Vestnik zoologii, **50**(2): 99–104, 2016
DOI 10.1515/vzoo-2016-0012

DE GRUYTER OPEN

UDC 595.122: [591.4+577.2] (477)

A NEW RECORD OF *CHAUNOCEPHALUS FEROX* (DIGENEA, ECHINOSTOMATIDAE) FROM *CICONIA NIGRA* IN UKRAINE INCLUDING MORPHOLOGICAL AND MOLECULAR DATA

O. B. Greben[1]*, O. Kudlai[1], E. N. Korol[1, 2], V. V. Kornyushin[1], I. B. Vasilkovska[3], V. V. Kobylinsky[3]

[1]*Schmalhausen Institute of Zoology, NAS of Ukraine, vul. B. Khmelnytskogo, 15, Kyiv, 01030 Ukraine*
[2]*National Museum of Natural History, NAS of Ukraine, vul. B. Khmelnytskogo, 15, Kyiv, 01030 Ukraine*
[3]*Kiev Zoo, Peremohy Ave, 32, Kyiv, 04116 Ukraine*
**E-mail: oksana-greben@yandex.ru*

A New Record of *Chaunocephalus ferox* (Digenea, Echinostomatidae) from *Ciconia nigra* in Ukraine Including Morphological and Molecular Data. Greben, O. B., Kudlai, O., Korol, E. M., Kornyushin, V. V., Vasilkovska, I. B., Kobylinsky, V. V. — Morphological and molecular data on the type-species of *Chaunocephalus* Dietz, 1910, *Chaunocephalus ferox* (Rudolphi, 1795) is provided based on material collected from the type-host, *Ciconia nigra* (Linnaeus), from Kiev Zoo, Ukraine.

Key words: *Chaunocephalus*, Echinostomatidae, Ciconiidae, morphology, rDNA sequence, Ukraine.

Introduction

Chaunocephalus Dietz, 1910 (Digenea: Echinostomatidae) is a small trematode genus, with 11 described species (Skrjabin & Bashkirova, 1956; Yamaguti, 1971; Ku et al., 1974; Mehra, 1980), i. e. *Chaunocephalus ferox* (Rudolphi, 1795) (type-species), *Ch. elongatus* Vrat, 1947, *Ch. gerardi* Gedoelst, 1913, *Ch. kirati* Vrat, 1947, *Ch. leucocephalus* Vrat, 1947, *Ch. odhneri* Vrat, 1947, *Ch. panduriformis* Travassos, 1922, *Ch. schulzi* Gnedina, 1941, *Ch. similiferox* Verma, 1936, *Chaunocephalus sinensis* Ku, Chiu, Li & Chu, 1974 and *Ch. travassosi* Vrat, 1947. They have been reported from a wide range of birds, mainly of the order Ciconiformes from Europe, Asia, South America and Australia (Kostadinova, 2005). Species of *Chaunocephalus* are characterised by possessing a body divided into bulbous forebody and shorter, narrow, subcylindrical hindbody and localisation into crypts in the intestinal wall of the definitive host. The intestinal nodular lesions associated with infections by species of *Chaunocephalus* can lead to debilitation and associated mortality (Santoro et al., 2013).

The type-species, *Ch. ferox*, originally described on the basis of material from *Ciconia nigra* (Linnaeus, 1758) has been reported mainly from ciconiid birds including the white stork *C. ciconia* (Linnaeus, 1758), bittern *Botaurus stellaris* (Linnaeus, 1758), black-neked stork *Ephippiorhynchus asiaticus* (Latham, 1970) and Asian open-billed stork *Anastomus oscitans* (Boddaert, 1783) (see Dietz, 1910; Bashkirova, 1941; Brglez, 1975; Iskova, 1985; Poonswad et al., 1992; Saad, 2009; Saidul et al., 2009). *Chaunocephalus ferox* was reported several times from *C. nigra* and *C. ciconia* in Ukraine (Kowalewski, 1896; Ivanitsky, 1928; Sergienko, 1968, 1978; Iskova, 1973; Smogorzhevskaya et al., 1978; Kornyushin et al., 2004). Although, this species has been recorded frequently, there are no genetic data available. This study presents an original morphological description and a partial sequence of 28S rRNA gene of *Ch. ferox* based on adult newly collected from the black stork, *C. nigra*, from the Kiev Zoo, Ukraine.

