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Morphological and molecular identification of *Neomoliniella longicorpa* gen. et sp. nov. (Digenea: Echinostomatidae) from the Eurasian coot *Fulica atra* (Aves: Rallidae): a taxonomic evaluation

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Abstract

The purpose of the research is the morphological and molecular identification of trematodes found in the intestines of migratory waterfowl with subsequent taxonomic evaluation.

Materials and methods. Mature specimens of trematodes were collected from the intestines of waterfowl (*Fulica atra* and *Aythya ferina*) shot during the ecological-faunistic monitoring in the southern part of the Primorsky krai. Preliminary morphological analyze revealed that trematodes belong to the Echinostomatidae family. The whole-mounts of worms from *F. atra* were prepared using standard methods with alum carmine. Divergent domains of 28S ribosomal RNA gene were sequenced and used to determine systematic position and relationships of discovered species of echinostome by reconstructing Bayesian and Maximum likelihood trees.

Results and discussion. Trematodes found in the coots' intestine, should be described as *Neomoliniella longicorpa* in the new genus Neomoliniella gen. et sp. nov., which is reliably confirmed by morphological and genetics data. Another cryptic echinostomatid species sister to Neomoliniella gen. nov., and probably representing the separate unknown genus, was demonstrated only on the phylogenetic reconstructions and confirmed by large genetic distances. Currently, this taxon is indicated as Echinostomatidae gen. sp. and more evidence is needed for its validation. We also report about the first detection of *Echinostoma paraulum* from *A. ferina* in the south of the Russian Far East. The biology, morphology of each life cycle stage of newly described species of worms should be investigated to test not only their scientific but also medical significance. For the first time in the history of the group, dichotomous keys for eight families of the superfamily Echinostomatoidea and, to date, the largest of all previously known for the family Echinostomatidae identification chart – dichotomous keys for the identification of 27 Palearctic genera were compiled.

Keywords: Echinostomatidae, 28S rRNA gene, taxonomy, keys, Russian Far East

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Научная статья

Морфологическая и молекулярная идентификация *Neomoliniella longicorpa* gen. et sp. nov. (Digenea: Echinostomatidae) из обыкновенной лысухи *Fulica atra* (Aves: Rallidae): таксономическая оценка

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Аннотация

Цель исследований – морфологическая и молекулярная идентификация трематод, обнаруженных в кишечнике мигрирующих водоплавающих птиц, с последующей таксономической оценкой.

Материалы и методы. Материалом служили половозрелые трематоды, изъятые из кишечника отстрелянных в ходе эколого-фаунистического мониторинга на юге Приморья водоплавающих птиц (*Fulica atra* и *Aythya ferina*). Предварительный морфологический анализ показал принадлежность трематод к семейству Echinostomatidae. Препараты гельминтов из *F. atra* готовили с использованием стандартной методики окрашивания в квасцовом кармине. Нуклеотидные последовательности доменов гена 28S рибосомной РНК были расшифрованы и использованы для установления систематического положения и филогенетических отношений обнаруженных эхиностом путем реконструкции байесовского дерева и дерева максимального правдоподобия.

Результаты и обсуждение. В кишечнике лысухи найдены трематоды, которых следует рассматривать в составе нового рода Neomoliniella как *Neomoliniella longicorpa* gen. et sp. nov., что подтверждается данными морфологии и генетики. Существование другого криптического вида эхиностоматид сестринского Neomoliniella gen. nov., и вероятно представляющего собой отдельный неизвестный род, продемонстрировано только на филогенетических реконструкциях и подтверждается значениями генетических дистанций. В настоящее время этот таксон обозначен как Echinostomatidae gen. sp. и для обоснования его валидности требуется больше доказательств. Нами впервые обнаружена *Echinostoma paraulum* в *A. ferina* на юге Дальнего Востока России. Особенности биологии и морфологии каждой стадии жизненного цикла описанных видов гельминтов должны быть исследованы для оценки не только их научной, но и медицинской значимости. Впервые в истории изучения группы были составлены: определительная таблица для восьми семейств надсемейства Echinostomatoidea и на сегодняшний день самая большая из всех ранее составленных для семейства Echinostomatidae таблица для определения 27 палеарктических родов.

Ключевые слова: Echinostomatidae, ген 28S рРНК, таксономия, определительная таблица, Дальний Восток России

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Прозрачность финансовой деятельности: в представленных материалах или методах авторы не имеют финансовой заинтересованности.

Конфликт интересов отсутствует.

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Introduction

The family Echinostomatidae Looss, 1899 exhibits substantial taxonomic diversity which is associated with a broad range of susceptible definitive host and cosmopolitan distribution. Most species parasitize poultry, wild birds and mammals. It is widely known that migratory birds of Primorsky Krai are carriers of parasitic infections which can lead to the death of poultry and have a direct impact on agriculture and human food safety. Moreover, echinostomiasis is difficult to control, because of the wide range of aquatic second intermediate hosts (snails, bivalves, crustaceans, fish, and amphibians) distributed in domestic and native environments.

There are 46 species of echinostomes that commonly infect birds in the Russian Far East [2]. Adult echinostomatids are characterized by significant morphological convergence and as a consequence inadequate species descriptions, poor diagnoses, extensive synonymy and species complexes (for example *Echinostoma revolutum*, *Echinoparyphium recurvatum*) [7, 12, 13]. That is why molecular techniques in the study of this group have achieved significant success. A variety of genetic markers have been developed and used effectively to differentiate among echinostome species irrespective of life cycle stage [7, 13, 18, 19, 21–23]. To date, however, there is insufficient data regarding levels of genetic variation and phylogenetic relationships of Asian echinostomes, especially from the Russian Far East. Furthermore, mature worms were obtained only in an experimental study of its life cycles [11], and since the second half of the 20th century, data on the helminth fauna of birds in the natural environment have not been updated.

