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Morphological and complete mitogenomic characterisation of the acanthocephalan *Polymorphus minutus* infecting the duck *Anas platyrhynchos*

Huda Sarwar[1,2], **Wen-ting Zhao**[1], **Caroline Jepkorir Kibet**[1], **Jiljí Sitko**[3] and **Pin Nie**[1,4]

[1] State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China;

[2] University of Chinese Academy of Sciences, Beijing, China;

[3] Comenius Museum, Přerov, Czech Republic;

[4] School of Marine Science and Engineering, Qingdao Agriculture University, Qingdao, China

Abstract: Morphological characteristics of the acanthocephalan *Polymorphus minutus* (Goeze, 1782), which was collected from the duck *Anas platyrhynchos* Linnaeus in the Czech Republic, are described. The mitochondrial (mt) genome of *P. minutus* was sequenced, with a total length of 14,149 bp, comprising 36 genes including 12 protein coding genes (PCGs), 22 transfer RNA (tRNA) genes and two ribosomal RNA genes (*rrnL* and *rrnS*). This genome is similar to the mt genomes of other syndermatan species. All these genes were encoded on the same DNA strand and in the same orientation. The overall nucleotide composition of the *P. minutus* mt genome was 38.2% T, 27.3% G, 26.2% A, and 8.3% C. The amino acid sequences of 12 PCGs for mt genomes of 28 platyzoans, including *P. minutus*, were used for phylogenetic analysis, and the resulting topology recovers *P. minutus* as sister to *Southwellina hispida* (Van Cleave, 1925), and the two taxa form a sister clade to *Centrorhynchus aluconis* (Müller, 1780) and *Plagiorhynchus transversus* (Rudolphi, 1819), which are all species in the Palaeacanthocephala, thus supporting the monophyly of this class.

Keywords: Acanthocephala, Palaeanthocephala, morphological characteristics, mitochondrial genome, Polymorphida

Acanthocephalans are widely distributed obligate endoparasites with a total of about 1,300 species, and they use arthropods, including insects and crustaceans, as intermediate hosts. They can be found in all classes of vertebrates as final hosts, or occasionally as paratenic hosts (Bush et al. 2000, Amin 2013). Four classes, i.e. Archiacanthocephala, Eoacanthocephala, Palaeacanthocephala, and Polyacanthocephala, are recognised in terms of morphological traits such as the position of the lacunar system, the shape and size of the proboscis, the number of hooks, ligament sacs in females, cement glands in males, and even host taxonomy and ecology (Bullock 1969, Amin 1985, 1987, Crompton and Nickol 1985). In the Palaeacanthocephala, three orders, Echinorhynchida, Polymorphida and Heteramorphida, are recognised, with the highest species richness representing 65% of all acanthocephalan species, and a wide range of definitive hosts, including fish, birds and mammals, and malacostracans such as isopods and amphipods serving as intermediate hosts in this particular class of acanthocephalans (Near et al. 1998, Amin 2013, Goater et al. 2014, Gazi et al. 2016).

Polymorphus minutus (Goeze, 1782) in the Polymorphida of the Palaeacanthocephala is reported in aquatic birds from the order Anseriformes, and its larval stages are frequently recorded in marine- and freshwater gammarids, although a wide spectrum of definitive bird hosts is reported in other orders, such as Galliformes, Gruiformes, Alciformes, Charadriiformes, etc. (Crompton and Harrison 1965, Grabda 1971, Sulgostowska 1997, Pojmańska et al. 2007). Surprisingly, *P. minutus* is reported occasionally in other vertebrates, including raccoon, *Procyon lotor* (Linnaeus), European water-shrew, *Neomys fodiens* (Pennant), Arctic fox, *Vulpes lagopus* (Linnaeus), and muskrat *Ondatra zibethicus* (Linnaeus) (Mituch 1964, Crompton and Harrison 1965, Rausch et al. 1990, Piróg et al. 2018).

Mitochondrial (mt) genomes represent a useful source of data to test the monophyly of Palaeacanthocephala and Archiacanthocephala, and the validity of Syndermata (Acanthocephala and Rotifera) was confirmed through mt genome analyses (Pan and Nie 2013, Sielaff et al. 2016). Numerous studies have found that acanthocephalans in the Palaeacanthocephala and Archiacanthocephala are both

Address for correspondence: P. Nie, School of Marine Science and Engineering, Qingdao Agricultural University, Qingdao, Shandong Province, 266109, China. E-mail addresses: pinnie@qau.edu.cn

monophyletic, as supported by morphological and molecular data (Monks 2001, Near 2002, García-Varela and Nadler 2006, Verweyen et al. 2011, Amin 2013, Gazi et al. 2016), but phylogenetic analysis based specifically on SSU rDNA data supported non-monophyly (Herlyn et al. 2003).

In this study, the ratio of the proboscis length to body length of *P. minutus* from *Anas platyrhynchos* Linnaeus was further studied, and its complete mt genome was sequenced for phylogenetic analyses. The mt genome was analysed and the phylogeny of acanthocephalans based on comparative analysis of available acanthocephalan complete mt genomes was constructed to provide a view on their phylogenetic relationships.

MATERIALS AND METHODS

Specimen collection and morphological examination

Specimens of *Polymorphus minutus* were collected from intestines of *Anas platyrhynchos* in Záhlinice, Czech Republic (49.2891N, 17.4798E). They were then washed with 0.9% saline and fixed in 95% alcohol for further use. For light microscopy, the specimens were stained with iron acetocarmine solution, destained in 70% hydrochloric acid alcohol, dehydrated in a series of gradually increasing percentages of ethanol, 70%, 80%, 95% and 100%, cleared in clove oil and mounted in Canada balsam. Measurements were taken under BH-2 Olympus microscope; length measurements are given before width, with the mean followed by the range. Specimens are deposited at the Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei Province, China (No. 201601).

DNA extraction

Prior to mtDNA extraction, parasites were washed thoroughly with water and then stored in 95% ethanol at -80 °C, extracted by using Abcam Mitochondrial DNA extraction kit (ab65321; Abcam Trading, Shanghai), with suspended tissues in 1x Cytosol Extraction Buffer followed by treatment with Mitochondrial Lysis buffer and Enzyme Mix (lyophilised) according to manufacturer's instruction.