Material and methods

Specimens for this study were obtained from *C. nigra* from the Kiev Zoo in February 2015. The examined bird entered Zoo in May, 2014 from Borodyanka District, Kyiv Region. Anthelminthic treatment was performed with Dectomax in a dose of 0.2 ml according to the manufacturer protocol. The bird died nine months after the treatment. After dissection, the intestine was removed and fixed in 96 % ethanol. Contracted digenean specimens were collected from the intestine and from the crypts in the intestinal walls. Specimens for light microscopical examination were stained with iron acetocarmine or Mayer's hematoxylin, dehydrated in a graded ethanol series, cleared in clove oil (after carmine staining) or methyl salicylate (after hematoxylin staining), and mounted in Canada balsam.

All measurements are in micrometres and presented in the text as the range followed by the mean in parentheses. The material is deposited in the collection of the I. I. Schmalhausen Institute of Zoology, National Academy of Sciences of Ukraine, Kyiv, Ukraine (Coll. No. Sl1-012). One hologenophore is deposited in the collection of the Institute of Parasitology (Coll. No. HCIP D-13), Biology Centre of the Czech Academy of Sciences, České Budéjovice, Czech Republic.

Total genomic DNA was isolated from an alcohol-fixed specimen of *Ch. ferox* according to the protocol described by Georgieva et al. (2013). Approximately 1,300 bp long fragment of the 28S rRNA gene was amplified using the forward primer LSU5 (5'-TAG GTC GAC CCG CTG AAY TTAAGC A-3') and the reverse primer 1500R (5'-GCT ATC CTG AGG GAA ACT TCG-3') (Tkach et al., 2003). PCR amplicons were purified using a QIAquick PCR purification Kit (Qiagen Ltd, UK), following manufacturer's instructions, sequenced directly with ABI BigDye chemistry (ABI Perkin-Elmer, UK), alcohol-precipitated and run on an ABI Prism 3130x1 automated sequencer. The original PCR primers as well as the internal primers 300F (5'-CAA GTA CCG TGA GGG AAA GTT G-3'), ECD2 (5'-CTT GGT CCG TGT TTC AAG ACG GG-3') and 900F (5'-CCG TCT TGA AAC ACG GAC CAA G-3') (Tkach et al., 2003) were used for sequencing. Contiguous sequence was assembled and edited using Sequencher™ ver. 4.1.1 (GeneCodes Corp.) and submitted to GenBank.

Results

A total of 45 specimens of *Ch. ferox* was collected from the black stork; 23 of those were localised in the cavity of the intestinal tract and 22 specimens were obtained from crypts in the intestinal wall (1–2 specimens in each crypt).

Family ECHINOSTOMATIDAE Dietz, 1909
Genus *Chaunocephalus* Dietz, 1909
Chaunocephalus ferox (Rudolphi, 1795)

Host: *Ciconia nigra* (Linnaeus, 1758) (Ciconiiformes, Ciconiidae).
Site in host: Intestine, crypts in the intestinal wall.
Localities: Kiev Zoo (originated from Borodianka, Kyiv Region).
Representative DNA sequence: KT447522 (partial 28S rRNA gene).

Description (fig. 1)

[Based on 8 contracted specimens from intestine]. Body elongate, divided into bulbous forebody (FO = 53–56 %) with maximum width 1,182–1,709 (1,452) and shorter narrow hindbody, with maximum width 595–1,279 (968). Body length 3,115–3,628 (3,417). Tegument armed with large spines.

Head collar reniform, strongly muscular, and well differentiated from body, 194–302 × 405–479 (245 × 448). Collar spines 27; four angle spines on each ventral lappet, larger than marginal spines, 120–148 × 20–25 (136 × 22); lateral spines in single row, 90–115 × 13–19 (102 × 15); dorsal spines in double row, 85–113 × 13–18 (99 ×15). Oral sucker terminal, transversely oval, 116–159 × 131–182 (133 × 155). Ventral sucker strongly muscular, with deep cavity, 502–690 × 497–614 (610 × 553). Sucker-ratio 1: 3.0–4.3 (1: 3.6). Prepharynx short, 69–75 (72). Pharynx muscular, elongate-oval, larger than oral sucker, 217–278 × 111–154 (244 × 136). Oesophagus 986–1,448 (1,168) long with saccular lateral diverticula. Intestinal bifurcation just anterior to ventral sucker. Caeca reach close to posterior extremity and open into excretory vesicle to form uroproct.

Testes tandem, post-ovarian, elongate-oval, entire; right testis 200–41 × 163–343 (299 × 232); left testis 258–355 × 165–364 (303 × 230). Post-testicular region 531–792 long, occupying 15–23 % of body length. Cirrus-sac small, elongate-oval, 181 × 146, antero-dorsal to ventral sucker. Internal seminal vesicle not observed. Genital pore median, closely anterior to ventral sucker.

