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ORIGINAL PAPER

Genetic diversity in the trypanorhynch cestode *Tentacularia coryphaenae* Bosc, 1797: evidence for a cosmopolitan distribution and low host specificity in the teleost intermediate host

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Abstract Partial large subunit (28S) rRNA gene (LSU) sequences were studied from *Tentacularia coryphaenae* (Cestoda: Trypanorhyncha) plerocercoids from the southern Java coast, Indonesia, collected from two different localities and five different host species. The teleost hosts belonged to four fish families with an overlapping depth range of 0–885 m. The LSU sequences were identical, demonstrating that all specimens belonged to the same species. They also corresponded to a sequence of *T. coryphaenae* from the Blue shark *Prionace glauca* in the North Atlantic, giving genetic evidence for the cosmopolitan distribution of the species. A 1,851 bp region of mitochondrial (mt) DNA (coding for partial cytochrome *c* oxidase subunit I (cox1), complete trnT and partial 16S ribosomal RNA) showed a very low level of intra-specific variation of 1%. Pairwise comparisons of published sequences for partial LSU rDNA and the same region of mtDNA demonstrated that the same regions varied by 8% in the mtDNA for two genotypes (G1 and G4) of *Echinococcus granulosus* (order Cyclophyllidea), at 16% in newly sequenced *Kotorella pronosoma* from the same trypanorhynch family and at 23% in *Grillotia pristiophori* from a different superfamily. The high genetic homogeneity in *T. coryphaenae* is explained by a constant gene flow between different regions and hosts along the Indonesian coast caused by extensive migrations of the second intermediate/paratenic and also the final hosts. Implications for the zoogeographical distribution, host specificity of the species and future research are discussed.

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Introduction

The cosmopolitan distribution of marine fish parasitic helminths continues to be of major interest. Though earlier morphological studies indicated a worldwide distribution for the marine fish nematode *Anisakis simplex*, recent molecular studies demonstrated that the morphotype consists of three cryptic or sibling species, *A. simplex* (sensu stricto), *A. pegreffii* and *A. simplex* C, having similar morphotypes but different genotypes and being separated by different host preferences and geographical distributions (Abe et al. 2005; Marques et al. 2006). *Anisakis simplex* (s.s.) is cosmopolitan and occurs especially in the North Atlantic and the Pacific Ocean, while *A. pegreffi* is most frequent in the Mediterranean Sea and the southern hemisphere and *A. simplex* C is most frequent at the Pacific coast of Canada and in the southern hemisphere (Marques et al. 2006; Mattiucci et al. 1997). Most recently, *A. pegreffii* was recorded together with *A. simplex* (s.s.) from the same teleost host in Japan (Abe et al. 2005). Genetic studies on the identity and the presence of siblings in other widely distributed marine fish helminths that utilize the marine food web for transmission into the final host, such as the acanthocephalans and cestodes, are completely missing.

Trypanorhynch cestodes are a cosmopolitan order of marine tapeworms with the majority of species occurring in tropical oceans, comprising 260 valid species (Palm 2004; Beveridge and Campbell 2005; Beveridge and Justine 2006).

They live in the stomach and intestine of elasmobranchs as final hosts Campbell and Beveridge 1994 and infect invertebrates and various teleosts as intermediate hosts. They have the advantage that the larvae and adults have the same scolex morphology, enabling accurate taxonomic diagnosis and detailed analysis of infection patterns all around the world based on morphology (Palm 2004). In most other cestodes, nematodes, acanthocephalans and also trematodes, the larvae lack species diagnostic features that would enable closely related species to be distinguished morphologically. Consequently, trypanorhynchs are the only larger taxon of marine fish helminths that are getting transferred through the marine food web for which the full range of final and second intermediate/paratenic hosts can be determined accurately. Hence, this order of parasitic flatworms can be used as a model taxon to study the evolution of fish parasitic helminths and their life cycles in marine ecosystems (Palm 2004; Palm and Klimpel 2007a).