Waterfowl (coots and ducks) were shot during the ecological-faunistic monitoring in the southern part of the Primorsky krai (Khasansky district). Preliminary morphological analyses revealed trematodes presumably belonging to the family Echinostomatidae. We detected damage to the intestinal mucosa, inflammation and duodenal erosion – characteristics of clinical pathology of echinostomiasis. The primary aim of this study was the morphological and molecular identification of trematodes found in the intestines of migratory waterfowl in southern Primorye with subsequent taxonomic evaluation.

Materials and methods

Sample collection

Ten adults of echinostome species were collected from the intestines of shot birds – two Eurasian coots *Fulica atra* Linnaeus, 1758 and one common pochard *Aythya ferina* (Linnaeus, 1758). All specimens were washed thoroughly in physiological saline, and then three and seven specimens were preserved in 70 and 96% ethanol for the morphological and molecular genetic analyses, respectively.

Morphological analysis

The whole-mounts of three trematodes from *F. atra* were prepared using standard methods: trematode specimens were stained in alum carmine, dehydrated in a graded ethanol series (75%, 80%, 96%), cleared in clove oil, and mounted in Canada balsam. The preparations were analyzed using ZEISS Primo Star (Carl Zeiss, Germany). Measurements were made for the whole-mounts using the software ZEISS AxioVision 4.8.1 (Carl Zeiss, Germany) in the Department of Cell Biology and Genetics of Far Eastern Federal University. Three specimens of *Neomoliniella longicorpa* Vainutis, Voronova, Andreev, gen. et sp. nov. isolated from Eurasian coot were deposited to the helminthological collection of the Somov Research Institute of Epidemiology and Microbiology, Vladivostok, Russia, with accession No. FECEN-2.

DNA sequence analyses

Genomic DNA was extracted for seven adult worms by the HotSHOT technique [26]. D1–D3 domains of the 28S rDNA were amplified by PCR in MiniAmp Plus thermal cycler (Thermo Scientific) using DreamTaq Green Master Mix (Thermo Scientific, Lithuania) and pairs of primers dig12 and 1500R [24]. No gDNA (i. e. negative) controls were included in each run. Cycling conditions consist of a preliminary denaturation at 94 ^{0}C for 3 min, followed by 35 cycles of denaturation at 94 ^{0}C for 30 s, annealing at 56 ^{0}C for 30 s, elongation at 72 ^{0}C for 2 min, and a final product extension at 72 ^{0}C for 7 min. Amplicons were enzymatically cleaned up with ExoSAP-IT PCR Product Cleanup Reagent from Thermo Scientific and then sequenced on Honor 1616 Genetic Analyzer (Superyears Company) using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) (as instructed by the manufacturer) and sequencing primers the same as those used for PCR. Contiguous sequences were assembled using Finch TV and MEGA 7.0 [16], and submitted to GenBank under accession numbers: OP389066, OP408063–OP408064 and OP410306–OP410309.

Sequence alignment and phylogenetic analyses

Seven partial 28S rDNA sequences generated in the study were aligned with sequences of representatives of the Echinostomatidae sensu stricto available in GenBank with the aid of ClustalW according to Tkach et al. [25]. The final, manually refined data matrix (positions which could not be unambiguously aligned were excluded from the analysis) compiled of 36 28S rDNA sequences (D1–D3) 1188 bp in length. Species names and accession numbers are indicated on the resulting phylogenetic tree. A sequence for Caballerotrema sp. (KT956941) was used as a root based on the topology of the phylogenetic tree of the comprehensive molecular phylogeny for the superfamily Echinostomatoidea presented by Tkach et al. [25]. Genetic divergence was estimated using genetic p-distance values, which were calculated by including all substitution types in MEGA 7.0. Phylogenetic relationships among taxa were reconstructed using Bayesian inference (BI) in MrBayes 3.2.7 software [10]. The MCMC algorithm was performed using two independent runs and sampled every 1000 generations, where generations were added until the average standard deviation of split frequencies was less than 0.01; 25% of generations were discarded as burn-in. jModeltest v. 2.1.5 software [6] was used to select the best nucleotide substitution model – TVM+G for the gene data matrix. Additional Maximum Likelihood (ML) analyses were performed with PhyML 3.0 [8] run on the ATGC bioinformatics platform (http://www.atgc-montpellier.fr) with a non-parametric bootstrap validation based on 1000 pseudo-replicates. In calculating maximum likelihood trees, values of G and substitution rate parameters we reset to those estimated by jModeltest and nucleotide frequencies used were empirical.

Results and discussion

Morphologically worms isolated from *F. atra* were described as the new species, they well differed from other closely related echinostomatids. Below we provided the comprehensive description of these worms.

Neomoliniella longicorpa Vainutis, Voronova, Andreev, gen. et sp. nov. (Fig. 1, table 1)

Generic diagnosis. Neomoliniella gen. nov. belongs to the family Echinostomatidae by several morphological characters: elongate body, presence of the head collar covered with the spines on the anterior end of the body, poorly developed oral sucker, preacetabular intestinal bifurcation, uterus pretesticular, tandem testes, vitellarium restricted in the hindbody.

Neomoliniella gen. nov. has the highest morphological affinity to the genus Moliniella Hubner, 1939 [9, 20] by the following features (Fig. 1): body elongate; reniform head collar; collar marginal spines form continuous row; both testes are elongate-oval, entire; vitellarium extends from the posterior end of body to the level posterior to the ventral sucker.