Ovary large, sinistral, pretesticular, transversely oval, 171–392 × 232–477 (281 × 338). Mehlis' gland strongly developed, dextral, immediately pretesticular, 222–410 × 280–572 (305 × 382). Laurer's canal and uterine seminal receptacle not observed. Uterus short with numerous eggs, 87–111 × 54–75 (99 × 60). Vitellarium extensive, follicles large, in whole

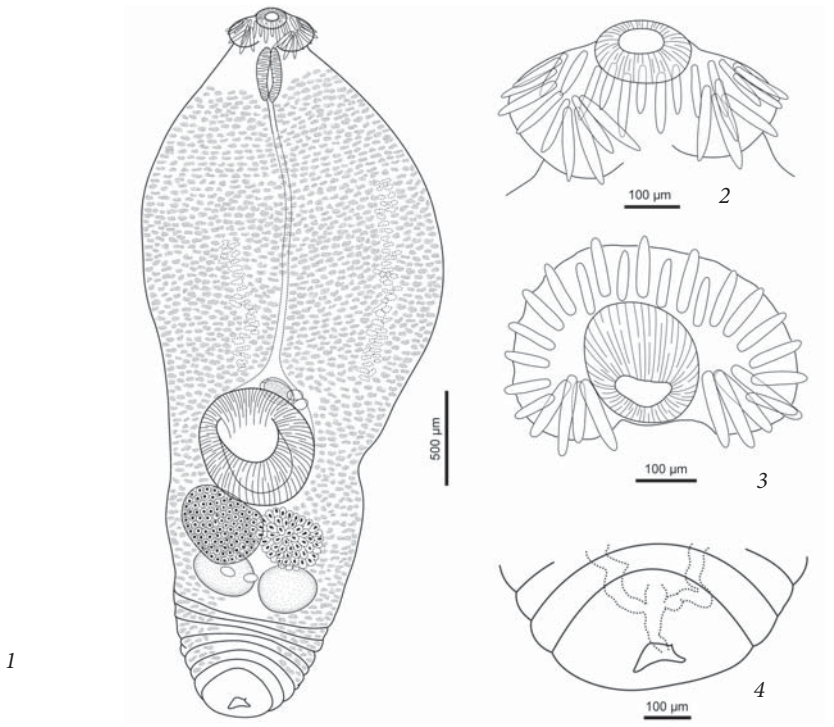

Fig. 1. *Chaunocephalus ferox*: *1* — ventral view; *2* — head collar; *3* — head collar of hologenophore; 4 — uroproct.

forebody and two lateral fields in hindbody, confluent in forebody. Excretory vesicle not observed; pore terminal.

Remaks

The material described above agrees well with the diagnosis of *Chaunocephalus* given by Kostadinova (2005). The presence of 27 collar spines in our specimens is consistent with four species in the genus, namely *Ch. ferox*, *Ch. gerardi*, *Ch. similiferox* and *Ch. sinensis* (table 1).

The present species differs from:

(i) *Ch. gerardi* in having a distinctly smaller collar (194–302 × 405–479 *vs* 530 × 830–1,000) and oral sucker (116–159 × 116–159 *vs* 380–400), shorter and narrower angle and marginal spines (120–148 × 20–25 *vs* 210–240 × 32; 90–115 × 13–19, 85–113 × 13–18 *vs* 130–135 × 21–30);

(ii) *Ch. similiferox* in having a narrower collar (405–479 *vs* 590–670), shorter and narrower angle spines (120–148 × 20–25 *vs* 190–210 × 35–45), smaller oral sucker (116–159 × 131–182 *vs* 210–230 × 300–330) and ovary (207–487 × 157–391 *vs* 400–500 × 500), and higher limits for the length and width of eggs (87–111 × 54–75 *vs* 67–109 × 42–65);

(iii) *Ch. sinensis* in having longer angle spines (120–148 *vs* 77–109), larger pharynx (217–278 × 111–154 *vs* 168–186 × 96–132), narrower Mehlis' gland (252–495 *vs* 537–645) and higher lower limits for ventral sucker and eggs, and low or higher upper limits for testes and ovary (table 1).