Evaluation of a host–parasite checklist for the trypanorhynchs revealed low ecological and phylogenetic host specificity in the final and especially in the teleost second intermediate or paratenic hosts (Palm 2004). Based on morphology, adults of the cosmopolitan *Tentacularia coryphaenae* Bosc, 1797 were recorded from 11 different elasmobranch species and the plerocercoids from over 80 species of larger pelagic or oceanic fishes and squids. The fish ecology, and not phylogeny, seems to be more important in determining the trypanorhynch infracommunity in teleost fish (Jakob and Palm 2006). However, the occurrence of cryptic or sibling species in anisakids (see above) suggests that siblings might also exist in the trypanorhynchs, particularly in a taxon such as *T. coryphaenae*. By using gel electrophoresis for loci, Sin et al. (1992) demonstrated significant variations in the allelic and phenotypic frequencies between *Hepatoxylon trichiuri* (Holten 1802) populations from 11 different teleost host species.

To date, no molecular genetic study was carried out on intra-specific genetic homogeneity within a cosmopolitan trypanorhynch species in the final or the intermediate host. Partial LSU rDNA was used among a diversity of taxa to recognize cryptic species including cestodes (e.g. Rosas-Valdez et al. 2004) and is used extensively in cestode phylogenetics (e.g. Olson et al. 2001). LSU rDNA domains D1–D3 sequences were obtained from *T. coryphaenae* plerocercoids from the southern Java coast and compared with a published sequence from an adult worm from the North Atlantic to confirm species identity. The specimens were collected from two different localities (Java, Bali) and five different host species belonging to four fish families with an overlapping depth range of 0–885 m. To detect intra-specific genetic variation, a 1,851 bp region of mitochondrial (mt) DNA (partial cytochrome *c* oxidase subunit I (cox1)), complete trnT and partial 16S ribosomal RNA) was sequenced. This fragment was chosen because relatively conserved primers could be designed from published mt genomes of platyhelminths for this region. To better evaluate the level of intra-specific variation within *T. coryphaenae*, pairwise comparisons of published sequences for partial LSU rDNA and the same region of mtDNA were carried out for other cestodes; these include representatives from the order Cyclophyllidea and Proteocephalidea. An outlook of further molecular genetic studies in this important group of cestodes for broader evolutionary studies of marine fish helminths is given and implications for host specificity and zoogeographical distribution of the species are discussed.

Materials and methods

Specimen collection and deposition Specimens of *T. coryphaenae* were isolated from the mesentery and the body cavity of *Coryphaena hippurus* L., 1758 (family Coryphaenidae, pelagic, oceanodromous, depth range 0–85 m), *Katsuwonus pelamys* L., 1758 (family Scombridae, pelagic, oceanodromous, 0–260 m), *Trichiurus lepturus* L., 1758 (family Trichiuridae, benthopelagic, 0–400 m), *Promethichthys prometheus* (Cuvier 1832) (family Gempylidae, benthopelagic, oceanodromous, 80–800 m (300–400 m common depth)) and *Lepidocybium flavobrunneum* (Smith 1843) (family Gempylidae, bathypelagic, oceanodromous, 200–885 m) (Froese and Pauly 2006). The specimens were collected in January 2003 (*K. pelamys*), July 2004 (*T. lepturus*) and February 2005 from Pelabuhan Ratu fish market, southern Java coast, Indonesia (6°59′13 S and 106°32′38 E) and in July 2006 (*L. flavobrunneum*) from Kedonganan fish market close to Denpasar, Bali, Indonesia (8°45′25 S and 115°10′05 E) (Fig. 1). Specimens were transferred and washed in saline solution, and fixed and stored in either 100% or 70% ethanol. Where possible, voucher specimens were designated for the sample used for the extraction of gDNA. The scolex was stained in acetic carmine and prepared as whole mount specimens in Canada balsam. The species was identified by using the method of Palm (2004).