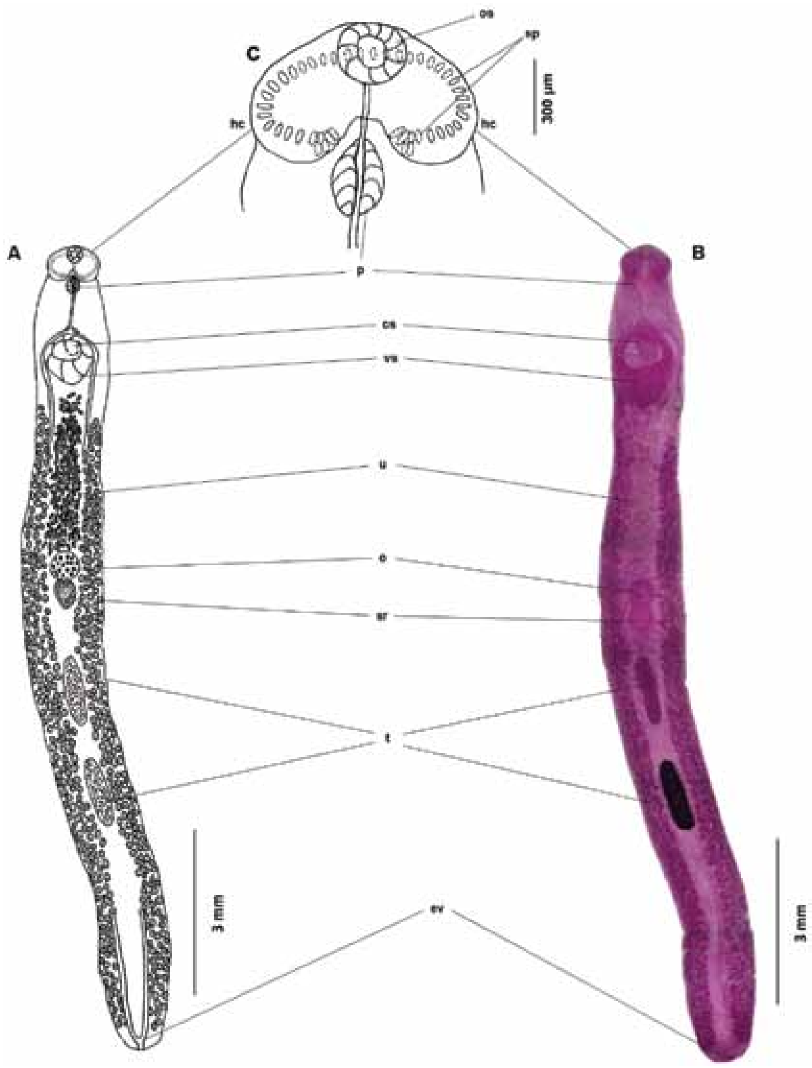

Fig. 1. Adults of *Neomoliniella longicorpa* gen. et sp. nov.:
A – entire worm (holotype), ventral view; B – microphotograph of the holotype; C – schematic image of the head collar. Abbreviations: cs – cirrus sac; ev – excretory vesicle; hc – head collar; o – ovary; os – oral sucker; p – pharynx; sp – spines; sr – seminal receptacle; t – testes; u – uterus; vs – ventral sucker

[**Рис. 1.** Взрослые особи *Neomoliniella longicorpa* gen. et sp. nov.:
A – червь целиком (голотип), вентрально; B – микрофотография голотипа; C – схематичное изображение головного воротника. Сокращения: cs – половая бурса; ev – экскреторный пузырь; hc – головной воротник; o – яичник; os – ротовая присоска; p – фаринкс; sp – шипы; sr – семяприемник; t – семенники; u – матка; vs – брюшная присоска]

The characters distinguishing Neomoliniella gen. nov. and Moliniella (from Hubner (1939) [9] and Odhner (1911) [20]) on the intergeneric level are as follows: the morphometric values of Neomoliniella gen. nov. do not overlap those of Moliniella (see Table 1), the minima values of Neomoliniella gen. nov. are larger than maxima values of Moliniella; internal corners of head collar each contain six spines in Neomoliniella gen. nov. vs. five spines in Moliniella; collar marginal spines ordered in one row in Neomoliniella gen. nov. vs. two rows in Moliniella; lateral fields of the vitellarium do not merge in posttesticular space and its posterior border do not reach the posterior end of body on some distance in Neomoliniella gen. nov. vs. follicles merge in posttesticular space in Moliniella; anterior border of vitellarium on the level between first and second fifth of uterus in Neomoliniella gen. nov. vs. in the midlevel or between first and second third of uterus in Moliniella.

Among other echinostomatids, the genera Echinostoma, Echinoparyphium, and

Echinodollfusia resemble Neomoliniella gen. nov. by a number of morphological characters. We performed the comparative morphological analysis of Neomoliniella gen. nov. with the species *Echinostoma revolutum*, *Echinoparyphium politum*, and *Echinodollfusia longiuscula* as the closest in morphology (shown in the Table 1), their common features are (based on the published data [1, 3, 17]): elongate body, tandem testes; reniform head collar, vitelline follicles do not merge in posterior end of body. The common morphological features differing these species from Neomoliniella gen. nov. are as follows: collar marginal spines ordered in double-row, vs. single row in Neomoliniella gen. nov. Particularly Neomoliniella gen. nov. differs from Echinodollfusia with smaller morphometric values, shorter hindbody, tapered eggs, cirrus sac dorsal to ventral sucker vs. preacetabular cirrus sac, 45 collar spines vs. 37–47 spines. Neomoliniella gen. nov. has the following differential characters in relation to Echinoparyphium: six spines in the ventral corners of the collar vs. four corner spines; larger size of body, oral and ventral suckers, internal organs, and eggs; ventral sucker in first fifth of body vs. on the border of first and second fourth of the body. Neomoliniella gen. nov. differs from the genus Echinostoma with: six corner spines vs. 3–5 corner spines; elongate-oval, entire testes vs. entire or lobed testes of irregular shape.

Type and only species. *N. longicorpa* Vainutis, Voronova, Andreev, gen. et sp. nov.

Etymology. The generic name "Neomoliniella" was given due to the high morphological similarity with other echinostomatid genus Moliniella.