The morphology of the present form generally agrees with the description of *Ch. ferox* by Dietz (1910) and description by Iskova (1985); the latter based on material from *C. ciconia* in Danube Delta. However, specimens from our collection exhibit substantially smaller length of the body that we consider is due to the fixation of the worms inside the host intestine. Notably, the size of angle spines and the width of the pharynx of our specimens lie outside the lower range of variation reported by Dietz (1910). Also the length of the prepharynx and the maximum width of the ventral sucker in our specimens are distinctly larger. Specimens re-

Table 1. Comparative metrical data for *Chaunocephalus* spp.

Species	*Chaunocephalus ferox*				*Ch. elongatus* Vrat, 1947	*Ch. gerardi* Gedoelst, 1913	*Ch. kirati* Vrat, 1947
Host	*Ciconia nigra* (L.)		*Ciconia nigra, Ciconia ciconia* (L.)	*Ciconia ciconia*	*Anhinga melanogaster* (Pennant, 1769)	*Anastomus lamelligerus Temminck, 1823*	*Anastomus oscitans* (Boddaert, 1783)
Origin	Ukraine		Central Europe	Ukraine	India	Belgian Congo	India
Source	Present study		Dietz (1910)	Iskova (1985)	Mehra (1980)	Skrjabin & Bashkirova (1956)	Mehra (1980)
Parameter	Range	Mean	Range	Range	Range	Range	Range
Body length	3,115–3,628	3,417	5,500–8,000	5,320–6,500	6,800	4,000–4,500	4,760
Body width in forebody	1,182–1,709	1,452	1,870–2,290	1,280–1,360	–	2,450–2,850	1,190
Body width in hindbody	595–1,279	968	–	800–900	–	650–1,200	1,020
Collar length	194–302	245	–	200	187	530	200
Collar width	405–479	448	–	450–500	255	830–1,000	420
Number of spines	27		27	27	25	27	26
Angle spines length	120–148	136	160–185	98–140	175–180	210–240	85
Angle spines width	20–25	22	34	22–28	38	32	20
Lateral spines length	90–115	102	–	56–62	88–93	130–135	65
Lateral spines width	13–19	15	–	21	23	21–30	15
Dorsal spines length	85–113	99	74–125	56–62	–	130–135	65
Dorsal spines width	13–18	15	–	21	–	21–30	15
Oral sucker length	116–159	133	–	120–130	–	–	–
Oral sucker width	131–182	155	130–150	140–160	136	380–400	136
Prepharynx length	69–75	72	27	–	0	–	85
Pharynx length	217–278	244	220	–	204	–	170
Pharynx width	111–154	136	170	112–128	136	–	136
Oesophagus length	986–1,448	1,168	–	–	1,700	–	1,730
Ventral sucker length	502–690	610	–	–	–	–	–
Ventral sucker width	497–614	553	430–530	440–460	–	560	425
Cirrus-sac length	181	–	–	–	120	–	221
Cirrus-sac width	146	–	–	–	108	–	135
Right testis length	200–410	299	300	260–280	200	–	370
Right testis width	163–343	232	240	240	136	–	220
Left testis length	258–355	303	300	260–280	170	–	220
Left testis width	165–364	230	240	240	136	–	150
Ovary length	171–392	281	310	340	170	–	220
Ovary width	232–477	338	370	360	204	–	200
Mehlis' gland length	222–410	305	–	–	–	–	–
Mehlis' gland width	280–572	382	–	–	–	–	–
Egg-length	87–111	99	88–92	88–96	93	–	–
Egg-width	54–75	60	53–57	48	48	–	–

ported by Iskova (1985) differ from ours in having distinctly shorter dorsal spines and smaller lower limits for the length of the angle spines and egg width (table 1).

The genus *Chaunocephalus* requires detail taxonomic revision to assess the validity of all species included with a special emphasis on these from India. Such a large variability of number of spines (table 1) makes the validity of the species somewhat doubtful.