DNA amplification and sequencing Total genomic DNA (gDNA) was extracted using DNeasy™ Tissue Kit (QIAGEN) for purification of DNA following the standard manufacturer-recommended protocol. Two µl gDNA was used as a template in 25 µl reactions using Ready-To-Go™ PCR beads (Amersham Pharmacia Biotech). Partial LSU rRNA (899–1,530 bp) was characterized for 5 specimens of *T. coryphaenae* from Indonesia. The region was amplified using ZX-1 (5′ ACC CGC

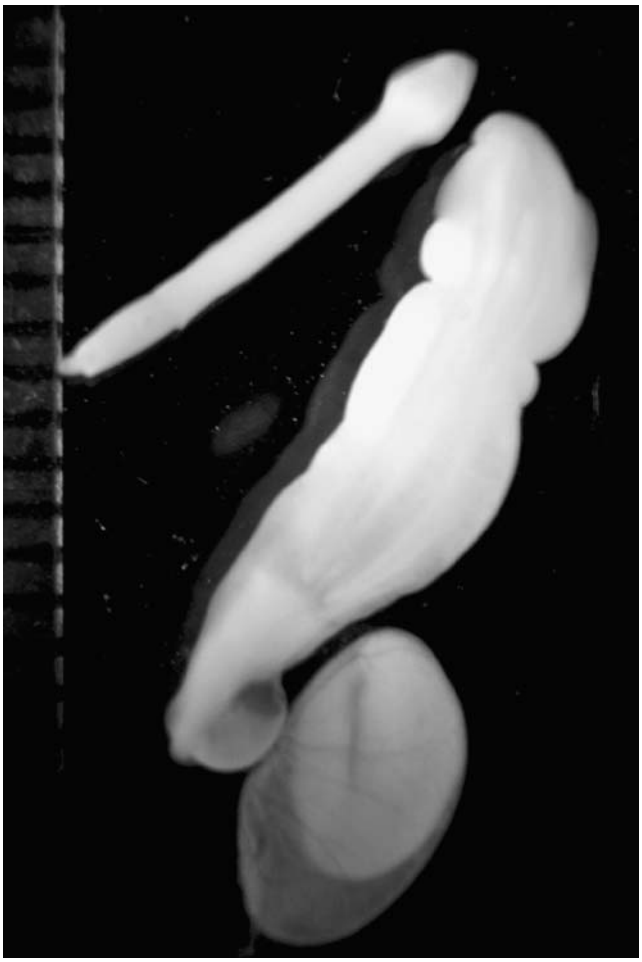

Fig. 1 Specimens of *T. coryphaenae* from *L. flavobrunneum*. Note the different total size ranges of the specimens. The large specimens were found surrounded by a large translucent host capsule while the small specimen occurred free in the body cavity (bars=1 mm)

TGA ATT TAA GCA TAT 3′) (modified from Van der Auwera et al. 1994) and 1500R (5′-GCT ATC CTG AGG GAA ACT TCG-3′) (Tkach et al. 2003) using the following cycling conditions: denaturation for 5 min at 95°C, followed by 40 cycles of 30 s at 95°C, 30 s at 55°C, 2 min at 72°C and 7 min extension at 72°C. PCR amplicons were either gel-excised using QIAquick™ Gel Extraction Kit (QIAGEN) or purified directly using QIAquick™ PCR Purification Kit (QIAGEN) following the standard manufacturer-recommended protocol, cycle-sequenced from both strands using ABI BigDye™ chemistry, alcohol-precipitated and run on an ABI 3730 DNA Analyser, Big Dye version 1.1. Products were sequenced using the two PCR primers and internal primers 300F (5′-CAA GTA CCG TGA GGG AAA GTT G-3′), ECD2 (5′-CTT GGT CCG TGT TTC AAG ACG GG-3′), 400R (5′-GCA GCT TGA CTA CAC CCG-3′) and 900F (5′-CCG TCT TGA AAC ACG GAC CAA G-3′). Partial mt fragments spanning cox1–16S rDNA (1,851 bp) were characterized for 3 specimens of *T. coryphaenae* and two reference trypanorhynchs, namely, *Kotorella pronosoma* from *Dasyatis sayi* (Lesueur 1817) from Deer Island, Gulf of Mexico, MS, USA (leg. R. Overstreet, date of collection: 19.04.1999) and *Grillotia pristiophori* from *Pristiophorus nudipinnis* (Günther 1870) from San Remo, Victoria, Australia (leg. I. Beveridge, date of collection: 01.01.2001). Fragments were amplified using Cyclocox1FA (5′-CAR CAT ATG TTT TGR TTT TTT GG-3′) and Cyclo16SRC (5′-GCC AGG TCG GTT CTT ATC TAT T-3′) using the following cycling conditions: denaturation for 3 min at 94°C, followed by 40 cycles of 30 s at 94°C, 30 s at 52°C, 3 min at 72°C and 7 min extension at 72°C. PCR amplicons were gel-excised using QIAquick™ Gel Extraction Kit (QIAGEN) following the standard manufacturer-recommended protocol. Products were cloned using TOPO TA Cloning® Kit with pCR®2.1-TOPO® vector (Invitrogen) following the manufacturer's instructions. Positive clones were grown for 15 h in 3 ml volumes of LB at 37°C at 200 rpm in a shaking incubator. Plasmid DNA was purified using QIAprep Spin Miniprep Kit (QIAGEN) following the standard manufacturer-recommended protocol and cycle-sequenced from both strands using M13 primers and internal primers Ten_cox1_F1 (5′-TTA TGC TTT TTT CCT ATG C-3′) and Ten_16S_R1 (5′-TTG GTA GAC CTC TTT GCC-3′). Contiguous sequences were assembled and edited using Sequencher™ (GeneCodes, Version 4.6) and sequence identity checked using the Basic Local Alignment Search Tool (BLAST) (http://www.ncbi.nih.gov/BLAST/).