Description. Body unarmed, elongate, regular in width, with slight widening in distal region of uterus, and tapering of forebody from level of intestinal bifurcation to head collar. Oral sucker small, with reniform head collar, bearing 45 ellipsoid spines, 0.066 mm long, with 22 spines per each side and one dorso-median spine: 33 marginal spines mainly in one row of which dorso-median spines in two rows, and 12 corner spines. On ventral side, six corner spines on each of internal corners of head collar, where two in lower row, and four – in upper. Subterminal oral sucker approximately 3–8 times smaller than ventral sucker. Respectively short pre-pharynx, 0.148 mm long, opening into muscular oval pharynx. Esophagus nearly two times longer than pre-pharynx. Intestinal bifurcation anterior to ventral sucker. Ventral sucker in first fifth of body, its posterior end cone-shaped. Anterior and posterior testes large, elongate-oval, entire; tandem, posterior to ovary, on border of middle and fourth fifth of body; distance between testes – 0.629 mm. Small cirrus sac dorsal to ventral sucker. Genital pore median or submedian, anterior to ventral sucker. Ovary round, anterior to similar in size, oval seminal receptacle. Uterus in second fifth of body, with numerous large eggs, extending to anterior border of ovarian region. Vitellarium extending from posterior end of body to border of first and second fifth of body, not reaching ventral sucker on some distance. Vitelline follicles numerous in both lateral fields, small, and round. Excretory vesicle Y-shaped, in posttesticular space. Excretory pore terminal.

Material examined

Host: *Fulica atra* delivered by Pankratov D. V.

Localization: isolated from intestine, collectors – Vainutis K. S., Andreev M. E.

Holotype. Russia: Khasansky district, Primorsky region, 5 October 2021, registration no. FECEN 2-1.

Paratypes. Registration no. FECEN 2-2, FECEN 2-3, same data as holotype.

Biology. The knowledge on life cycle is incomplete. The only definitive host known is *Fulica atra*.

Distribution. Khasansky district, Primorsky region.

Etymology. The specific name "longicorpa" was given after the elongated body of this worm.

Echinostomatidae is the one of the most speciose and widespread families of trematodes. The reverse side of this species richness is the great difficulty in their identification due to the great phenotypic similarity of adult forms. At first glance, it was rather complicated to distinguish the samples from the intestines of two coots based on the morphology. Only with the use of genetic data, we were able to make final conclusions on the taxonomy and relationships of the studied worms. The 28S rRNA gene was the marker of choice, generating a high resolution of the phylogenetic signal. Both ML and BI analyses of the Echinostomatidae resulted in consensus trees with similar well supported topologies (Fig. 2). It is possible to distinguish four successively brunched complex clades, which include following genera: Echinoparyphium +

[Table 1] Таблица 1

Morphometric values (mm) of echinostomatids resembling *Neomoliniella longicorpa* gen. et sp. nov.
[Морфометрические показатели (мм) эхиностоматид схожих с *Neomoliniella longicorpa* gen. et sp. nov.]

Features [Признак]	*Neomoliniella longicorpa* Vainutis, Voronova, Andreev, gen. et sp. nov.			*Moliniella anceps* [9]	*Moliniella nilotica* [20]	*Echinodollfusia longiuscula* [17]	*Echinoparyphium politum* [3]	*Echinostoma revolutum* [1]
	Holotype [Голотип]	Range [Диапазон] (n = 3)	Mean [Среднее]					
Body length [Длина тела]	14.886	10.679–14.886	12.76	3.1–4.5	2.25–3.5	12.85–21.06	5.2	6.8–12
Body width [Ширина тела]	0.68	0.59–0.70	0.066	0.44–0.52	0.45	0.581–0.855	0.38	0.88–2.0
Oral sucker [Ротовая присоска]	0.242 × 0.274	0.167–0.251 × 0.221–0.36	0.22 × 0.285	0.08–0.12 × 0.08–0.12	0.04 × 0.18	0.265–0.374 × 0.343–0.39	0.09–0.11 в диаметре [in diameter]	0.138–0.341 × 0.198–0.358
Ventral sucker [Брюшная присоска]	0.936 × 1.012	0.936–1.481 × 1.006–1.252	1.243 × 1.09	0.21–0.26 × 0.21–0.26	0.06 × 0.026	0.515–0.581 × 0.507–0.581	0.34–0.37 × 0.37	0.68–1.32 × 0.64–1.84
Pre-pharynx [Префаринкс]	0.231	0.085–0.231	0.148	-	-	-	-	0.077–0.121
Pharynx [Фаринкс]	0.316 × 0.231	0.278–0.316 × 0.231–0.265	0.298 × 0.25	0.06–0.08 ширина [width]	-	0.133–0.195 × 0.172–0.216	0.17 × 0.08	0.14–0.32 × 0.099–0.253
Head collar [Головной воротник]	0.609 × 0.9	0.452–0.679 × 0.791–0.974	0.58 × 0.888	0.26–0.3 ширина [width]	-	0.518–0.675 ширина [width]	0.26–0.28 ширина [width]	0.44–0.825
Cirrus sac [Половая бурса]	0.536 × 0.428	0.463–0.536 × 0.406–0.557	0.511 × 0.464	0.15 длина [length]	-	-	0.25 длина [length]	0.473–0.605 × 0.034–0.0407
Anterior testis [Передний семенник]	1.263 × 0.349	1.064–1.599 × 0.349–0.473	1.309 × 0.404	0.3–0.38 × 0.14–0.21	-	0.475–0.787 × 0.499–0.577	-	0.52–1.40 × 0.40–0.84
Posterior testis [Задний семенник]	1.258 × 0.402	1.258–1.33 × 0.402–0.494	1.296 × 0.434	0.3–0.38 × 0.14–0.21	-	0.522–0.863 × 0.483–0.577	-	0.52–1.62 × 0.36–0.76
Ovary [Яичник]	0.505 × 0.52	0.381–0.505 × 0.374–0.52	0.454 × 0.442	0.14–0.18 × 0.1–0.12	-	0.218–0.273 × 0.269–0.339	-	0.76–0.605 × 0.62–0.638
Seminal receptacle [Семяприемник]	0.535 × 0.423	0.312–0.535 × 0.252–0.46	0.42 × 0.378	-	-	-	-	-
Eggs [Яйца]	0.159 × 0.032	0.106–0.159 × 0.032–0.056	0.127 × 0.047	0.096–0.1 × 0.065–0.078	0.08–0.09 × 0.063–0.066	0.109–0.132 × 0.062–0.078	0.11–0.13 × 0.062–0.074	0.099–0.132 × 0.05–0.073
Vitelline follicles [Желточные фолликулы]	0.099 × 0.123	0.074–0.099 × 0.059–0.123	0.085 × 0.082	-	-	-	-	-
Suckers' length ratio [Соотношение длины присосок]	1 : 3.87	1 : 3.87–7.85	1 : 5.65	1:2.2	-	-	-	-
Suckers'width ratio [Соотношение ширины присосок]	1 : 3.69	1 : 2.79–5.67	1 : 3.82	1:2.2	-	65–68 %	-	-