Amplification and sequencing

Initially, fragments of genes, including *cox1*, *rrnL*, *nad4* and *rrnS*, were amplified with degenerated primers. Based on these conserved regions, specific primers were designed to amplify long fragments using LA taq (TaKaRa) in a 25 ml volume of reaction mixture containing 0.5 µM of each primer 2.5 mM of dNTP mixture, 0.8 µg Template, TaKaRa LA Taq (5 units/µl) 0.25 µl and 10 × LA PCR Buffer ll (Mg^{2+}plus) 2.5 µl with the following amplification conditions for above four partial gene fragments: 94 °C for 2 min; 35 cycles of 94 °C for 30 s, 50 °C for 30 s, annealing at 72 °C for 45 s; extension at 72 °C for 10 min, and the following conditions for long PCR products: initial denaturation at 94 °C for 3 min; 34 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s; extension at 72 °C for 10 min. PCR products were purified using omega gel Extraction Kit (omega) and ligated in pMD18-T vector (TaKaRa), then transformed into competent *Escherichia coli* strain top10. The recombinant clones were selected and sequenced using a Big Dye Terminators Cycle-Sequencing Kit (ABi) in both directions by primer walking method. Sequence data were anatomised using the seqMan program from DNASTAR (http://www.DNASTAR.com/). The complete mt genome sequence of *P. minutus* was submitted to the GenBank database with accession number MN646175. The sequence of primers and the size of PCR products are shown in Table 1.

Gene annotation and alignment

The protein-coding genes (PCGs) of *P. minutus* in the mt genome were analysed by NCBI ORF Finder. The two ribosomal RNA genes, *rrn*S and *rrn*L, were confirmed based on the comparison of gene boundaries with other acanthocephalan mt genomes. The secondary structures of 22 tRNA genes were identified and drawn by tRNAScan-SE program (Lowe and Eddy 1997), DOGMA (Wyman et al. 2004), ARWEN (Laslett and Canbäck 2008) and MITOS (available at http://mitos.bioinf.uni-leipzig.de) (Bernt et al. 2013) and reconfirmed manually. Nucleotide composition data such as A + T content were used for drawing graph in R 3.6.1. Relative synonymous codon usage (RSCU) for 12 PCGs of 10 acanthocephalans was drawn in ggplot2. BLASTn analysis was performed by using mt genome data of 28 platyzoans from

Table 1. PCR primers used for cloning mitochondrial genome of *Polymorphus minutus* (Goeze, 1782).

Primer	DNA sequence (5'–3')	Product size	Reference
cox1F-661	AGTTCTAATCATAARGATATYGG	680 bp	Folmer et al. (1994)
cox1R-661	TAAACTTCAGGGTGACCAAAAAATCA		
PMAcan-rrnLF	GACYGTRCTWAGGTAGCRTRATC	320 bp	Gazi et al. (2015)
PMAcan-rrnLR	AWRDRATRATCCAACATCGAGGTA		
PMnad4-F	CCTAARGTKCATGTNGARGC	220 bp	This study
PMnad4-R	GVAMTACHGAHGARTAHGCHAC		
PMrrnS-F	GATTAGAWACCYDKGTAR	280 bp	This study
PMrrnS-R	TGACGGGCRATATGTACT		
cox1-rrnLF	GGAGGGGTAGGGTGAACTATG	2.1 kb	This study
cox1-rrnLR	CAAGGATGATCCAACATCGAGGTA		
rrnL-nad4F	GACTGTGCTAAGGTAGCATAATA	4.0 kb	This study
rrnL-nad4R	CTAACCAACTCACCTCCCCCC		
nad4-rrnSF	TTTATGGCGGATACGGGGGGAG	5.2 kb	This study
nad4-rrnSR	ATCCTAACCAAGTCCCCCTGCC		
rrnS-cox1F	TAGATACCTGTGTAGTCCTATGAAA	3.5 kb	This study
rrnS-cox1R	GCAGCAGCCAAAACAGGAATA		

Table 2. The species names and their mitochondrial genome GenBank accession numbers used in phylogenetic analyses in this study (29 species).

Species name	Class	Accession No.	Species name	Class	Accession No.
Platyhelminthes (15 species)			Syndermata (13 species)		
Benedenia seriolae	Monogenea	NC_014291.1	*Oncicola luehei*	Acanthocephala	NC_016754.1
Benedenia hoshinai	Monogenea	NC_014591.1	*Macracanthorhynchus hirudinaceus*	Acanthocephala	FR856886.2
Gyrodactylus salaris	Monogenea	NC_008815.1	*Leptorhynchoides thecatus*	Acanthocephala	NC_006892.1
Gyrodactylus gurleyi	Monogenea	KU659806.1	*Echinorhynchus truttae*	Acanthocephala	FR856883.1
Gyrodactylus nyanzae	Monogenea	MG970256.1	*Southwellina hispida*	Acanthocephala	KJ869251.1
Microcotyle sebastis	Monogenea	NC_009055.1	***Polymorphus minutus****	Acanthocephala	**MN646175**
Polylabris halichoeres	Monogenea	NC_016057.1	*Centrorhynchus aluconis*	Acanthocephala	KT592357.1
Fasciola hepatica	Trematoda	NC_002546.1	*Plagiorhynchus transversus*	Acanthocephala	KT447549.1
Brachycladium goliath	Trematoda	KR703278.1	*Paratenuisentis ambiguus*	Acanthocephala	FR856885.2
Fasciola gigantica	Trematoda	MH621335.1	*Pallisentis celatus*	Acanthocephala	JQ943583.1
Paragonimus westermani	Trematoda	AF219379.2	*Polyacanthorhynchus caballeroi*	Acanthocephala	KT592358.1
Taenia solium	Cestoda	NC_004022.1	*Philodina citrina*	Rotifera	FR856884.1
Cladotaenia vulture	Cestoda	KU559932.1	*Rotaria rotatoria*	Rotifera	NC_013568.1
Versteria mustelae	Cestoda	MK681866.1	Arthropoda – outgroup		
Benedenia seriolae	Monogenea	NC_014291.1	*Limulus polyphemus*	Merostomata	NC_003057.1

* indicating the species whose mitochondrial genome was sequenced in the present study.

NCBI. The complete list of mt genomes and GenBank accession numbers are given in Table 2.