Alignment Alignments were performed using CLUSTAL_X (Thompson et al. 1997) followed by minor adjustments by eye using MacClade (Maddison and Maddison 1992; Version 4.1). The newly generated LSU sequences were combined with the LSU of *T. coryphaenae* from the Blue shark *Prionace glauca* L., 1758 from Montauk, NY, USA. Additional alignments of partial LSU rDNA were made for a variety of cestode species available on GenBank where multiple individuals had been characterized. The newly generated mt fragments for trypanorhynchs were aligned against each other and similar alignments for the same mt fragment were aligned within and between species of *Echinococcus* and *Taenia*. The full lists of alignments for the LSU and mt DNA including the GenBank accession numbers are detailed in Tables 1 and 2.

Pairwise comparison With the alignments in Tables 1 and 2, pairs of taxa were compared and scored for numbers and proportion of base changes to give an estimate of genetic similarity within and between genera for each molecular marker. Differences and scores were determined using PAUP* (Swofford 2002).

Table 1 Results of pairwise comparisons within and between species for partial (D1–D3) LSU rDNA sequences, expressed as percent difference with source of data including the GenBank accession numbers

Taxon	Total alignment (bp)	Number comparisons	Proportion difference (%)	GenBank accession numbers
Within species				
Trypanorhyncha				
Tentacularia coryphaenae	848	6	0.00	EF095265–EF095269 (Indonesia), AF286976 (USA)
Tetraphyllidea				
Phyllobothrium delphini	552	8	0.00	AY741599–AY741606
Monorygma grimaldi	621	8	0.00	AY741591–AY741598
Clistobothrium cf. *montaukensis*	633	13	0.00	AF126069, AF382071–AF382082, AF286957
Proetocephalidea				
Peltidocotyle lenha	992	3	0.00	AJ238834, AJ238836–AJ238837
Within genus				
Cyclophyllidea				
Paranoplocephala spp.	1,295–1,315	16	0.01–0.02	AY569742, AY569744, AY569747, AY569748, AY569750, AY569752, AY569755, AY569756, AY569757, AY569759, AY569761, AY569762, AY569764, AY569766, AY569768, AY569774
Anoplocephaloides spp.	1,299–1,331	7	0.01–0.06	AY569742, AY569745, AY569747–AY569748, AY569750, AY569752, AY569755–AY569757, AY569759, AY569761–AY569762, AY569764, AY569766–AY569767, AY569774
Proetocephalidea				
Proteocephalus spp.	962–986	18	0.01–0.17	AF026116, AJ275062, AJ275228, AJ275230, AJ275233, AJ275234, AJ388594, AJ388599, AJ388606, AJ388609, AJ388610, AJ388616, AJ388622, AJ388626, AJ388633, AJ388635, AJ388636, AJ388638
Corallobothrium spp.	991–1,000	3	0.02–0.08	AJ583450, AY548162, AY548163