Hypoderaeum (I), Moliniella (II), Echinostoma + Neoacanthoparyphium (III), Neomoliniella gen. nov. + Echinostomatidae gen. sp. + Patagifer + Artyfechinostomum (IV). The latter clade (IV) divided into two subclades, from which basal was for *Artyfechinostomum sufrartyfex* and another subclade was represented by monophyletic generic groups, two of which were sister and with reliable support were formed by newly sequenced worms from *Fulica atra*: *Neomoliniella longicorpa* sp. nov. and Echinostomatidae gen. sp. The trematode from *Aythya ferina* clustered with *Echinostoma paraulum* on one branch and gave a 100% similarity match with this species in BLAST.

No intragenera variation was detected for newly described Neomoliniella and Echinostomatidae gen. sp. Calculated genetic differences between species within genera represented on the tree (intragenus variability) ranged from a minimum of 0.06% for Patagifer to a maximum of 0.6% for Echinostoma. Sequence divergence between *Neomoliniella longicorpa*, Echinostomatidae gen. sp. and echinostomatids genera included into the analyses ranged from 1.5 to 3.6% that perfectly falls within the level of intergeneric variation (0.6–3.7%, table 2). Some authors based on 28S sequences during detailed pairwise comparisons of genetic distances between Echinostomatidae genera indicated following ranges 0.6–6.8% [21] and 0.9–8.7% [11]. Fixed ranges naturally depend on the groups of comparison and length of the marker sequences, so reciprocal fitness and/or overlapping of marginal values, which has been seen in this study, is the important factor that influences validity of the final suggestions.

Table 2 [Таблица 2]

Average values of genetic *p*-distances between generic groups of echinostomes included into the analysis
[Средние значения генетических *p*-дистанций между родами эхиностом, включенными в анализ]

№	Name [Род]	1	2	3	4	5	6	7	8	9	10
1	Artyfechinostomum		0.0049	0.0043	0.0048	0.0044	0.0051	0.0052	0.0055	0.0057	0.0047
2	Echinoparyphium	0.036		0.0052	0.0056	0.0053	0.0016	0.0023	0.0046	0.0048	0.0054
3	Echinostoma	0.030	0.030		0.0050	0.0049	0.0052	0.0049	0.0046	0.0044	0.0040
4	**Neomoliniella gen. nov.**	**0.028**	**0.035**	**0.032**		0.0033	0.0055	0.0057	0.0049	0.0052	0.0038
5	**Echinostomatidae gen. sp.**	**0.030**	**0.032**	**0.030**	**0.015**		0.0051	0.0056	0.0045	0.0050	0.0034
6	Euparyphium	0.037	0.006	0.030	**0.036**	**0.032**		0.0029	0.0048	0.0047	0.0050
7	Hypoderaeum	0.035	0.009	0.029	**0.034**	**0.034**	0.010		0.0047	0.0051	0.0053
8	Moliniella	0.035	0.026	0.031	**0.031**	**0.032**	0.026	0.027		0.0051	0.0042
9	Neoacanthoparyphium	0.035	0.028	0.026	**0.033**	**0.029**	0.027	0.030	0.032		0.0046
10	Patagifer	0.027	0.027	0.022	**0.017**	**0.015**	0.026	0.028	0.024	0.024	

Note [Примечание]. SD values are shown above the diagonal
[Значения среднеквадратического отклонения показаны над диагональю]

Latter phylogenetic study performed by Tkach et al. [25] based on the molecular genetic data revealed eight valid families belonging to the superfamily Echinostomatoidea: Echinostomatidae, Echinochasmidae Odhner, 1910, Caballerotrematidae Tkach, Kudlai & Kostadinova, 2016, Himasthlidae, Cyclocoelidae, Fasciolidae, Philophthalmidae, and Psilostomidae. That allows us to prepare complete dichotomous keys (table 3) for distinguishing the above families considering the keys earlier provided as follows: for the subfamilies Echinostomatinae, Echinochasminae, and Himasthlinae of which latter two were later raised to family status [4]; for the echinostomatoid families Echinostomatidae, Philophthalmidae, Fasciolidae, and Psilostomidae [14, 15]; for the families Caballerotrematidae and Echinochasmidae [25].

The whole study resulted into making the dichotomous keys (table 4) to 27 Palearctic genera of the family Echinostomatidae including the newly described Neomoliniella gen. nov. and to those noted in the several famous publications [14, 21, 25].

Conclusions

Our knowledge of the parasitic fauna of migratory birds is far from complete. Results