Phylogenetic analysis

Phylogenetic analyses were conducted in PhyloSuite based on amino acid sequences of 12 PCGs for 28 platyzoans mt genomes including 11 acanthocephalan species along with the new mt genome of *P. minutus*, with *Limulus polyfemus* (Linnaeus) (Arthropoda) chosen as outgroup. Amino acid sequences for 12 PCGs were translated using MitoTool, and were aligned using the GINS-i algorithm performed with MAFFT (Katoh and Standley 2013). Alignments were refined using Gblocks (Talavera and Castresana 2007), concatenated in PhyloSuite (Zhang et al. 2018) and then analysed phylogenetically with Bayesian Interference (BI) and Maximum likelihood (ML) methods. ML analysis was performed via RAxML 7.0.3 using the MtArt model (G + I + F) for all genes with 1,000 bootstrap replicates (Stamatakis 2006). BI analysis was performed by MrBayes 3.2.6 with parameters of Partition models for all genes (LG + I + G + F) and 2×10^7 generations with 0.25 burn-in fraction. Phylogenetic trees were visualised and annotated by iTOL.

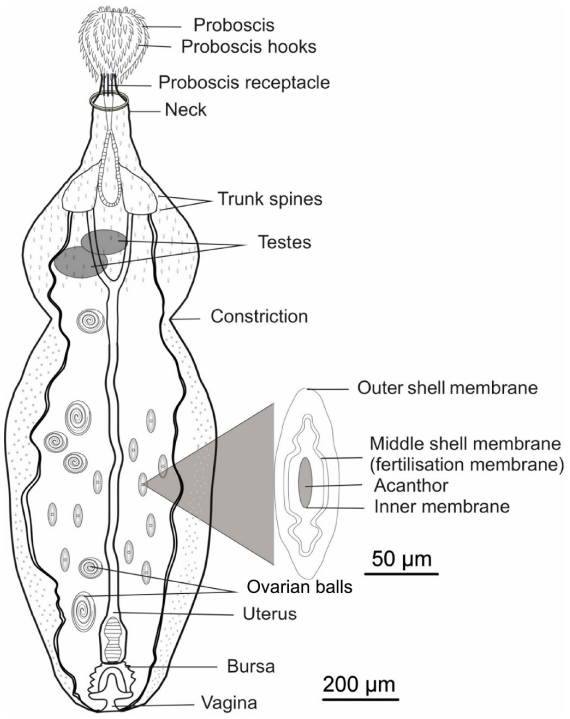

Fig. 1. Line drawing representation of whole *Polymorphus minutus* (Goeze, 1782) from *Anas platyrhynchus* Linnaeus. Elliptical egg with polar prolongations of middle membrane is showed via magnification.

RESULTS AND DISCUSSION

Morphological description of *Polymorphus minutus*

General: Parasites slightly oval, elongate, with prominent constriction observed at half body length, maximum width usually at middle of trunk. Anterior body part, before constriction, partially covered with numerous small spines 10–15 µm long. Proboscis oval, armed with 15 to 17 longitudinal rows of hooks, each with 8–10 hooks. Length of hooks smaller at base of proboscis. Proboscis receptacle much elongated and sac-like. Numerous eggs and ovarian balls present within trunk of mature females (Fig. 1).

Male (n = 3): Body 3.3–5.6 mm (4.1 mm) long, with maximum width 740–900 µm (830 µm) at middle of trunk. Proboscis 194–280 µm (240 µm) long and 132–170 µm (153 µm) wide; anterior hooks 50–90 µm (70 µm) long; medial hooks 60–80 µm (70 µm) long; posterior hooks 50–60 µm (55 µm) long. Neck 250–320 µm (284 µm) long and 100–150 µm (110 µm) wide. Proboscis receptacle 500–770 µm (656 µm) in length and 103–131 µm (119 µm) in width (Fig. 1).

Female (n = 20): Body 2.4–4.6 mm (3.2 mm) long, with maximum width 630–900 µm (749 µm) at middle of trunk. Proboscis 186–280 µm (228 µm) long and 110–170 µm (148 µm) wide; anterior hooks 50–90 µm (72 µm) long; medial hooks 55–86 µm (73 µm) long; posterior hooks

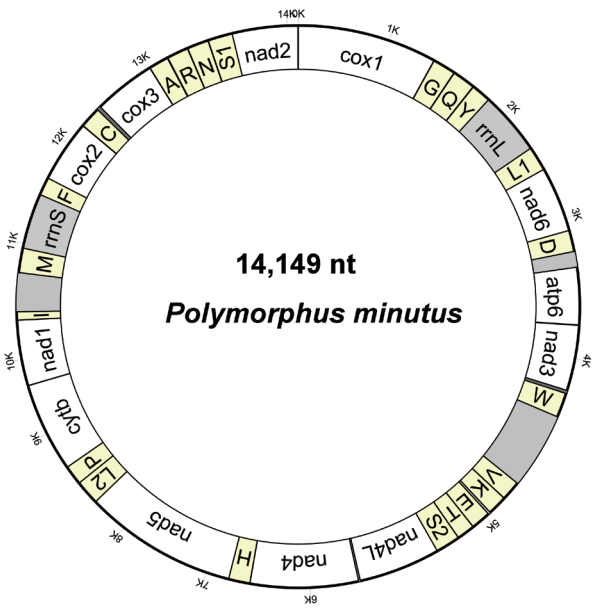

Fig. 2. Circular representation of the complete mitochondrial genome of *Polymorphus minutus* (Goeze, 1782) from *Anas platyrhynchos* Linnaeus.

40–70 µm (53 µm) long. Neck 220–320 µm (271 µm) long and 100–150 µm (110 µm) wide. Proboscis receptacle 550–850 µm (669 µm) in length and 80–150 µm (120 µm) in width. Eggs 65–119 µm (96 µm) long and 22–35 µm (30 µm) wide (Fig. 1).

