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PHYLOGENETIC POSITION OF *POLYMORPHUS PHIPPSI* KOSTYLEW, 1922 AND *POLYMORPHUS MAGNUS* SKRJABIN, 1913 (PALAEACANTHOCEPHALA, POLYMORPHIDAE) ASCERTAINED ON THE BASIS OF MOLECULAR DATA

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Polymorphidae is a family of acanthocephalans, obligatory parasites with a complex life cycle involving arthropods as intermediate hosts and vertebrates of different taxa as definitive hosts. The current taxonomy of Polymorphidae seems to be equivocal. Its type genus *Polymorphus* has been shown to be polyphyletic based on molecular data. We obtained partial sequences of 28S rDNA gene and *cox1* mitochondrial gene of two species of this genus, *Polymorphus phippsi* and *P. magnus*, and used them in a reconstruction of the polymorphid phylogeny. As a result, *P. magnus* was included into the same clade as the type species of the genus, *P. minutus*, while *P. phippsi* appeared to be close to *Profilicollis* spp. The position of *P. phippsi* agrees with the polyphyly of *Polymorphus* but does not correspond to its taxonomic status based on described phenotypic characters.

Key words: Palaeacanthocephala, Polymorphidae, *Polymorphus magnus, Polymorphus phippsi,* molecular phylogeny, taxonomy

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Acanthocephala, so-called thorny-headed worms, is a small phylum of obligatory endoparasites related to the Syndermata (Ahlrichs, 1997). To date only ca. 1300 species of acanthocephalans have been described (Khokhlova, 1986; Amin, 2013; Smales, 2015).

Also known as thorny-headed worms, acanthocephalans have a complex life cycle involving the intermediate host (an arthropod) and the definitive host (a vertebrate) (Khokhlova, 1986). It was shown that several species use paratenic hosts (Sharpilo, Salamatin, 2005). Classes and major orders of the phylum Acanthocephala were considered as monophyletic taxa with strong support by molecular and morphological data (Garcia-Varela et al., 2000; Amin, 2013; Monks, 2001). However, the composition of families and genera based on morphological characters is ambiguous (Amin, 2013). Data of molecular phylogeny are contrary to taxonomy based on phenotypic characters, especially in regard to family Polymorphidae (Garcia-Varela et al., 2013).

Polymorphidae is a species-rich family related to the class Palaeacanthocephala. Its type genus *Polymorphus* Lühe, 1911 demonstrated polyphyly (Garcia-Varela et al., 2013). An assemblage of new molecular data from other *Polymorphus* spp. can yield to changes in polymorphid phylogenetic tree topology. In this study we obtained new sequences of two widespread highly pathogenic for definitive hosts (water birds) *Polymorphus* spp. – *P. phippsi* Kostylew, 1922 and *P. magnus* Skrjabin, 1913, and added them to the phylogenetic reconstruction of the Polymorphidae.

MATERIALS AND METHODS

Larvae of *P. phippsi* 2-3 were collected from naturally infected amphipods *Gammarus setosus* (Luvenga, White Sea, Russia, 11.07.2019) and juvenile *P. phippsi* 1 were collected from Herring gull *Larus argentatus* (Dalnye Zelentsy, Barents Sea, Russia, 2001) and identified using Uspenskaya (1963) guide. A subadult specimen of *P. magnus* was isolated from Slaty-backed gull *Larus schistisagus* experimentally infected with cystacanths from naturally infected *G. lacustris* collected in the tundra lake situated close to the mouth of Chaun-Pucheveem river in area of Chaun research station (Chaun Bay coast, East-Siberian sea, Russia, July 2019). Species identification was carried out basing on Khokhlova (1986) guide and our experience.