Results

LSU rDNA All *T. coryphaenae* sequences were identical. This demonstrates that the studied specimens from the elasmobranch and five teleosts belonging to four fish families with a depth range from 0 to 885 m and sampled in different years (from 2003 to 2006), different regions (Java/Bali) and two different Oceans (Atlantic/Indian) belong to the same trypanorhynch species. Comparison of genetic differences of the same LSU-region in other cestodes also revealed no intra-specific variation within the same species in *Phyllobothrium delphini*, *Monorygma grimaldi* and *Clistobothrium* cf. *montaukensis* (order Tetraphyllidae, marine) and *Peltidocotyle lenha* (Proteocephalidae, freshwater). A comparison of several species within the same genus showed genetic differences of 1–2% and 1–6% in *Paranoplocephala* spp. and *Anoplocephaloides* spp. (both Cyclophyllidea, terrestrial), respectively and 1–17% and 2–8% in *Proteocephalus* spp. and *Corallobothrium* (both Proteocephalidea, freshwater), respectively (Table 1).

mtDNA Pairwise comparison between larval specimens of *T. coryphaenae* obtained from *C. hippurus*, *K. pelamys* and *L. flavobrunneum* revealed very low genetic variability (Table 2). A total of 14–21 positions differed among the specimens with a 1% difference between the specimens. Comparison of the genetic differences of the studied mtDNA region between cox1 and 16S of *T. coryphaenae* with other trypanorhynchs (*Kotorella*, same family, and *Grillotia*, different superfamilies) revealed a 16–23% difference. Comparison within other genera from other orders of cestodes revealed a variation of 8% within the same species (*Echinococcus granulosus*) and of 10% within the genus *Echinococcus* and 13–17% in *Taenia* (both Cyclophyllidea, terrestrial). The mtDNA data for *T. coryphaenae* demonstrate that the studied specimens from the three teleost hosts and from the two Indonesian regions

Table 2 Results of pairwise comparisons within and between species for the cox1–trnT–16S fragment of mtDNA, expressed as percent difference with source of data [GenBank accession numbers]

Taxon/isolate [GenBank accession numbers]; pairwise comparison	Total alignment (bp)	Number difference (bp)	Proportion difference (%)	Notes
Tentacularia; Trypanorhyncha				
T. coryphaenae/HP16 [EF095270] vs *T. coryphaenae*/HP24 [EF095271]	1,851	14	0.01	Within species
T. coryphaenae/HP16 [EF095270] vs *T. coryphaenae*/HP25 [EF095272]	1,851	21	0.01	Within species
T. coryphaenae/HP24 [EF095271] vs *T. coryphaenae*/HP25 [EF095272]	1,851	15	0.01	Within species
T. coryphaenae (each of above) vs *Kotorellapronosoma* [EF103923]	1,840	296–299	0.16	Between species
T. coryphaenae (each of above) vs *Grillotiapristiophori* [EF103924]	1,828	426–427	0.23	Between species
Kotorellapronosoma [EF103923] vs *Grillotiapristiophori* [EF103924]	1,827	421	0.23	Between species
Echinococcus; Cyclophyllidea				
E. granulosus G1 [AF297617] vs *E. granulosus* G4 [AF346403]	1,858	156	0.08	Within species
E. granulosus G1 [AF297617] vs *E. multilocularis* [AB018440]	1,858	186	0.10	Between species
E. granulosus G4 [AF346403] vs *E. multilocularis* [AB018440]	1,862	186	0.10	Between species
Taenia; Cyclophyllidea				
T. solium [AB086256] vs *T. asiatica* [AF445798]	1,864	247	0.13	Between species
T. solium [AB086256] vs *T. crassiceps* [AF216699]	1,848	305	0.17	Between species
T. asiatica [AF445798] vs *T. crassiceps* [AF216699]	1,848	301	0.16	Between species

belonged to the same species and have a very low level of genetic variation.

Discussion

Examination of LSU rDNA and mtDNA of *T. coryphaenae* revealed only few differences among the studied specimens from five different teleost hosts and different localities. The LSU sequences between the adult *T. coryphaenae* from the North Atlantic and the larvae from Indonesia were identical, confirming that both materials belonged to the same species.