The description of the proboscis observed in the present study is compatible with that of worms obtained from *Spatula querquedula* (Linnaeus), Kashmir, India, characterised by Bhattacharya (2007). The length of anterior hooks of our specimens is consistent with that from specimens in waterfowl (length 67–79 µm in McDonald 1988), *S. querquedula*, Kashmir (length 58.5 µm in Bhattacharya 2007) and *Procyon lotor* (Linnaeus), Europe (length 52–66 µm in Piróg et al. 2018). In contrast, slight differences exist in the proboscis size, proboscis/body length ratio, and hook length (Crompton and Harrison 1965, McDonald 1988, Bhattacharya 2007, Piróg et al. 2018). The ratio of the proboscis length to body length is most notable, with the ratio being 1 : 10–21 (females) and 1 : 12–23 (males) in our study, but 1 : 6 in *P. minutus* from *P. lotor* (see Piróg et al. 2018). Differences are also noticed when comparing measurements of the proboscis between the data obtained in the present study and worms in *P. lotor*,

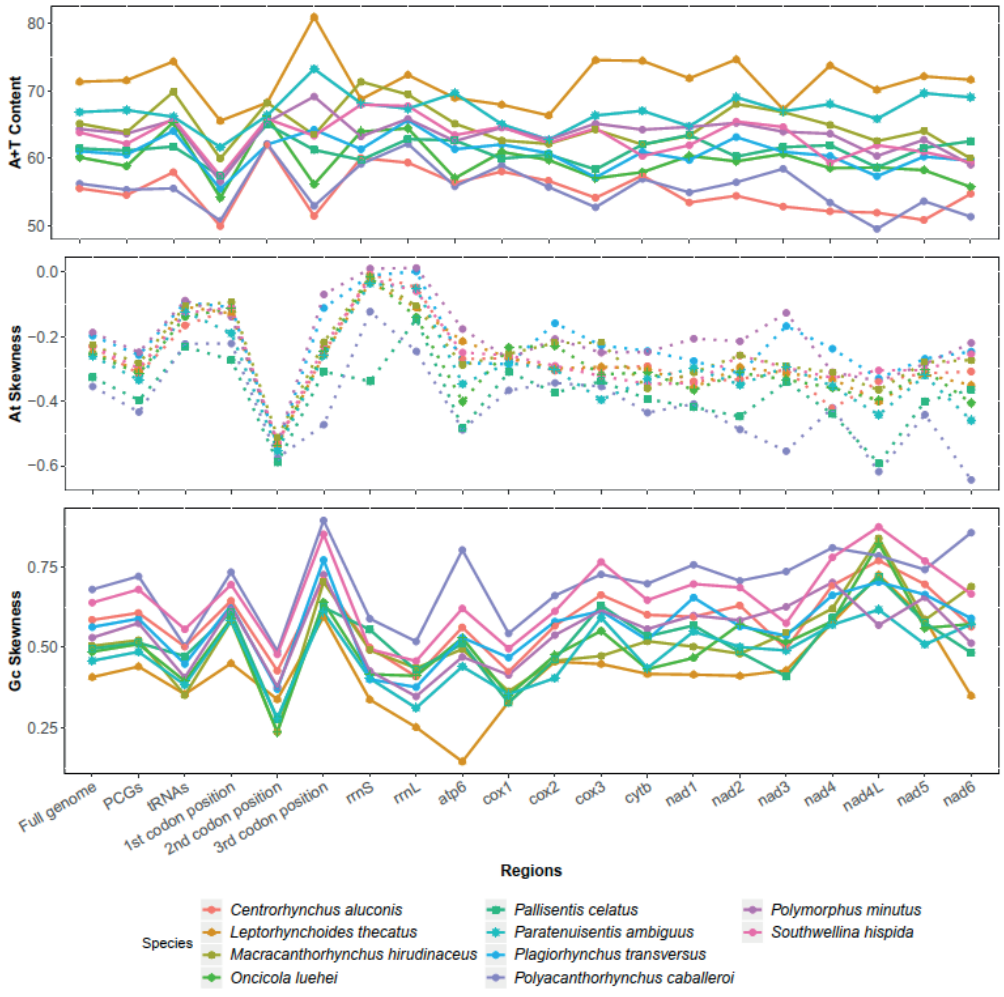

Fig. 3. A + T content and skewness of individual elements of 10 species of acanthocephalans.

i.e., 410–480 ×150 µm (Piróg et al. 2018). Furthermore, Piróg et al. (2018) did not observe decrease of hook size from the anterior to the posterior ones as observed in the present study.

Mitochondrial genome of *Polymorphus minutus*

The mt genome of *P. minutus* contains a total of 36 genes, including 12 PCGs (atp6, cytb, cox1–3, nad1–6 and nad4L), 22 transfer RNAs (tRNAs) and 2 ribosomal RNAs (rRNAs), which are on the same strand and encoded in the same direction. Gene content and gene order of PCGs are consistent with those in other mt genomes in acanthocephalans (Fig. 2) (Gissi et al. 2008). The length of mt genome in *P. minutus*, being 14,149 bp long, falls within the size range of other acanthocephalans (13,574–15,144 bp). The A + T content, which is 64.4% in *P. minutus*, is similar to that in mt genomes of other acanthocephalans, i.e., 63.9% in *Southwellina hispida* (Van Cleave, 1925) (14,742 bp in size) and 64.9% in *Macracanthorhynchus hirudinaceus* (Pallas, 1781) (14,282 bp), and 55.6% in *Centrorhynchus aluconis* (Müller, 1780) (15,144 bp), even 71.46% in *Leptorhynchoides thecatus* (Linton, 1891) (13,888 bp). The nucleotide composition in *P. minutus* is biased towards T, with higher T content (39.8% T) in 12 PCGs corresponding to relatively high frequency of T-rich codons (Fig. 3; Supplementary Table 1) (Steinauer et al. 2005, Gazi et al. 2012, Weber et al. 2013). Unequal usage of synonymous codon is contributing factor describing nucleotide composition toward A + T content. Complete descriptions of A + T content and AT skewness of acanthocephalans are compared for the first time. Nucleotide skewness of the mt genome (including all elements) in *P. minutus* exhibits a pattern as observed in mt genomes of other acanthocephalans (Fig. 3).

The 12 PCGs (excluding *atp8*) were distinguished in the *P. minutus* mt genome. The *nad5* (1,647 bp) and *cox1* (1,536 bp) are largest in size, while *nad4L* (270 bp) is the smallest. The usage of start and termination codons among acanthocephalan members varies greatly depending on genes, and there is no remarkable diagnostic pattern. The start codons, such as ATG, GTG, ATA, and stop codons, including TAG, TAA, T (incomplete) were observed in the mt genome of *P. minutus*. Truncated stop codons are common in other acanthocephalans and among bilaterian mitochondrial PCGs, which would probably be completed through post transcriptional polyadenylation (Ojala et al. 1981, Steinauer et al. 2005, Pan and Nie 2013, Gazi et al. 2012, 2016). Details of initiation and termination codons of 12 PCGs are shown in Supplementary Table 2.