The acanthocephalans were fixed in 96% ethanol, stained in Bömer's hematoxylin (except for *P. phippsi* 1) and mounted in Canada balsam. Before staining, pieces of tissue were cut off and DNA was extracted from them using Chelex-100 procedure (De Lamballerie et al., 1992). Polymerase chain reactions (PCR) were performed with 5X ScreenMix (Evrogen, Moscow). Partial CDS of 28s rRNA were amplified using two overlapping PCR fragments of 2000–2500 bp. Primers for amplicon 1 were forward 5'-CAAGTACCGTGAGGGAAAGTTGCGC and reverse 5'-CTTCTCCAAC(T/G) TCAGTCTTCAA; amplicon 2 forward 5'-CTAAGGAGTGTGTAACAACTCACC and reverse 5'-CTTCGCAATGATAGGAAGAGCC (Garcia-Varela, Nadler, 2005). Partial CDS of cytochrome-oxydase I were amplified using forward primer 5'-AGTTCTAATCATAA(R)GATAT(Y)GG and reverse 5'-TAAACTTCAGGGTGACCAAAAAATCA (Folmer et al., 1994). PCR cycling parameters included denaturation at 94ºC for 3 min, followed by 35 cycles of denaturation at 94ºC for 1 min, annealing at 65ºC(28s)/60º(CO1) for 1 min, and elongation at 72ºC for 2 min, followed by final elongation at 72ºC for 4 min. PCR products were sequenced using Sanger's technology in the resource center "Development of molecular and cellular technology" of St. Petersburg State University. Initial sequence analysis and reads assemblage was performed using ChromasPro 1.42.

We used 28S rDNA and CO1 mtDNA alignments from Garcia-Varela et al. (2013) as a template (table 1). Both amplicons of 28s rDNA were added to the corresponding alignment and then aligned in SeaView 4 (Gouy et al., 2010). The same procedure was performed for both CO1 sequences. CO1 alignment was trimmed according to the shortest sequence, 588 bp in length. Alignment of 28S rDNA was trimmed according to the common length of two overlapping amplicons from *P. phippsi* samples, so that its length was 2966 bp. We counted optimal substitution models and phylogenetic distances using MEGA 11.0.11 (Tamura et al., 2021) for each separate alignment. After that we created a concatenate matrix using SeaView 4.

For *P. phippsi* 1 CO1 sequence and fragments of 28S rDNA for *P. phippsi* 3 and *P. magnus* was replaced with missing data, such as 28S rDNA – for *P. phippsi* 3 and *P. magnus* (table 1).

Table 1. Source information

Species	**Host**	**Locality**	**28s rDNA GenBank №**	**CO1 GenBank №**
Andracantha gravida	*Phalacrocorax auritus*	Yucatán, México	EU267814	EU267822
Arhythmorhynchus frassoni 1	*Uca spinicarpa*	Yucatán, México	JX442176	EU189484
Arhythmorhynchus frassoni 2	*Eudocimus albus*	Sinaloa, México	JX442177	JX442188
Bolbosoma turbinella	*Eschrichtius robustus*	Monterey Bay, California, USA	JX442178	JX442189
Bolbosoma sp.	*Callorhinus ursinus*	St. Paul Island, Alaska, USA	JX442179	JX442190
Corynosoma australe	*Phocarctos hookeri*	New Zealand	JX442180	JX442191
Corynosoma enhydri	*Enhydra lutris*	Monterey Bay, California, USA	AY829107	DQ089719
Corynosoma magdaleni	*Phoca hispida saimensis*	Lake Saimaa, Finland	EU267815	EF467872
Corynosoma obtuscens	*Callorhinus ursinus*	St. Paul Island, Alaska, USA	JX442181	JX442192
Corynosoma strumosum	*Phoca vitulina*	Monterey Bay, California, USA	EU267816	EF467870
Corynosoma validum	*Callorhinus ursinus*	St. Paul Island, Alaska, USA	JX442182	JX442193
Ibirhynchus dimorpha	*Eudocimus albus*	Veracruz, México	GQ981437	GQ981438
Hexaglandula corynosoma	*Nyctanassa violacea*	Veracruz, México	EU267817	EF467869