While identical LSU rDNA sequences among different specimens within the same species can be observed also in other cestode genera belonging to the tetraphyllideans and proteocephalideans, the 1% variation of the mtDNA between the three larvae from the Indonesian fish was much lower than the 8% variation that can be observed in the terrestrial cyclophyllidean *E. granulosus*. Though the *T. coryphaenae* specimens were collected from different fish living in very different habitats and from two localities about 1,000 km apart, they show very high genetic homogeneity. This might be explained by a constant gene flow between different regions and hosts facilitated by extensive migrations of the second intermediate/paratenic and also the final hosts (see below). The comparison of the genetic variability of the mt DNA within the trypanorhynchs (*Tentacularia* vs *Kotorella* from the same trypanorhynch family and *Grillotia* from a different superfamily) was higher (16–23%) than those observed for the terrestrial cyclophyllideans *Echinococcus* spp. (10%) and *Taenia* spp. (13–17%). A reason for this can be seen in the different taxonomic levels that were compared, with the observed genetic variation ranging within a species (1–8% in *T. coryphaenae* and *E. granulosus*), genus (10% and 13–17% in *Echinococcus* spp. and *Taenia* spp.) and among genera belonging to the same family (16%) or different superfamilies of the trypanorhynchs (23%).

The present study demonstrates that the plerocercoids from five different teleost hosts from the Indonesian coast are genetically identical (LSU) or closely corresponding (mtDNA). The occurrence of the same trypanorhynch species in shallow water pelagic and in deepwater bathy-

pelagic fish belonging to four different families (all of the order Perciformes) with a depth range between 0 and 885 m confirms the low phylogenetic host specificity in the second intermediate or paratenic host (euryxenic according to Euzet and Combes 1980 and Bray 1987), especially considering the occurrence of the species (based on morphology) also in, for example, beryciform, gadiform, lophiiform, salmoniform and pleuronectiform fish (Palm 2004). According to Jakob and Palm (2006), a common feeding ecology, and not phylogeny, has the greatest influence on the infestation patterns in the teleost intermediate host. The authors recorded *T. coryphaenae* from the epipelagic and bathypelagic aulopiform *Alepisaurus ferox* from the same region, migrating from near the surface to below 1,000 m and described extensive vertical distribution patterns of tentacullariid trypanorhynchs at the southern Java coast (*T. coryphaenae*, *Mixonybelinia lepturi*). Such movements can be confirmed on the basis of the genetic identification of *T. coryphaenae* in fish hosts with an overlapping depth range between 0 and 885 m from the Atlantic and Indian Ocean collected in different years. Extensive migration of the second and paratenic intermediate hosts greatly facilitates the dispersal of a coastal to epipelagic *T. coryphaenae* (based on the final host distribution) horizontally and vertically into the deep-sea (also see Palm and Klimpel 2007b).

T. coryphaenae was recorded in the adult form from 11 shark species and in the larval form from several elasmobranchs and over 60 different teleost hosts worldwide. The main final hosts are carcharhiniforms with a further record in the lamniform *Carcharodon carcharias* (great white shark). The plerocercoid was collected from different squaliforms and a rajiform, and from several squid and teleost hosts. Though oceanic scombrids are within the typical host range for this parasite, a wide range of other pelagic, but also benthic and benthopelagic fish become infected with this parasite (see Palm 2004). The lack of genetic variation in the sampled specimens suggests a cosmopolitan distribution and that host records of *T. coryphaenae* also from other carcharhinid and sphyrnid sharks (as based on morphology) might belong to the same species. Utilizing the classification scheme for host specificity that was introduced by Euzet and Combes (1980) and extended by Bray (1987), *T. coryphaenae* would be characterized as a predominantly stenoxenic species in the final host.

The purpose of this study was to provide the first genetic identification of the morphotype of *T. coryphaenae* from different intermediate hosts and regions. LSU rDNA and mtDNA (cox1–16S) both appeared useful to confirm the species identity. Further molecular studies, including *T. coryphaenae* from the Pacific and from other intermediate hosts, are needed to confirm and validate further the given results above, to search for sibling species within the *T. coryphaenae* morphotype and to genetically characterize the sequence variation within this broadly distributed trypanorhynch species. The search for other, more variable and independently evolving genes (regions) will enable a better understanding of the genotypic and phenotypic complexity (Palm and Jakob, submitted for publication) within this group of tapeworms. This will enable the comparison of *T. coryphaenae* to other trypanorhynchs that have different life cycles, which probably underlie a different rate of genetic change dictated by ecology and/or phylogeny.