The PCGs of the *P. minutus* mt genome are fundamentally encoded by T-rich codons (more than two Ts in a triplet), as for some other invertebrates, including acanthocephalans, nematodes, molluscs, dipteran insects and rotifers (Okimoto et al. 1992, Kurabayashi and Ueshima 2000, Lessinger et al. 2000, Steinauer et al. 2005, Min and Park 2009). In the *P. minutus* mt genome, valine (encoded by GTN; 15.99%), leucine 2 (encoded by TTR; 12.96%) and glycine (encoded by GGN; 11.44%) were the most common, accounting for 40.4% of total amino acid components (Supplementary Table 3). Valine (encoded by GTN) was most abundant (15.99%) of total amino acid composition in the *P. minutus* mt genome, which is also observed in *C. aluconis* (17.67%), *Polyacanthorhynchus caballeroi* Diaz-Ungria et Rodrigo, 1960 (19.68%), *Oncicola luehei* (Travassos, 1917) (18.24%), *M. hirudinaceus* (15.48%) and *S. hispida* (16.46%) (Supplementary Table 3, Supplementary Figure).

The *rrnL* gene is 917 bp in length flanked by *trnY* and *trnL1*, similar to *L. thecatus* and *O. luehei*. The *rrnS* gene is 581 bp in length, and is situated between *trnM* and *trnF*. Twenty two tRNA genes, ranging in size from 48 bp to 61 bp, folded into cloverleaf-like secondary structure with the exception of *trnS1* and *trnQ*, which lacks a dihydrouridine (DHU) arm, whereas the remaining 20 tRNAs lack TΨC arm and hold a TV-replacement loop instead (Fig. 4), similar to those in other acanthocephalan mt genomes reported so far (Gazi et al. 2016).

Pattern of the mitochondrial gene order

The gene arrays of four species, *S. hispida*, *P. minutus*, *C. aluconis* and *Plagiorhynchus transversus* (Rudolphi, 1819) in the order Polymorphida are almost identical. Three tRNA genes, *trnV*, *trnS1*, *trnS2*, were translocated in *P. minutus* (Polymorphidae) and *S. hispida* (Polymorphidae), while only *trnS2* was transferred in the mt genomes of *P. minutus* (Polymorphidae) and *P. caballeroi* (Polyacanthocephala). The results are contrary to the previous reports that same gene arrangements of *P. caballeroi* in relation to *Pallisentis celatus* (Van Cleave, 1928) reveal the sister relationship between the Eoacanthocephala and Polyacanthocephala (Fig. 5) (Gazi et al. 2016).

The specific gene order in species in the Polymorphida further reveals the monophyletic foundation concordant with the monophyletic cluster recovered from genome sequence analysis (Gazi et al. 2016). Three conserved gene clusters, *nad2-cox1-trnG*, *trnY-rrnL-trnL2-nad6*, and *nad4L-nad4-trnH-nad5*, exist among all members of the Lemniscea (Bdelloida + Acanthocephala), in spite of the striking difference in the arrangement of tRNAs (Fig. 5). The tRNA translocations are also observed in nematodes, molluscs, tunicates and crustacean arthropods (Boore et al. 2004, Tang and Hyman 2005, Kilpert and Podsiadlowski 2006, Vallès and Boore 2006, Stach et al. 2010, Hyman et al. 2011).

Phylogeny of the Acanthocephala

ML and BI methods were used to determine evolutionary relationship among the classes in the Acanthocephala by constructing mt genome phylogeny from amino acid sequences of 12 PCGs, although *Echinorhynchus truttae* Schrank, 1788 lacks *nad4* and *nad4L* in the GenBank database, from 28 species belonging to the Platyzoa, including the newly sequenced mt genome of *P. minutus*. The generated trees with identical topologies had minor dissimilarities in supporting values for the Polymorphida (Fig. 6). The basic position of four classes in the Acanthocephala, was consistent with previously published phylogenetic studies: monophyletic Archiacanthocephala, the earliest

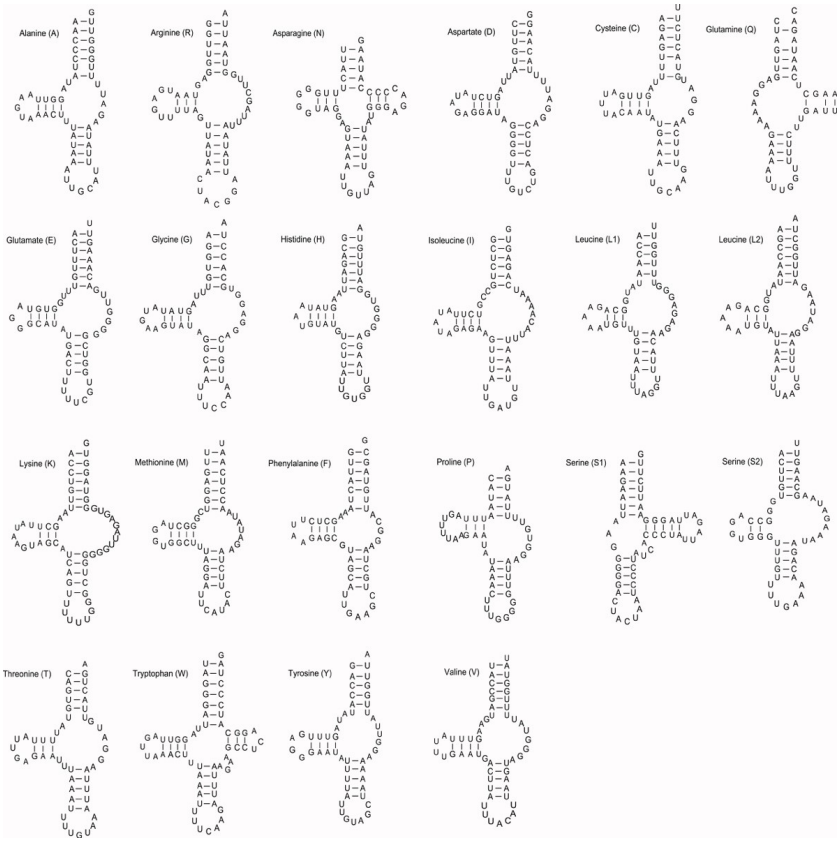

Fig. 4. The predicted secondary structure of 22 tRNAs of *Polymorphus minutus* (Goeze, 1782) from *Anas platyrhynchos* Linnaeus.