Polymorphus brevis 1	*Nycticorax nycticorax*	Michoacán, México	AY829105	DQ089717
Polymorphus brevis 2	*Nycticorax nycticorax*	Michoacán, México	JX442183	JX442194
Polymorphus minutus	*Gammarus pulex*	Dijon, France	EU267819	EF467865
Polymorphus obtusus	*Ahythya affinis*	Baja California Sur, México	JX442184	JX442195
Polymorphus trochus	*Fulica america*	Sinaloa, México	JX442185	JX442196
Profilicollis altmani	*Enhydra lutris*	Monterey Bay, California, USA	AY829108	DQ089720
Profilicollis botulus 1	*Somateria mollissima*	Denmark	EU267818	EF467862
Profilicollis botulus 2	*Anas platyrhynchos*	USA	AY829109	DQ089721
Profilicollis bullocki	*Emerita analoga*	Caleta Lenga, Chile	JX442186	JX442197
Pseudocorynosoma constrictum	*Anas clypeata*	Estado de México, México	EU267812	EU267820
Pseudocorynosoma anatarium	*Bucephala albeola*	Durango, México	EU267813	EU267821
Pseudocorynosoma sp.	*Oxyura jamaicensis*	Durango, México	JX442187	JX442198
Southwellina hispida 1	*ND*	Hallai, USA	EU267810	EF467866
Southwellina hispida 2	*Tigrisoma mexicanum*	Veracruz, México	EU267811	EF467867
Centrorhynchus sp.	*Falco peregrinus*	California, USA	AY829104	DQ089716
Gorgorhynchoides bullocki	*Eugerres plumieri*	Quintana Roo, México	AY829103	DQ089715
Plagiorhynchus cylindraceus	*Porcilio saber*	Dijon, France	AY829102	DQ089724
Polymorphus phippsi 1 (This study)	*Larus argentata*	Dalnye Zelentsy, Barents sea, Russia	OK235421 (1st amplicon)	-
Polymorphus phippsi 2 (This study)	*Gammarus setosus*	Luvenga, White sea, Russia	-	OL676091
Polymorphus phippsi 3 (This study)	*Gammarus setosus*	Luvenga, White sea, Russia	ON667751 (2nd amplicon)	OL676687
Polymorphus magnus (This study)	*Larus schistisagus*	Chaun, Chukotka, Russia	-	OL689013

Phylogenetic relationships were determined by maximum-likelihood analysis in CIPRES Science Gateway platform (Miller et al., 2010) with RaxML-HPC black box tool and by Bayesian analysis in MrBayes 3.2.7a (Ronquist et al., 2012). The following parameters were specified in Bayesian tree search: partitioned model (GTR+G and GTR+G+I for 28S rDNA and CO1, respectively) and setting outgroup identical to the taxset from Garcia-Varela et al. (2013).

RESULTS AND DISCUSSION

Interspecific genetic divergence between 28S rDNA sequences of *P. phippsi* 1 and *P. phippsi* 2 (0.028) was greater than that between the sequences from samples of one species (*Arhytmorhynchus frassoni* 0.016*, Southwellina hispida* 0.003, *Polymorphus brevis* 0.002) but smaller than the divergence between different congeneric species (average for *Corynosoma* spp. 0.06, for *Profilicollis* spp. 0.04). Divergence between *P. phippsi* 2 and *P. phippsi* 3 (0.008) was smaller than that between the sequences from samples of one species (*Arhytmorhynchus frassoni* 0.193 COI*, Southwellina hispida* 0.048, *Polymorphus brevis* 0.014). All the sequences from samples of *P. phippsi* were grouped together in a well-supported clade (fig. 1). We can conclude that the three samples were cophenetic. Overall topologies of our tree and the tree from the previous research (Garcia-Varela et al., 2013) were congruent. Bayesian and maximum-likelihood trees yielded the same topology (fig. 1). The clade containing *P. phippsi* sequences grouped with the clade containing *Profilicollis bullocki* and *Pr. altmani*, with *Pr. botulus* being basal to this clade. *P. magnus* grouped in one clade with *P. obtusus*, while *P. minutus* was basal to this clade.

The position of *P. phippsi* in our phylogenetic reconstructions supports the polyphyly of *Polymorphus* postulated by Garcia-Varela et al. (2013). The position of *P. magnus* corresponds to its accepted taxonomic status (Khokhlova, 1986). Thus, it is likely that assemblage of new molecular data can outline only more contradictions with accepted taxonomy of Polymorphidae. In this case the necessity of taxonomic characters revision, including additional data of comparative morphology, can appear.

