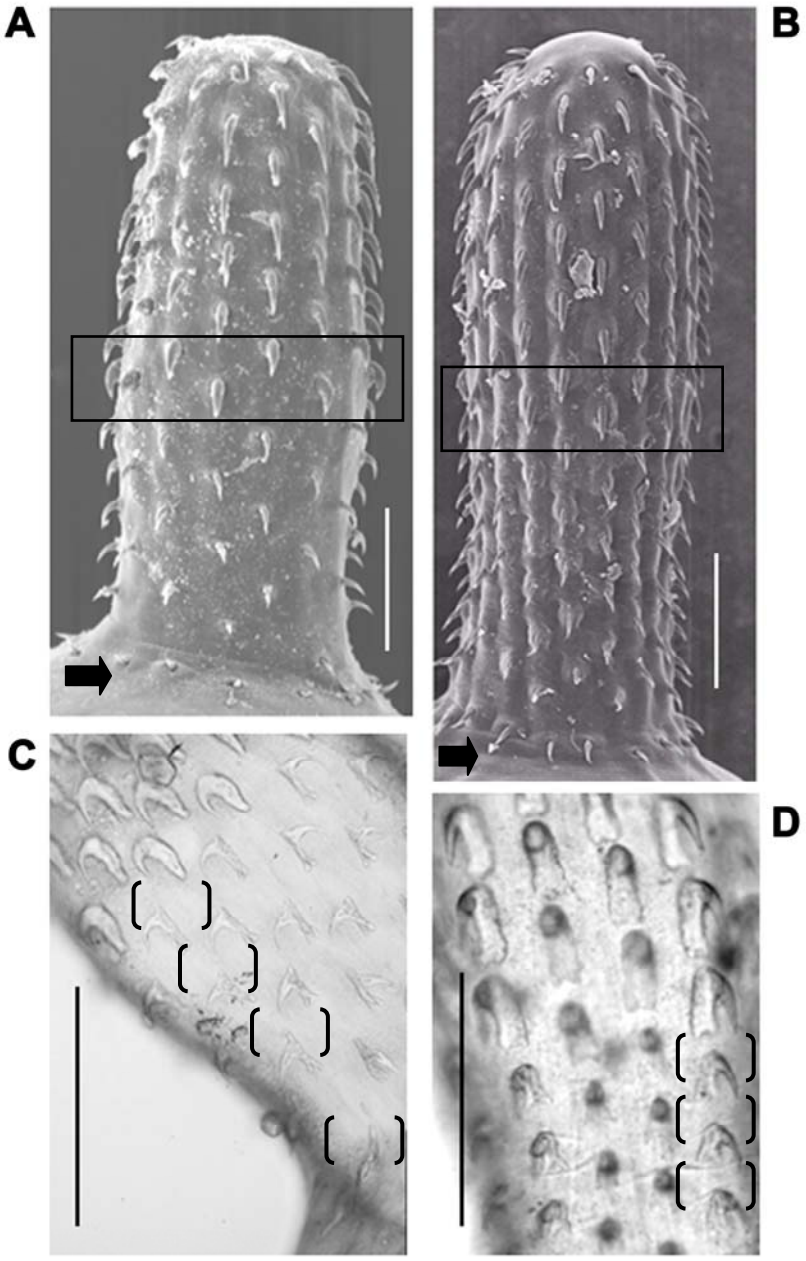

Fig. 5. *Pomphorhynchus tereticollis* – male from chub (*Squalius cephalus*) from the Olšava River, Slovakia (A, C) and *Pomphorhynchus laevis* - female from chub from the Rokytná River, Czech Republic (B, D). Scanning electron micrographs of the proboscis (A, B); photomicrograph of the middle part of the proboscis of live specimen (C, D). Bars = 100 µm.
Rectangles indicate the proboscis area with the stoutest hooks Nos 5 and 6 (A) and without stoutest hooks (B); arrow indicates the last hooks located on the bulbus (A) and on the proboscis (B); brackets indicate the proximal part of bases of 7^{th} – 10^{th} hooks armed with the projections (C) and the proximal part of the base of 7^{th} – 9^{th} hooks without any projections (D).