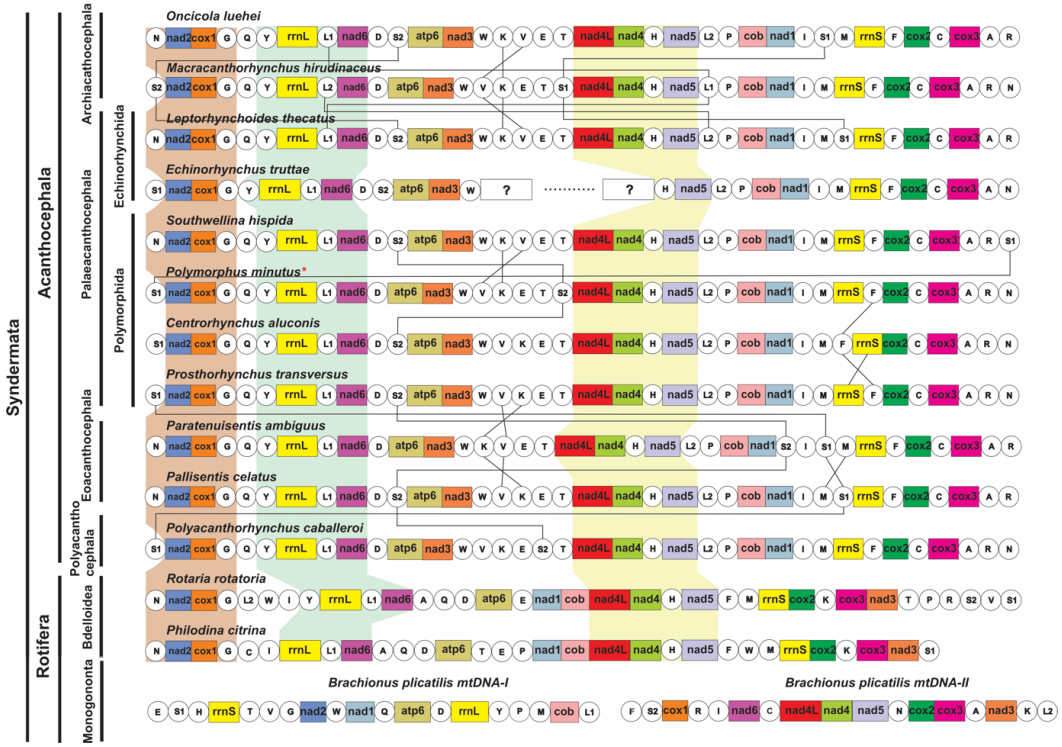

Fig. 5. Comparison of the linearised mitochondrial genome arrangements for 14 syndermatans, including 11 acanthocephalans and 3 rotiferan species. All genes are transcribed in the same direction from left to right. The asterisk (*) indicates the sequence of *Polymorphus minutus* (Goeze, 1782) from *Anas platyrhynchos* Linnaeus, which is newly characterised in this study. Boxes with (?) symbolise sequence regions with no gene annotation provided in previous studies. The shadowed regions highlighted in different colours represent common gene clusters. The tRNAs are shown in single letter abbreviations of an amino acid code.

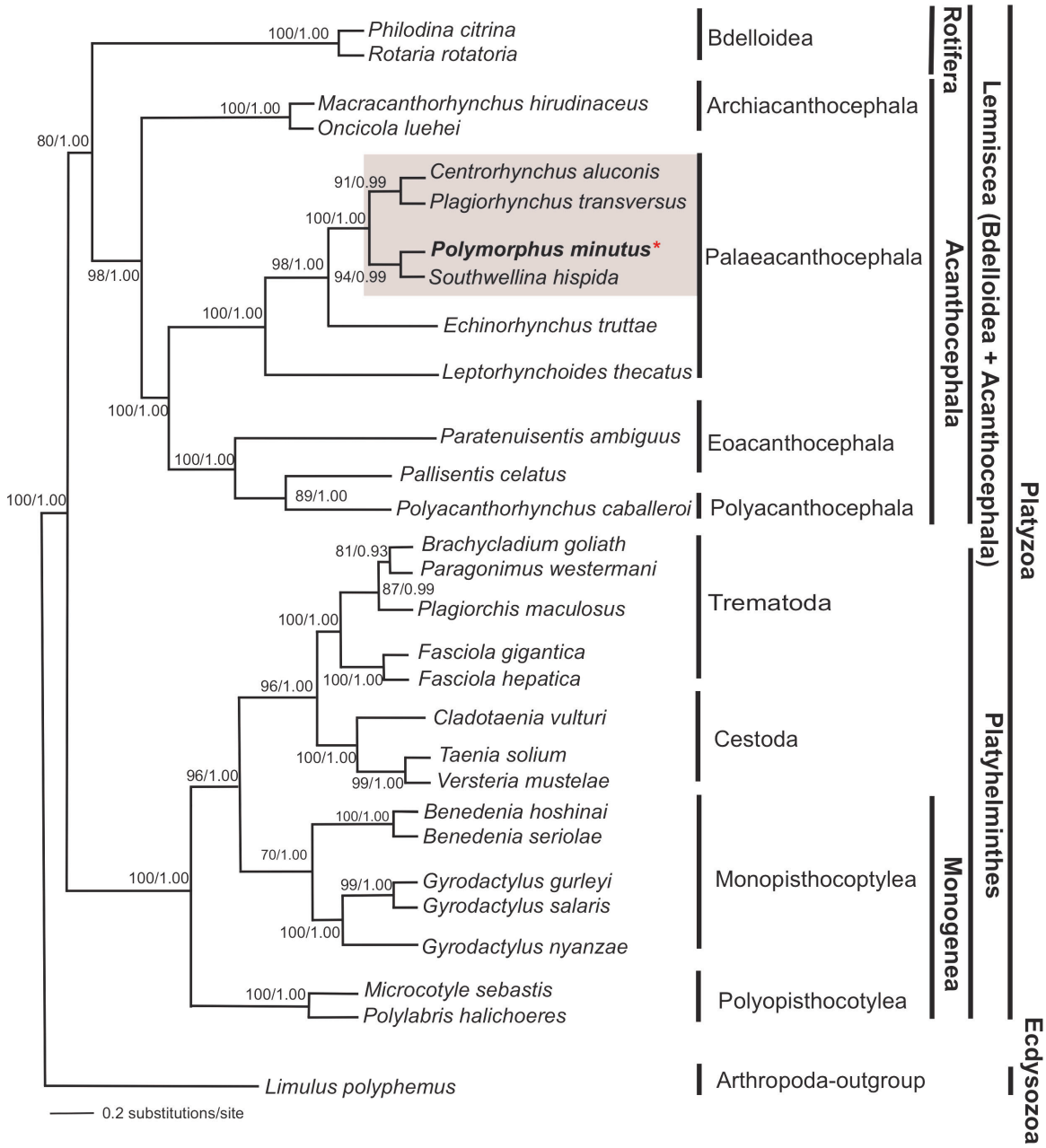

Fig. 6. Phylogenetic tree resulting from maximum likelihood for 12 protein-coding genes for 28 Platyzoan and 1 Arthropoda (outgroup) mitochondrial genomes. The asterisk (*) in red indicates the sequence of *Polymorphus minutus* (Goeze, 1782) from *Anas platyrhynchos* Linnaeus. The values indicated near the branches are the bootstrap percentages (BP) and Bayesian posterior probability (BPP) values for Maximum likelihood and Bayesian analysis, respectively.