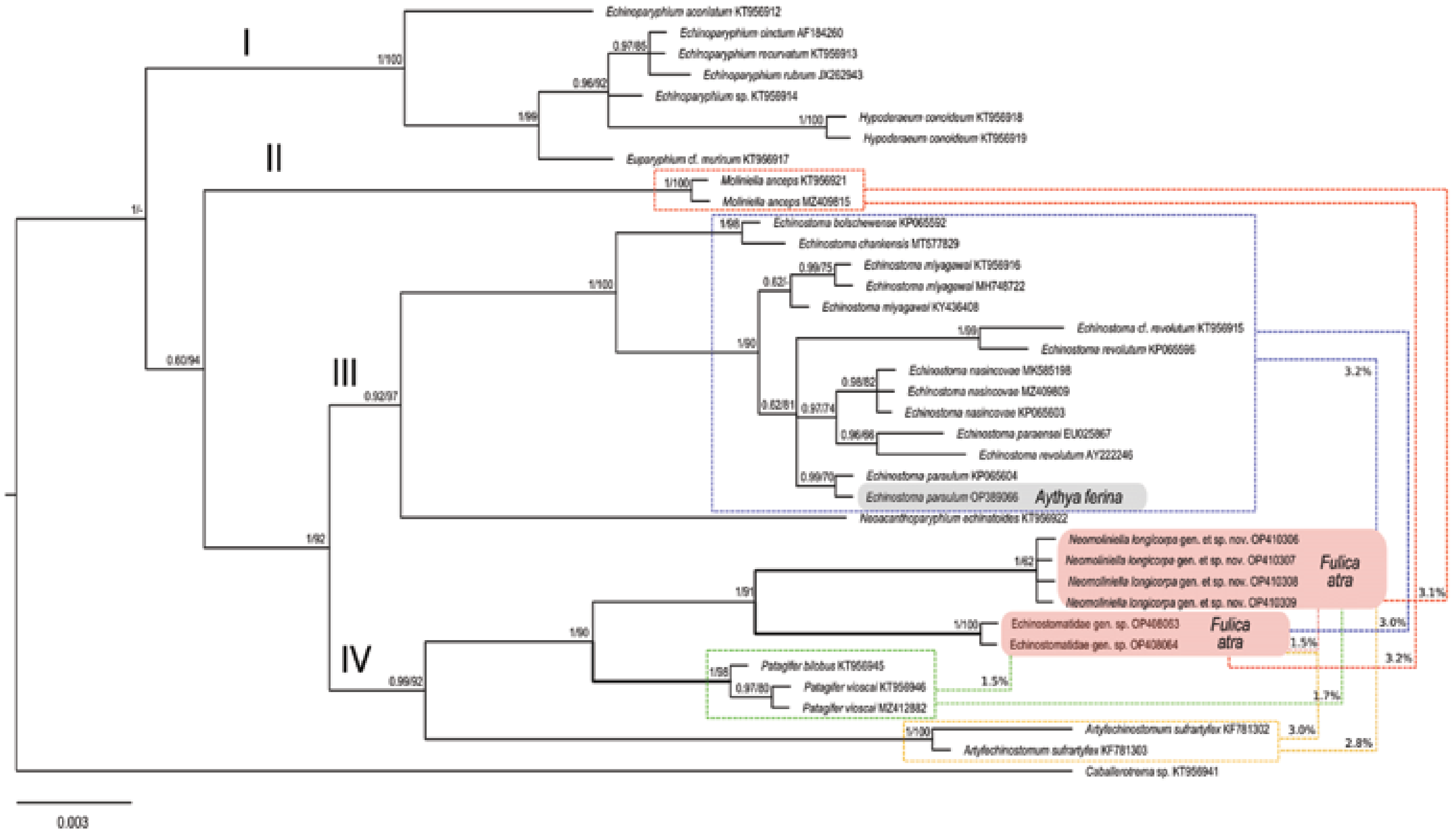

Fig. 2. Phylogenetic relationships of the Echinostomatidae family resulting from Bayesian inference (BI) and Maximum Likelihood (ML) analyses based on the partial sequences of the nuclear 28S rRNA gene (D1–D3). Bayesian inference posterior probability values and Maximum Likelihood bootstrap values associated with the branches are shown as BI/ML; support values lower than 50 (ML) are not shown. Four clades are indicated by Roman numerals. Newly generated sequences are in color boxes. Intergeneric distances (in %) between Neomoliniella, Echinostomatidae gen. sp., Moliniella, Echinostoma, Petagifer, and Artyfechinostomum are indicated at the intersection of dotted lines. The scale bar indicates the number of substitutions per site

[Рис. 2. Филогенетические отношения семейства Echinostomatidae, рассчитанные с помощью байесовского вывода (BI) и методом максимального правдоподобия (ML) на основе частичных последовательностей ядерного гена 28S рРНК (домены D1–D3). Значения апостериорной вероятности байесовского вывода и бутстреп-оценка максимального правдоподобия на ветвях показаны – BI/ML; значения поддержки ниже 50 % (ML) не показаны. Четыре клады обозначены римскими цифрами. Полученные последовательности выделены цветом. Межродовые расстояния (в %) между Neomoliniella, Echinostomatidae gen. sp., Moliniella, Echinostoma, Petagifer и Artyfechinostomum указаны на пересечении пунктирных линий. Шкала с указанием числа замен на сайт] obtained in this study once again confirm the importance of using molecular data in the biodiversity researches. Earlier reported about only three species of *Echinostoma*: *E. chloropodis* (Zeder, 1800), *E. grandis* Baschkirova, 1946 and *E. ralli* Yamaguti, 1934 found in the intestines of the Eurasian coots in the south of the Russian Far East. Based on phylogenetic reconstructions and large genetic distances the new genus Neomoliniella should be established. In the genus Neomoliniella gen. et sp. nov. we described the only species *N. longicorpa*, which well differs morphologically from other echinostomatids. Another cryptic echinostomatid species, sister to Neomoliniella gen. nov., was detected using the genetic data, and probably represents the separate unknown genus. Currently this taxon is indicated as Echinostomatidae gen. sp. and more evidence is needed to confirm its validity. Representatives of the family Echinostomatidae are believed to have evolved as avian parasites with several secondary host-switching events and changes in transmission patterns that eventually led to parasitism in other vertebrates, including humans [4, 22]. The pathogenicity of echinostomatids for humans strongly depends on the intensity of invasion and a number of associated pathogenetic factors. The biology, morphology of each life cycle stage (cercaria, metacercaria) of newly described species of worms should be investigated to test not only their scientific but also medical significance.

Dichotomous keys for eight families of the superfamily Echinostomatoidea and, to date, the largest of all previously known for the family Echinostomatidae identification chart – dichotomous keys for the identification of 27 Palearctic genera were compiled.