Remarks

Rudolphi (1809) listed *Echinorhynchus tereticollis* Rud. as the parasite of *P. flessus* (syn. *Pleuronectes flessus*) from Greifswald, and mentioned also the other fish hosts *Myoxocephalus scorpius* (L.) (syn. *Cottus scorpius*), *Lota lota* (L.) (syn. *Gadus lota*), *Zoarces viviparous* (L.) (syn. *Blennius viviparus)*, *Anguilla anguilla* (L.) (syn. *Muraena anguilla*), *Gymnocephalus cernua* (L.) (syn. *Perca cernua*), *Perca fluviatilis* L.*,* various cyprinids, and *Salmo trutta* L. Later, Rudolphi (1819) enlarged the list of fish hosts by *Acipenser sturio* L., *Silurus glanis* L., *Merlangius merlangus* (L.) (syn. *Gadus merlangus*), *Huso huso* (L.) (syn. *Accipenser huso*), and *Cottus gobio* L. from Greifswald and Vienna. The only original Rudolphi's material is represented by a single set of 56 juvenile acanthocephalans from *P. flessus* from the vicinity of Greifswald (Gryphiae), and eight juvenile specimens were used for the redescription. As no adult specimens were among the type material, we collected new fresh material from type-host fish *P. flessus* caught in the Baltic Sea near Stralsund. Morphological characters of these acanthocephalans fit well with those of type specimens of *P. tereticollis* as well as with

those of *P. tereticollis* described by Meyer (1932 – 1933). Later, additional three congeneric species have been described from European teleosts. *Pomphorhynchus kostylevi* Petrochenko, 1956, described from *Capoeta capoeta sevangi* DeFilippi (Cyprinidae), endemic to Lake Sevan (Armenia), is characterised by a relatively long proboscis (940 μm) and large eggs (126 – 129 × 23 μm); *Pomphorhynchus bosniacus* Kiskároly et Čanković, 1967, described from *Barbus barbus* (L.) from the River Sava, possesses a small bulbus (300 – 700 μm), long proboscis hooks (42 – 61 μm) and small eggs (83 – 95 × 15 – 18 μm) (Petrochenko, 1956; Kiskároly & Čanković, 1967). The latter species was reported from various other fishes (e.g. *Salmo ohridanus* Steindachner and *Acipenser ruthenus* L.) from lakes and rivers of the Balkan (Kakacheva-Avramova, 1973; Hristovski *et al*., 1999; Cakić *et al*., 2008). The last European species, *P. intermedius* from Baltic *P. flessus*, closely resembles *P. tereticollis* and both species have been considered as synonyms of *P. laevis* by Amin *et al*. (2003).

Another *Pomphorhynchus* species, *P. spindletruncatus* Amin, Abdullah et Mhaisen, 2003, described from cyprinids *Aspius vorax* (Heckel) and *Barbus xanthopterus* (Heckel) from Northern Iran by Amin et al. (2003), has been recently reported also from six other cyprinid fishes and the marsh frog *Pelophylax ridibundus* (Pallas) (Ranidae) in the Asian part of Turkey (Heckmann *et al*., 2010). It differs from *P. tereticollis* by a ribbed proboscis surface, a wider (3.6 – 4.1 mm) and spindle-shaped trunk, a shorter proboscis (520 – 750 μm), a longer (167 – 232 μm) and