branching clade of the Acanthocepala and monophyly of the Eoacanthocephala and Polyacanthocephala are rejected. It is notable to mention that the Palaeacanthocephala has close relationship with the Eoacanthocephala, which was concordant with previous findings based on morphological and molecular data such as SSU rDNA, LSUrDNA and mtDNA *cox1* (García-Varela et al. 2000, 2002, García-Varela and Nadler 2005, 2006, Verweyen et al. 2011, Gazi et al. 2012, 2015, 2016). *Polymorphus minutus* clusters with *S. hispida* forming a sister clade to *C. aluconis* and *P. transversus* in the Palaeacanthocephala.

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REFERENCES

Amin O.M. 1985: Classification. In: D.W.T. Crompton and B.B. Nickol (Eds.), Biology of the Acanthocephala. Cambridge University Press, London, pp. 27–71.

Amin O.M. 1987: Key to the families and subfamilies of Acanthocephala, with the erection of a new class (Polyacanthocephala) and a new order (Polyacanthorhynchida). J. Parasitol. 73: 1216–1219.

Amin O.M. 2013: Classification of the Acanthocephala. Folia Parasitol. 60: 273–305.

Bernt M., Bleidorn C., Braband A., Dambach J., Donath A., Fritzsch G., Golombek A., Hadrys H., Jühling F., Meusemann K., Middendor M., Misof B., Perseke M., Podsiadlowski L., von Reumont B., Schierwater B., Schlegel M., Schrödl M., Simon S., Stadler P.F., Stöger I., Struck T.H. 2013: A comprehensive analysis of bilaterian mitochondrial genomes and phylogeny. Mol. Phylogenet. Evol. 69: 352–364.

Bhattacharya S.B. 2007: Handbook on Indian Acanthocephala. Zool. Surv. India, Kolkata, 225 pp.

Boore J., Medina M., Rosenberg L.A. 2004: Complete sequences of the highly rearranged molluscan mitochondrial genomes of the scaphopod *Graptacme eborea* and the bivalve *Mytilus edulis*. Mol. Biol. Evol. 21: 1492–1503.

Bullock W.L. 1969: Morphological features as tools and pitfalls in acanthocephalan systematics. In: G.L. Schmidt (Ed.), Problems in Systematics of Parasites. University Park Press, Baltimore, pp. 9–45.

Bush A.O., Jacqueline C.F., Esch G.W., Seed J.R. 2000: Parasitism: Diversity and Ecology of Animal Parasites. Cambridge University Press, Cambridge, 566 pp.

Crompton D.W.T., Harrison J.G. 1965: Observations on *Polymorphus minutus* (Goeze, 1782) (Acanthocephala) from a wildfowl reserve in Kent. Parasitology 55: 345–355.

Crompton D.W.T., Nickol B.B. 1985: Biology of the Acanthocephala. Adv. Parasitol. 5: 205–246.

Folmer O., Black M., Hoeh W., Lutz R., Vrijenhoek R. 1994: DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol 3: 294–299.

García-Varela M., Cummings M.P., Pérez-Ponce de León G., Gardner S.L., Laclette J.P. 2002: Phylogenetic analysis based on 18S ribosomal RNA gene sequences supports the existence of class Polyacanthocephala (Acanthocephala). Mol. Phylogenet. Evol. 23: 288–292.

García-Varela M., Nadler S.A. 2005: Phylogenetic relationships of Palaeacanthocephala (Acanthocephala) inferred from SSU and LSU rDNA gene sequences. J. Parasitol. 91: 1401–1409.

García-Varela M., Nadler S.A. 2006: Phylogenetic relationships among Syndermata inferred from nuclear and mitochondrial gene sequences. Mol. Phylogenet. Evol. 40: 61–72.

García-Varela M., Pérez–Ponce de León G., de la Torre P., Cummings M.P., Sarma SS., Laclette J.P. 2000: Phylogenetic relationships of Acanthocephala based on analysis of 18S ribosomal RNA gene sequences. J. Mol. Evol. 50: 532–540.

Gazi M., Kim J., García-Varela M., Park C., Littlewood D.T.J., Park J.K. 2016: Mitogenomic phylogeny of Acanthocephala reveals novel class relationships. Zool. Scr. 45: 437–454.

Gazi M., Kim J., Park J.K. 2015: The complete mitochondrial genome sequence of *Southwellina hispida* supports monophyly of Palaeacanthocephala (Acanthocephala: Polymorphida). Parasitol. Int. 64: 64–68.

Gazi M., Sultana T., Min G.S., Park Y.C., García-Varela M., Nadler S.A., Park J.K. 2012: The complete mitochondrial genome sequence of *Oncicola luehei* (Acanthocephala: Archiacanthocephala) and its phylogenetic position within Syndermata. Parasitol. Int. 61: 307–316.

Gissi C., Iannelli F., Pesole G. 2008: Evolution of the mitochondrial genome of Metazoa as exemplified by comparison of congeneric species. Heredity 101: 301–320.

Goater T.M., Goater C., Esch G. 2014: Parasitism: the Diversity and Ecology of Animal Parasites. Cambridge University Press, Cambridge, 497 pp.

Grabda J. 1971: [Catalogue of parasitic fauna of Poland. Part II. Parasites of cyclostomes and fishes.] PWN, Warszawa, 40 pp. (In Polish.)

Herlyn H., Piskurek O., Schmitz J., Ehlers U., Zischler H. 2003: The syndermatan phylogeny and the evolution of acanthocephalan endoparasitism as inferred from 18S rDNA sequences. Mol. Phylogenet. Evol. 26: 155–164.