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References

Abe N, Ohya N, Yanagiguchi R (2005) Molecular characterization of *Anisakis pegreffii* larvae in Pacific cod in Japan. J Helminthol 79:303–306

Beveridge I, Campbell RA (2005) Three new genera of trypanorhynch cestodes from Australian elasmobranch fishes. Syst Parasitol 60:211–224

Beveridge I, Justine JL (2006) Gilquiniid cestodes (Trypanorhyncha) from elasmobranch fishes off New Caledonia with descriptions of two new genera and a new species. Syst Parasitol 65:235–249

Bray RA (1987) A revision of the family Zoogonidae Odhner, 1902 (Platyhelminthes: Digenea): subfamily Lepidophyllinae and comments on some aspects of biology. Syst Parasitol 9:83–123

Campbell RA, Beveridge I (1994) Order Trypanorhyncha Diesing, 1863. In: Khalil LF, Jones A, Bray RA (eds) Keys to the cestode parasites of vertebrates. CAB International, Wallingford, pp 51–148

Euzet L, Combes C (1980) Les problèmes de l'espèce chez les animaux parasites. In: Bocquet C, Génermont J, Lamotte M (eds) Les problèmes de l'espèce dans les règne animale. Mémoires de la Société Zoologique Française 40:239–285

Froese R, Pauly D (eds) (2006) Fishbase. World Wide Web electronic publication. http://www.fishbase.org, version (05/2006)

Jakob E, Palm HW (2006) Parasites of commercially important fish species from the southern Java coast, Indonesia, including the distribution pattern of trypanorhynch cestodes. Verhandlungen der Gesellschaft für Ichthyologie 5:165–191

Maddison WP, Maddison DR (1992) MacClade: analysis of phylogeny and character evolution. Sinauer Associates, Sunderland, Massachusetts

Marques JF, Cabral HN, Busi M, D'Amelio S (2006) Molecular identification of *Anisakis* species from Pleuronectiformes off the Portuguese coast. J Helminthol 80:47–51

Mattiucci S, Nascetti G, Clanchi R, Paggi L, Arduino P, Margolis L, Brattey J, Webb SC, D'Amelio S, Orecchia P, Bullini L (1997) Genetic and ecological data on the *Anisakis simplex* complex with evidence for a new species (Nematoda, Ascaridoidea, Anisakidae). J Parasitol 83:401–416

Olson PD, Littlewood DTJ, Bray RA, Mariaux J (2001) Interrelationships and evolution of the tapeworms (Platyhelminthes: Cestoda). Mol Phylogenet Evol 19:443–467

Palm HW (2004) The Trypanorhyncha Diesing, 1863. PKSPL-IPB Press, Bogor, x+710 pp

Palm HW, Klimpel S (2007a) Evolution of parasitic life in the ocean. Trends Parasitol (in press)

Palm HW, Klimpel S (2007b) Metazoan fish parasites of *Macrourus berglax* Lacepède, 1801 and other rattails in the North Atlantic: exploration of the deep-sea from the continental shelf. Deep Sea Res II (in press)

Rosas-Valdez R, Choudhury A, de Leon GP (2004) Phylogenetic analysis of Corallobothriinae (Cestoda: Proteocephalidea) from north American ictalurid fishes, using partial sequences of the 28S ribosomal gene. J Parasitol 90:1123–1127

Sin FYT, Waterman PB, Blair D (1992) Morphological and enzyme variations of marine tapeworms, *Hepatoxylon trichiuri* (Cestoda: Trypanorhyncha) and *H. megacephalum*, off Kaikoura coast, New Zealand. J Nat Hist 26:465–477

Swofford DL (2002) PAUP* 4.0: Phylogenetic Analysis Using Parsimony (*and other methods). Sinauer Associates, Sunderland, MA

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882

Tkach VV, Littlewood DTJ, Olson PD, Kinsella JM, Swiderski Z (2003) Molecular phylogenetic analysis of the Microphalloidea Ward, 1901 (Trematoda: Digenea). Syst Parasitol 56:1–15

Van der Auwera G, Chapelle S, De Wachter R (1994) Structure of the large ribosomal subunit RNA of *Phytophthora megasperma*, and phylogeny of the oomycetes. FEBS Lett 338:133–136