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Table 3 [Таблица 3]

Dichotomous keys to eight families of the superfamily Echinostomatoidea which validity was supported with genetic data]

[Определительная таблица восьми семейств надсемейства Echinostomatoidea, валидность которых была подтверждена генетическими данными]

1(8) Head collar absent	
2(3) Ventral sucker absent. Caeca forming cyclocoel. Uterus extending from posterior end of body to intestinal bifurcation	Cyclocoelidae Stossich, 1903 Synonym: Bothrigastridae Dollfus, 1948 Type genus: *Cyclocoelum* Brandes, 1892
3(2) Ventral sucker presents. Caeca separate, not forming cyclocoel. Uterus between gonads and ventral sucker	
4(5) Testes and ovary highly branched, dendritic, except for *Parafasciolopsis* (ovary entire) and *Protofasciola* (testes and ovary entire)	Fasciolidae Railliet, 1895 Type genus: *Fasciola* Linnaeus, 1758
5(4) Testes and ovary entire or lobate, round, oval, or irregular	
6(7) Pharynx well developed, oval, equal to oral sucker or smaller. Uterus pretesticular or lateral to testes; uterine coils may or may not over-reach caeca laterally	Philophthalmidae Looss, 1899 Synonym: Ommatobrephidae Poche, 1926; Parorchidae Skrjabin, 1965 Type genus: *Philophthalmus* Looss, 1899
7(6) Pharynx muscular, subglobular, occasionally absent or rudimentary (Apopharynginae). Uterus very short to long, intercaecal, pre-ovarian	Psilostomidae Looss, 1900 Type genus: *Psilostomum* Looss, 1899
8(1) Head collar present or rudimentary	
9(12) Cirrus sac entirely in forebody or dorsal to ventral sucker.	
10(11) Collar spines in single or double row interrupted ventrally and uninterrupted dorsally; or two anteriorly directed retractile proboscides armed with spines, one on each side of oral sucker (Rhopalias)	Echinostomatidae Looss, 1899 Synonyms: Rhopaliidae Looss, 1899; Chaunocephalidae Travassos, 1922; Cathaemasiidae Fuhrmann, 1928; Nephrostominae Mendheim, 1943; Balfouriidae Travassos, 1951; Ribeiroiinae Travassos, 1951; Echinocollidae Odening, 1961 Type genus: *Echinostoma* Rudolphi, 1809
11(10) Collar spines usually in single row interrupted dorsally	Echinochasmidae Odhner, 1910 Synonym: Saakotrematidae Odening, 1962 Type genus: *Echinochasmus* Dietz, 1909
12(9) Cirrus sac reaches into hindbody	
13(14) Collar reniform with distinct or indistinct ventral ridge. Intestinal bifurcation anterior to ventral sucker. Cirrus long, tubular, spined	Himasthlidae Odhner, 1910 Type genus: *Himasthla* Dietz, 1909
14(13) Collar very wide and dorso-ventrally flattened, ventral ridge absent. Intestinal bifurcation dorsal to ventral sucker. Cirrus short, muscular, smooth	Caballerotrematidae Tkach, Kudlai & Kostadinova, 2016 Type and only genus: *Caballerotrema* Prudhoe, 1960

Table 4 [Таблица 4]

Dichotomous keys to 27 Palearctic genera of the family Echinostomatidae
[Определительная таблица 27 Палеарктических родов семейства Echinostomatidae]]

1(4) Head collar rudimentary or absent	
2(3) Head collar rudimentary, occasionally indistinct; collar spines small, similar in size to tegumental spines, in two groups ventro-lateral to oral sucker	*Cathaemasia* Looss, 1899 Type species: *Cathaemasia hians* (Rudolphi, 1809) Looss, 1899
3(2) Rudimentary collar and spines not observed	*Pseudocathaemasioides* Saito & Fukumoto, 1972 Type species: *Pseudocathaemasioides cerylis* Saito & Fukumoto, 1972
4(1) Head collar muscular, with one or two crowns of spines, interrupted ventrally; collar spines larger than tegumental spines	
5(50) Body not subdivided into regions	
6(7) Oral suckers completely reduced, only muscular pharynx present in anterior end of body	*Pegosomum* Ratz, 1903 Type species: *Pegosomum saginatum* (Ratz, 1898) Ratz, 1903
7(6) Oral sucker presents	
8(13) Head collar bilobed	
9(12) Head collar with deep narrow dorsal incision and wider ventral notch. Elongate-oval testes in third quarter of body. Cirrus sac present	
10(11) Collar with wide ventral notch and shallow dorsal depression. Collar spines conical, not interrupted dorsally. Uterus long	*Nephrostomum* Dietz, 1909 Type species: *Nephrostomum ramosum* (Sonsino, 1895) Dietz, 1909
11(10) Collar distinctly bilobed. Collar spines rod-shaped, interrupted dorsally. Uterus short	*Patagifer* Dietz, 1909 Type species: *Patagifer bilobus* (Rudolphi, 1819) Dietz, 1909
12(9) Head collar with deep mid-dorsal and mid-ventral indentations. Subspherical testes close to posterior extremity. Cirrus sac absent	*Pelmatostomum* Dietz, 1909 Type species: *Pelmatostomum episemum* Dietz, 1909
13(8) Head collar of different shape	
14(17) Head collar poorly developed, transversely oval	
15(16) Cirrus sac posterior to posterior margin of ventral sucker	*Hypoderaeum* Dietz, 1909 Type species: *Hypoderaeum conoideum* (Bloch, 1782) Dietz, 1909
16(15) Cirrus sac at mid-level of ventral sucker	*Skrjabinophora* Baschkirova, 1941 Type species: *Skrjabinophora eroliae* Baschkirova, 1941
17(14) Head collar reniform	
18(23) Parasites with long, filamentous body	
19(20) Hindbody conspicuously attenuated between ventral sucker and ovary. Dorsal collar spines in single row. Ovary post-equatorial. Post-testicular field short. Internal seminal vesicle tubular, coiled	*Longicollia* Bychowskaja-Pawlowskaja, 1953 Type and only species: *Longicollia echinata* Bychovskaja-Pavlovskaja, 1953
20(19) Hindbody of uniform width. Dorsal collar spines in double row. Ovary pre-equtorial. Post-testicular field long to very long. Internal seminal vesicle saccular	
21(22) Collar spines 37-43. Lateral collar spines in double row. Testes pre-equatorial. Internal seminal vesicle unipartite. Cirrus unarmed. Uterus very long, winding. Vitelline fields non-confluent	*Echinodollfusia* Skrjabin et Baschkirova, 1956 Type species: *Echinodollfusia stenon* (Dollfus, 1950) Skrjabin & Baschkirova, 1956
22(21) Collar spines 27. Lateral collar spines in single row. Testes pre-equatorial. Internal seminal vesicle bipartite. Cirrus armed. Uterus short, coiled. Vitelline fields confluent posterior to testes	*Baschkirovitrema* Skrjabin, 1944 Type species: *Bashkirovitrema incrassatum* (Diesing, 1850) Skrjabin, 1944