Table 1. Continued

Species	*Ch. leucocephalus* Vrat, 1947	*Ch. odhneri* Vrat, 1947	*Ch. panduriformis* Travassos, 1922	*Ch. schulzi* Gnedina, 1941	*Ch. similiferox* Verma, 1936	*Ch. sinensis* Ku, Chiu, Li & Chu, 1974	*Ch. travassosi* Vrat, 1947
Host	*Mycteria leucocephala* (Pennant, 1769)	*Anastomus oscitans*	*Ciconia maguari* (Gmelin, 1789)	*Ciconia nigra*	*Ephippiorhynchus asiaticus* (Latham, 1790), Indian gull (according to Verma)	*Ciconia nigra*	*Antigone antigone* (L.)
Origin	India	India	Brazil	Russia	India	China	India
Source	Mehra (1980)	Mehra (1980)	Skrjabin & Bashkirova (1956)	Skrjabin & Bashkirova (1956)	Skrjabin & Bashkirova (1956)	Ku, Chiu, Li & Chu (1974)	Mehra (1980)
Parameter	Range	Range	Range	Range	Range	Range	Range
Body length	8,040	5,491	6,000–10,000	4,820	4,410–8,800	4,850–6,600	6,760
Body width in forebody	4,160	2,920	1,200–4,000	1,380	3,000–4,000	636–1,750	3,480
Body width in hindbody	1,360	1,020	–	–	700–1,000	–	–
Collar length	150	187	–	270	–	–	170
Collar width	750	560	870	440	590–670	412–448	660
Number of spines	29	26	23–26	29	27	27	28
Angle spines length	325	100	230	83–133	190–210	77–109	153
Angle spines width	38	25	–	–	35–45	18–23	25
Lateral spines length	–	75	120	98	83–120	–	100
Lateral spines width	–	15	–	14	18–20	–	18
Dorsal spines length	–	75	–	–	83–120	–	100
Dorsal spines width	–	15	–	–	18–20	–	18
Oral sucker length	–	–	260	121	210–230	150–182	250
Oral sucker width	290	102	220	103	300–330	136–173	170
Prepharynx length	117	204	–	–	–	72–77	85
Pharynx length	250	255	290	220	–	168–186	374
Pharynx width	170	221	260	121	250	96–132	340
Oesophagus length	4,500	380	3,000–4,000	–	–	–	3,650
Ventral sucker length	800	595	700	–	506	466–591	540
Ventral sucker width	600	544	–	570–580	547	376–502	490
Cirrus-sac length	204	–	–	311	170	223	510
Cirrus-sac width	119	–	–	294	200	99	187
Right testis length	220	510		207	420–460	295–394	420
Right testis width	500	340	340–430	276	290–330	161–287	290
Left testis length	200	340	–	207	300–330	269–403	340
Left testis width	120	222	340–430	224	210–250	179–287	250
Ovary length	–	–	–	432	400–500	448–645	578
Ovary width	600	510	500	449	500	358–518	470
Mehlis' gland length	935	830	–	536	–	305–466	850
Mehlis' gland width	395	440	–	259	–	537–645	320
Egg-length	–	110	120	103	67–109	70–108	80
Egg-width	–	55	63	69	42–65	59–73	50

Recently, the sequence of *Ch. ferox* generated in our study was used in the comprehensive molecular phylogeny of the Echinostomatoidea Looss, 1899 (Tkach et al., 2016). The analyses show no support for the subfamily Chaunocephalinae Travassos, 1922, which resulted in a synonymisation of the Chaunocephalinae with the Echinostomatidae (*sensu stricto*). We believe the sequence provided by our study will be also useful for the molecular identification of the life-cycle stages of the species in the future studies.

References

Bashkirova, E. Y. 1941. Bird echinostomatids in the USSR with a review of their life-cycles. *Trudy Bashkirskoi Nauchnoissledovatel'skoi Veterinarnoy Opitnoy Stantsii*, **3**, 243–300 [In Russian].

Brglez, J. 1975. First record of *Chaunocephalus ferox* (Rudolphi, 1795) Dietz, 1909 and *Echinoparyphium petrowi* Nevostrueva, 1953 (Trematoda, Echinostomatidae) in Yugoslavia. *Acta Parasitologica Iugoslavica*, **6** (2), 81–84.

Dietz, E. 1910. Die Echinostomiden der Vögel. *Zoologische Jahrbücher*, **12**, 256–512.

Georgieva, S., Selbach, C., Faltýnková, A., Soldánová, M., Sures, B., Skírnisson, K., Kostadinova, A. 2013. New cryptic species of the '*revolutum*' group of *Echinostoma* (Digenea: Echinostomatidae) revealed by molecular and morphological data. *Parasites* & *Vectors,* 6, 64.

Iskova, N. I. 1973. Data on trematode fauna of the Columbiformes and Ciconiiformes in the Crimea and Northern Black Sea area. *Parasity, parasitozy ta shlyahy yich likvidacii*. Naukova dumka, Kiyv, 62–65 [In Ukrainian].

Iskova, N. I. 1985. Trematodes. Echinostomata. Naukova dumka, Kiev, 1–200. (*Fauna Ukrainy, Vol. 34, is. 4*) [In Russian].