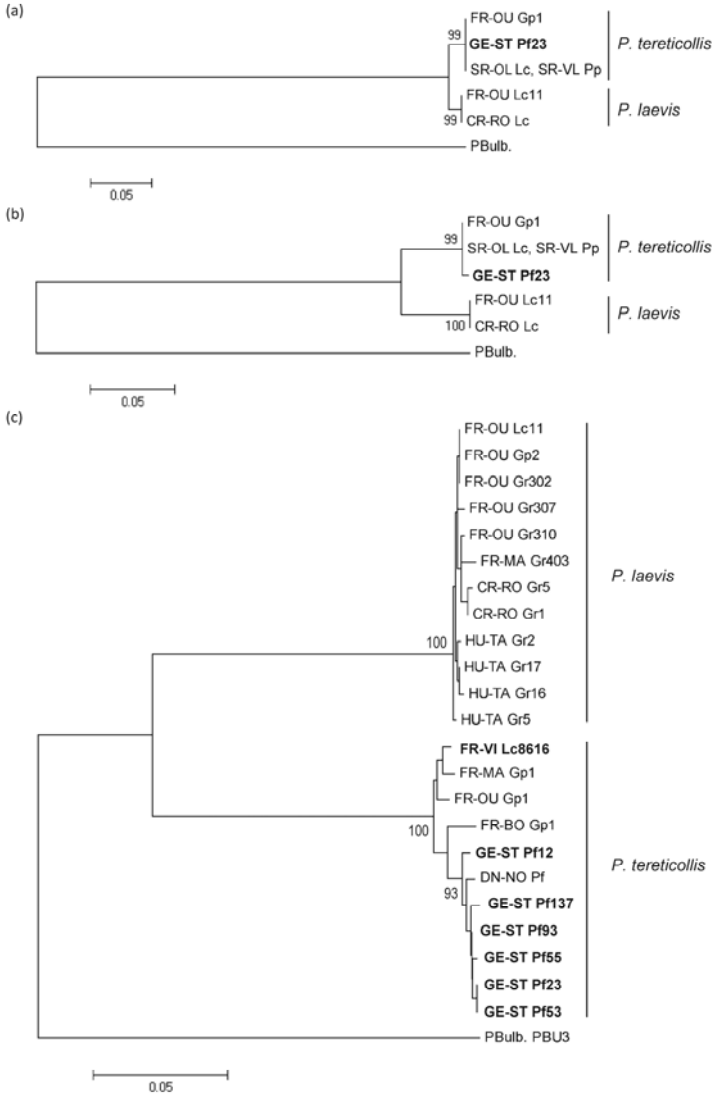

Fig. 6. Phylogenetic tree with maximum likelihood analysis displaying the relationships between *Pomphorhynchus laevis* and *Pomphorhynchus tereticollis* based on ITS1 (a), ITS2 (b) and partial COI (c) sequences. Labels refer to country of collection (CR, Czech Republic; DN, Denmark; FR, France; GE, Germany – near type locality; HU, Hungaria; SR, Slovak Republic) followed by the locality/river, and isolate (see Table 1 for abbreviations). Sequences in bold are new ones, while others were taken from the GenBank database. Bootstrap values are indicated.

wider (160 – 297 µm) bulb, a longer neck (255 – 315 µm), and smaller eggs (70 – 75 × 11 – 15 µm) in egg-bearing females, and in larger testes (670 – 142 × 300 – 110 µm) in males (Heckmann *et al*., 2010). However, *P. spindletruncatus* possesses some characters similar to those of *P. tereticollis*, namely the posteriorly incomplete muscular wall of the proboscis receptacle, the occurrence of markedly stout (robust) hooks in the middle part of the proboscis, and the last hooks located at the posterior-most end of the proboscis (Amin *et al*., 2003; Heckmann *et al*., 2010).

Molecular characterization

Comparisons of sequences from the type host and near type locality to those already available in GenBank, were based on 257 – 283 bp fragments (ITS1), 272 – 277 bp fragments (ITS2) and 610 bp fragments (COI). Since Neighbor-Joining (NJ) analysis and Maximum Likelihood (ML) analysis gave the same bootstrap consensus tree topology, only the latter one is presented (Fig. 6). Levels of the genetic divergence and the tree topology reveal the clustering of *P. laevis* specimens in two groups (Table 2, Fig. 6). Stralsund isolates cluster with some *P. laevis* isolates from near the Baltic Sea (Northern Øresund, Denmark), and from freshwaters of France and Slovak Republic (see Table 1 for hosts and localities). Since the specimens from the type host and locality of *P. tereticollis*, which are morphologically identical with paratypes of this species, are genetically closely related with some *P. laevis* specimens, the latter isolates are considered to be conspecific with *P. tereticollis*. This cluster of *P. tereticollis* sequences shares fixed differences compared to the other *P. laevis* sequences, in particular several deletions in the ITS1 region. This size polymorphism of ITS1 can be considered as diagnostic character, being based on sequences from 56 more individuals (data not shown). The whole ITS1 gene can be amplified for molecular discrimination of *P. tereticollis* and *P. laevis* by a simple PCR assay, with the BD1 forward primer (5'GTCGTAACAAGGTTTCCGTA3') (Králová-Hromadová *et al*., 2003), and a reverse primer designed in a conserved 3' region of the 5.8S rRNA (AC/ITS1r : 5'TTGCGAGCCAAGTGATTCAC3'), at 50°C annealing temperature. The amplification products from *P. tereticollis* and *P. laevis* specimens can be visualized in a 2 % agarose gel at about 350 bp and 320 bp respectively (Franceschi *et al*., 2008).

Discussion

The present results have demonstrated the morphological congruence between the type specimens of *P. tereticollis* and worms from the same host species and region. All acanthocephalans possess the last hooks localised at the anterior part of the bulbus or rarely at the posterior-most end of the proboscis and hooks Nos. 5 or 6 are markedly stout (robust), thus being distinct from surrounding hooks. The worms also share a similar size of their body parts, genital organs and proboscis hooks (type juveniles are smaller due to their developmental stage). These facts suggest that the flounder parasites from the Baltic coast near Stralsund belong to *P. tereticollis*.