Continuation of the table 4 [Продолжение таблицы 4]

23(18) Parasites with elongate or oval body	
24(29) Uterus well developed, with numerous eggs	
25(28) Margins of hindbody saw-toothed, each projection with spine	
26(27) Parasitic in heterotherms; eggs large, embryonated, or containing developed miracidium	*Prionosomoides* Teixeira de Freitas & Dobbin, 1967 Type species: *Prionosomoides scalaris* Teixeira de Freitas & Dobbin, 1967
27(26) Parasitic in homoiotherms; eggs non-embryonated	*Edietziana* Ozdikmen, 2013 Type species: *Edietziana serratum* (Diesing, 1850) Ozdikmen, 2013
28(25) Margins of hindbody not with saw-toothed projections	*Echinostoma* Rudolphi, 1809 Type species: *Echinostoma revolutum* (Fröhlich, 1802) Looss, 1899
29(24) Uterus poorly developed, with few eggs	
30(33) Testes usually transversely elongate, tandem, oblique or at same horizontal level.	
31(32) Head collar with 27 spines	*Petasiger* Dietz, 1909 Type species: *Petasiger exaeretus* Dietz, 1909
32(31) Head collar with 19 spines	*Neopetasiger* Baschkirova, 1941 Type species: *Neopetasiger neocomensis* (Fuhrmann, 1927) Baschkirova, 1941
33(30) Testes commonly longitudinally elongated, tandem or symmetrical	
34(35) Testes round, symmetrical	*Parallelotestis* Belopolskaya, 1954 Type species: *Parallelotestis horridus* Belopolskaja, 1954
35(34) Testes elongate or of irregular shape, tandem	
36(39) Anterior borders of vitellarium closer to posterior extremity	
37(38) Dorsal aboral collar spines longer than oral. Intestinal bifurcation halfway between pharynx and ventral sucker. Posttesticular field shorter. Cirrus unarmed	*Euparyphium* Dietz, 1909 Type species:
38(37) Dorsal aboral collar spines shorter than oral. Intestinal bifurcation just anterior to ventral sucker. Post-testicular field very long. Cirrus armed	*Isthmiophora* Lühe, 1909 Type species: *Isthmiophora melis* (Schrank, 1788) Lühe, 1909
39(36) Vitellarium anteriorly at level of ventral sucker or at some distance from it	
40(43) Collar spines in single row	
41(42) Collar spines 31 or 33, in single row, of which eight spines in ventral corners, not interrupted dorsally	*Dietziella* Skrjabin & Bashkirova, 1956 Type species: *Dietziella deparcum* (Dietz, 1909) Skrjabin & Bashkirova, 1956
42(41) Collar spines small, 20, 22, or 24, in single row, interrupted dorsally	*Ignavia* Teixeira de Freitas, 1948 Type species: *Ignavia venusta* Teixeira de Freitas, 1948
43(40) Collar spines in double row; exclusion – Neomoliniella gen. nov. with single rows of lateral and double row of dorsal spines	
44(47) Four spines in ventral corners of collar. Ventral sucker on border of first and second fourth of body or somewhat biased posteriorly	
45(46) Body small to medium. Collar spines 29-45. Difference in size between angle and marginal spines smaller. Dorsal spines long, with aboral longer than oral. Ovary pre-equatorial, smaller than testes. Eggs small, more than ten	*Echinoparyphium* Dietz, 1909 Type species: *Echinoparyphium elegans* (Looss, 1899) Dietz, 1909

End of table 4 [Окончание таблицы 4]

46(45) Body minute. Collar spines 49-59. Angle spines 2.5-3 times as long as marginal spines. Dorsal spines very small, of equal size. Ovary postequatorial, larger than testes. One or two eggs, larger than gonads	*Neoacanthoparyphium* Yamaguti, 1958 Type species: *Neoacanthoparyphium petrowi* (Nevostrueva, 1953) Yamaguti, 1958
47(44) Five or six spines in ventral corners of collar. Ventral sucker on border of first and second fifth of body	
48(49) Ventral collar corners each containing five spines. Collar marginal spines ordered in two rows. Lateral fields of the vitellarium merge in posttesticular space. Anterior border of vitellarium in the midlevel or between first and second third of uterus	*Moliniella* Hübner, 1939 Type species: *Moliniella anceps* (Molin, 1859) Hübner, 1939
49(48) Ventral collar corners each containing six spines. Collar marginal spines ordered mainly in one row. Lateral fields of the vitellarium do not merge in posttesticular space and its posterior border do not reach the posterior end of body on some distance. Anterior border of vitellarium on the level between first and second fifth of uterus	*Neomoliniella* gen. nov. Type and only species: *N. longicorpa* sp. nov.
50(5) Body subdivided into regions, forebody and hindbody of different shape	
51(52) Body divided into two regions that considerably differ in width. Collar reniform. No papillae on hindbody. Uroproct present	*Chaunocephalus* Dietz, 1909 Type species: *Chaunocephalus ferox* (Rudolphi, 1795) Dietz, 1909
52(51) Body divided into spatulate, dorso-ventrally flattened forebody and elongate, cylindrical hindbody. Collar disc-like. Two contractile median papillae present in mid-region of hindbody	*Sodalis* Kowalewski, 1902 Type species: *Sodalis spatulatus* (Rudolphi, 1819) Kowalewski, 1902

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Andreev Mark E. – work with literature sources and parasitological material, preparing whole mounts of biological samples for microscopic observation, microscopic study of preparations, drawings implementation, obtaining molecular data for analysis, data analysis.

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