Hyman B.C., Lewis S.C., Tang S., Wu Z. 2011: Rampant gene rearrangement and haplotype hypervariation among nematode mitochondrial genomes. Genetica 139: 611–615.

Katoh K., Standley D.M. 2013: MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30: 772–780.

Kilpert F., Podsiadlowski L. 2006: The complete mitochondrial genome of the common sea slater, *Ligia oceanica* (Crustacea, Isopoda) bears a novel gene order and unusual control region features. BMC Genomics 7: 241.

Kurabayashi A., Ueshima R. 2000: Complete sequence of the mitochondrial DNA of the primitive opisthobranch gastropod *Pupa strigosa*: systematic implication of the genome organization. Mol. Biol. Evol. 17: 266–277.

Laslett D., Canbäck B. 2008: ARWEN: A program to detect tRNA genes in metazoan mitochondrial nucleotide sequences. Bioinformatics 24: 172–175.

Lessinger A.C., Martins Junqueira A.C., Lemos T.A., Kemper E.L., da Silva F.R., Vettore A.L., Arruda P., Azeredo-Espin A.M. 2000: The mitochondrial genome of the primary screwworm fly *Cochliomyia hominivorax* (Diptera: Calliphoridae). Insect Mol. Biol. 9: 521–529.

Lowe T.M., Eddy S.R. 1997: tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucl. Acids Res. 25: 955–964.

McDonald M.E. 1988: Key to Acanthocephala reported in Waterfowl. U.S. Department of the Interior, Fish and Wildlife Service. U.S. Fish and Wildlife Service, Washington, D.C., 692 pp.

Min G.S., Park J.K. 2009: Eurotatorian paraphyly: revisiting phylogenetic relationships based on the complete mitochondrial genome sequence of *Rotaria rotatoria* (Bdelloidea: Rotifera: Syndermata). BMC Genomics 10: 533.

Mituch J. 1964: Beitrag zur Erkenntnis der Helmintenfauna der Gattung *Neomys* (Insectivora) in der Slovakei. Studia Helminthologia 1: 83–104.

Monks S. 2001: Phylogeny of the Acanthocephala based on morphological characters. Syst. Parasitol. 48: 81–116.

Near T.J. 2002: Acanthocephalan phylogeny and the evolution of parasitism. Integr. Comp. Biol. 42: 668–677.

Near T.J, Garey J.R., Nadler S.A. 1998: Phylogenetic relationships of the Acanthocephala inferred from 18S ribosomal DNA sequences. Mol. Phylogenet. Evol. 10: 287–298.

Ojala D., Montoya J., Attardi G. 1981: tRNA punctuation model of RNA processing in human mitochondria. Nature 290: 470–474.

Okimoto R., Macfarlane J.L., Clary D.O., Wolstenholme D.R. 1992: The mitochondrial genomes of two nematodes, *Caenorhabditis elegans* and *Ascaris suum*. Genetics 130: 471–498.

Pan T.S., Nie P. 2013: The complete mitochondrial genome of *Pallisentis celatus* (Acanthocephala) with phylogenetic analysis of acanthocephalans and rotifers. Folia Parasitol. 60: 181–191.

Piróg A., Kuśmierek N., Popiołek M. 2018: The occurrence of avian acanthocephalan *Polymorphus minutus* (Goeze, 1782) in raccoons (*Procyon lotor* L.) introduced to Europe. Ann. Parasitol. 64: 249–252.

Pojmańska T., Niewiadomska K., Okulewicz A. 2007: [Parasitic helminths in Poland. Species, hosts, Terra Incognita.] Polish Parasitological Society, Warszawa, 18 pp. (In Polish, with summary in English.)

Rausch R.L., Fay F.H., Williamson F.S.L. 1990: The ecology of *Echinococcus multilocularis* (Cestoda: Taeniidae) on St. Lawrence Island, Alaska. II. Helminth populations in the definitive host. Ann. Parasitol. Hum. Comp. 65: 131–140.

Sielaff M., Schmidt H., Struck T.H., Rosenkranz D., Welch M.D.B., Hankeln T., Herlyn H. 2016: Phylogeny of Syndermata (syn. Rotifera): mitochondrial gene order verifies epizoic Seisonidea as sister to endoparasitic Acanthocephala within monophyletic Hemirotifera. Mol. Phylogenet. Evol. 96: 79–92.

Stach T., Braband A., Podsiadlowski L. 2010: Erosion of phylogenetic signal in tunicate mitochondrial genomes on different levels of analysis. Mol. Phylogenet. Evol. 55: 860–870.

Stamatakis A. 2006: RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690.

Steinauer M.L., Nickol B.B., Broughton R., Ortí G. 2005: First sequenced mitochondrial genome from the phylum Acanthocephala (*Leptorhynchoides thecatus*) and its phylogenetic position within Metazoa. J. Mol. Evol. 60: 706–715.

Sulgostowska T. 1997: [Catalogue of parasite fauna of Poland. IV. Parasites of birds. Acanthocephala.] Polskie Towarzystwo Parazytologiczne, Warszawa, 29 pp. (In Polish.)

Talavera G., Castresana J. 2007: Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst. Biol. 56: 564–577.

Tang S., Hyman B. 2005: Rolling circle amplification of complete nematode mitochondrial genomes. J. Nematol. 37: 236–241.

Vallès Y., Boore J.L. 2006: Lophotrochozoan mitochondrial genomes. Integr. Comp. Biol. 46: 544–557.

Verweyen L., Klimpel S., Palm H. 2011: Molecular phylogeny of the Acanthocephala (class Palaeacanthocephala) with a paraphyletic assemblage of the orders Polymorphida and Echinorhynchida. PLoS One 6: e28285.

Weber M., Wey-Fabrizius A.R., Podsiadlowski L., Witek A., Schill R.O., Sugar L., Herlyn H., Hankeln T. 2013: Phylogenetic analyses of endoparasitic Acanthocephala based on mitochondrial genomes suggest secondary loss of sensory organs. Mol. Phylogenet. Evol. 66: 182–189.

Wyman S.K., Jansen R., Boore J. 2004: Automatic annotation of organellar genomes with DOGMA. Bioinformatics 20: 3252–3255.

Zhang D., Gao F.L., Li, W.X., Jakovlić I., Zou H., Zhang J., Wang G.T. 2018: PhyloSuite: an integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Mol. Ecol. Resour. 20: 348–355.

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