The most important species-specific feature of *P. tereticollis* is the existence of proximal projections of the basal parts of the proboscis hooks located on the posterior proboscis half, well visible in the newly collected specimens (Figs. 4, 5). This morphological character was also used by Meyer (1932 – 1933) to distinguish *P. tereticollis* from *P. laevis*, which has no proximal protrusions of bases of relevant hooks. This trait characterizing *P. tereticollis*, however, varied in size and shape among worms parasitizing individual fish, and some specimens possessed the projections of hook bases less developed, i.e. identical to those of *P. intermedius* as shown by Engelbrecht (1957). Therefore, the latter species is considered to be a synonym of *P. tereticollis*.

Due to the fact that the most important differential feature - the shape of the hook bases - is visible only in live worms and due to the overall similarity of *P. laevis* and *P. tereticollis*, all acanthocephalans found in freshwater and brackish fishes throughout the Palaearctic region were considered to be the former species for a long time (e.g. Moravec & Scholz, 1991; Chibani *et al*., 2004; Kennedy, 2006). However, the present data allows us to confirm the

Table 2. Sequence similarity of *Pomphorhynchus* isolates (see Table 1), given as percentage nucleotide identity. Length of compared fragments: 257 – 283 bp of ITS1, 272 – 277 bp of ITS2, and 610 bp of COI. Specimens of *P. tereticollis* misidentified as *P. laevis* are labelled with an asterisk.

	Range of sequence similarity in % (sample size)		
Pomphorhynchus sample	ITS1	ITS2	COI
P. tereticollis Germany	100 (4)	100 (4)	99.1 – 100 (6)
P. tereticollis Germany / *P. laevis** France	100 (4/1)	99.6 (4/1)	97.6 – 98.3 (6/4)
P. tereticollis Germany / *P. laevis** Slovakia	100 (4/1)	99.6 (4/1)	–
P. tereticollis Germany / *P. laevis** Denmark	–	–	99.0 – 99.5 (6/1)
P. tereticollis Germany / *P. laevis* France	88.6 (4/1)	90.9 (4/1)	80.0 – 80.7 (6/6)
P. tereticollis Germany / *P. laevis* Czech Republic	88.6 (4/1)	90.9 (4/1)	79.9 – 80.4 (6/2)
P. tereticollis Germany / *P. laevis* Hungary	–	–	80.2 – 80.7 (6/4)

occurrence of both *P. laevis* and *P. tereticollis* in several freshwater fishes, amphipod intermediate hosts, and fluvial localities. New *Pomphorhynchus* specimens from flounder, which are morphologically identical with the type material of *P. tereticollis*, are genetically closely related (almost identical) with previously identified *P. laevis* specimens from freshwater chub *Squalius cephalus* (L.), minnow *Phoxinus phoxinus* (L.), and *Gammarus pulex* (L.) from France and Slovakia, and from flounder in Northern Øresund, Denmark (Køie, 1999; Køie, pers. comm.). Therefore, the latter isolates are considered to be conspecific with *P. tereticollis*. This means that *P. tereticollis* parasitizing flounder and fluvial hosts represent a single morphologically and genetically homogeneous group occurring throughout Europe, together with its more common congener *P. laevis*. In some localities, e.g. in the Rivers Ouche and Tille, France, they can occur sympatrically also in barbel *Barbus barbus* (L.) (Perrot-Minnot, unpublished results).

The resurrection of *P. tereticollis* as a species distinct from *P. laevis* calls for a complete re-evaluation of their respective biogeography and ecology. A phylogeographic study including the geographic distribution of several lineages of *P. laevis* and *P. tereticollis* throughout Europe is currently in progress. Ecological studies addressing the range of hosts used by *P. laevis* and *P. tereticollis* in the local community where they occur in sympatry should also be conducted. More generally, our study also illustrates taxonomic difficulties in studies on acanthocephalans (a 'systematist's nightmare'; Kennedy, 2006) as they have low anatomical diversity but can exhibit a rather considerable intraspecific variation in the only hard structures they possess, the hooks on their proboscis (Kennedy, 2006). Coupling morphological and genetic studies is therefore necessary to clarify the taxonomic status of other suspected cases of cryptic species, such as *Echinorhynchus gadi* Zoega in Müller, 1776 and *Leptorhynchoides thecatus* (Linton, 1981) (Wayland *et al*., 2005; Steinauer *et al*., 2006).

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