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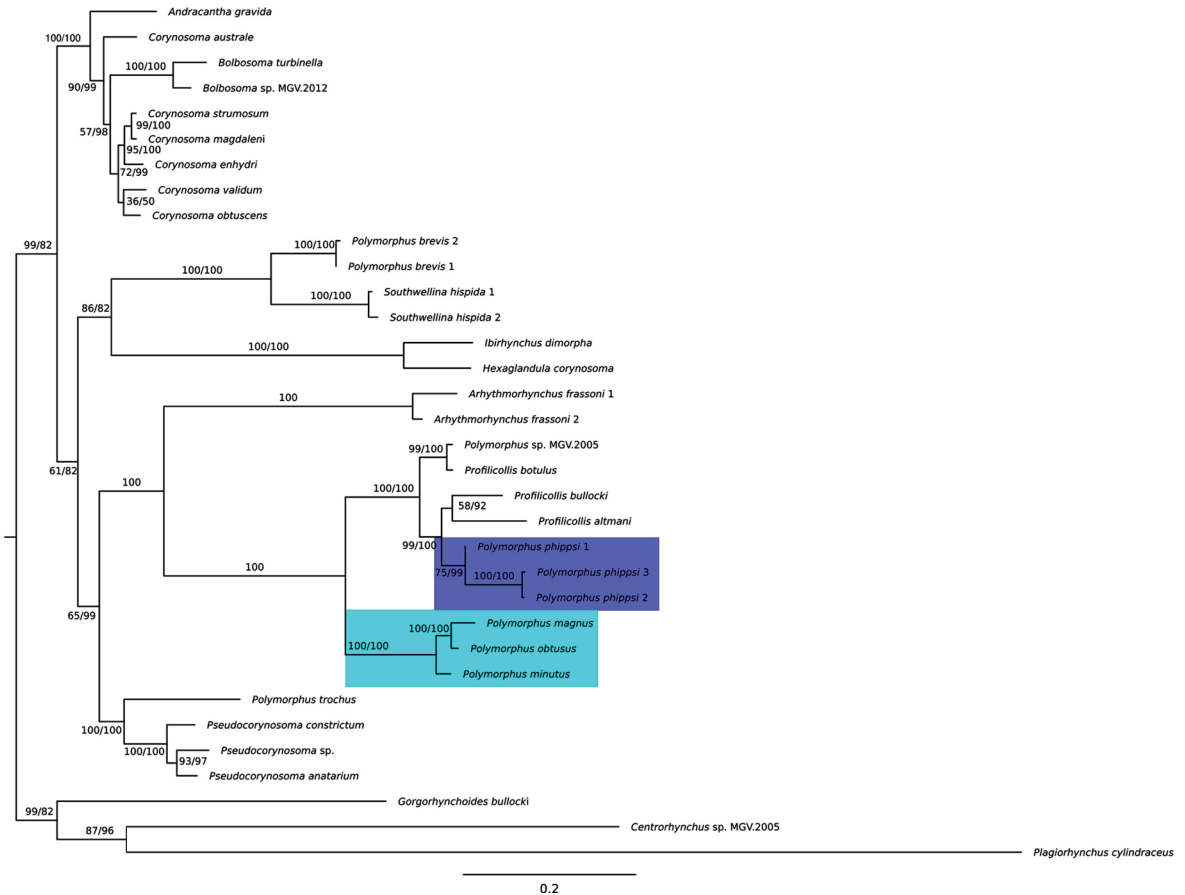

Figure 1. Phylogenetic relationships inferred from combined 28S rDNA + COI data set. Numbers near internal nodes show ML/MP bootstrap clade frequencies (percents) and posterior probabilities clade frequencies (percents). Clade containing *Polymorphus phippsi* sequences is highlighted in violet and *Polymorphus magnus* – in blue.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

CONFLICT OF INTEREST

This work does not contain any studies involving human and animal subjects.

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ФИЛОГЕНЕТИЧЕСКОЕ ПОЛОЖЕНИЕ *POLYMORPHUS PHIPPSI* KOSTYLEW, 1922 И *POLYMORPHUS MAGNUS* SKRJABIN, 1913 (PALAEACANTHOCEPHALA, POLYMORPHIDAE) ПО ДАННЫМ МОЛЕКУЛЯРНОЙ ФИЛОГЕНИИ

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Ключевые слова: Palaeacanthocephala, Polymorphidae, *Polymorphus magnus*, *Polymorphus phippsi,* молекулярная филогения, таксономия

РЕЗЮМЕ

Сем. Polymorphidae входит в состав класса Palaeacanthocephala типа Acanthocephala (скребни) – облигатных паразитов позвоночных, использующих членистоногих в качестве промежуточных хозяев. Таксономия семейства на данный момент является предметом дискуссий. Согласно данным молекулярной филогении, типовой род семейства Polymorphidae – *Polymorphus* Lühe, 1911 – полифилетический. Мы получили последовательности генов 28S рДНК и CO1 митохондриальной ДНК и включили их в филогенетическую реконструкцию семейства Polymorphidae. Согласно полученным данным, *P. magnus* группируется в одну кладу с типовым видом рода – *P. minutus* Lühe, 1911, но при этом *P. phippsi* оказался в одной кладе с представителями рода *Profilicollis* Meyer, 1931. Филогенетическое положение *P. phippsi* согласуется с данными о полифилии рода *Polymorphus* и, соответственно, противоречит принятому в настоящее время таксономическому статусу данного вида.