Ivanitsky, S. V. 1928. On the trematode fauna of vertebrate of Ukraine: based on 26 UHE. *Veterynarne dilo*, **2**, 30–48 [In Russian].

Kornyushin, V. V., Smogorzhevskaya, L. A., Iskova, N. I. 2004. Circulation of helminthes in colonies of Pelicaniformes and Ciconiiformes in the south of Ukraine. *Branta*, **7**, 241–277 [In Russian].

Kostadinova, A. 2005. Family Echinostomatidae Looss, 1899. *In*: Jones, A., Bray, R. A. & Gibson, D. I., eds. *Keys to the Trematoda*, Vol. 2. CAB International and The Natural History Museum, Wallingford, 9–64.

Kowalewski, M. 1896. Materyaly do fauny helmintologicznej pasorzytniczej polskiej. II. *Spraw. Kom. fizyogr. Akad. Umiej. w Krakowie*, **32**, 251–258.

Ku, C-T., Chiu, C-C., Li, M-M., Chu, H., 1974. Echinostome fauna from the intestine of the black stork (*Ciconia nigra*) of China. *Acta Zoologica Sinica*, **20** (1), 27–34.

Mehra, H. R. 1980. Plathelminthes. Vol. 1. Trematoda. *In*: Ananthakrisnnan, T. H., ed. *The fauna of India and the adjacent countries*. Government of India, New Delhi, 1–418.

Poonswad, P., Chatikavanij, P., Thamavit, W. 1992. Chaunocephalosis in a wild population of Asian open-billed storks in Thailand. *Journal of Wildlife Diseases*, **28** (3), 460–466.

Saad, A. I. 2009. First record of two digenetic trematodes; *Chaunocephalus ferox* (Rudolphi, 1795) Dietz, 1909 and *Cathaemasia hains* (Rudolphi, 1809) Looss, 1899 in Egypt and role of the migratory birds in introducing of new parasites to Egyptian fauna. *J. Egypt. Ger. Soc. Zool.*, **58 D**, 85–99.

Saidul, I., Anjan, T., Taibur, R., Nabaneel, B. 2009. Observations on the nodular fluke *Chaunocephalus ferox* (Rudolphi, 1795), Dietz, 1909 infection in an open billed stork (*Anastomus oscitans*) from Kaziranga National Park, Assam. *Journal of Veterinary Parasitology*, **23** (2), 143–145.

Santoro, M., Degli Uberti, B., Galiero, G., Di Prisco, F., D'Alessio, N., Veneziano, V. 2013. *Chaunocephalus ferox* (Digenea: Echinostomatidae) infection associated with fatal cachexia in a white stork (*Ciconia alba*). *Helminthologia*, **50** (3), 181–184.

Sergienko, V. I. 1968. *Fauna of flatworm and nemathelminthes of water wader birds from the river basin of upper Dnestr*. PhD thesis. Lvov, 1–24 [In Russian].

Sergienko, V. I. 1978. The collection of trematodes (Trematoda, Rud., 1808), parasitizing in birds. *In:* Pasternak, S. I. *Katalog muzeynich fondiv*. Zbirnik naukovich prac. Naukova Dumka, Kyiv, 169–181 [In Ukrainian].

Skrjabin, K. I., Bashkirova, E. Y. 1956. *Family Echinostomatidae. Osnovy Trematodologii*, **12**, 53–930 [In Russian].

Smogorzhevskaya, L. A., Iskova, N. I., Kornyushin, V. V., Shalimova, A. N. 1978. Materials to fauna of helminths of *birds* from the *Black Sea Biosphere Reserve. In*: Voinstvensky, M. A. et Myakushko, V. K. *50 years of Black Sea Biosphere Reserve*. Naukova Dumka, Kyiv, 141–152 [In Russian].

Tkach, V. V., Littlewood, D. T. J., Olson, P. D., Kinsella, J. M., Swiderski, Z. 2003. Molecular phylogenetic analysis of the Microphalloidea Ward, 1901 (Trematoda: Digenea). *Systematic Parasitology*, **56**, 1–15.

Tkach, V. V., Kudlai, O., Kostadinova, A. 2016. Molecular phylogeny and systematics of the Echinostomatoidea Looss, 1899 (Platyhelminthes: Digenea). *International Journal for Parasitology*, **46** (3), 171–185.

Yamaguti, S. 1971. *Synopsis of Digenetic Trematodes of Vertebrates*. Keigaku Publishing, Tokyo, 1–1074.

Received 22 December 2015
Accepted 23 February 2016