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Echinoparyphium limosorum n. sp. (Trematoda: Echinostomatidae) from Black-tailed Godwit, *Limosa limosa* (Aves, Charadriiformes) in Slovakia

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Summary

Echinostomatid trematode *Echinoparyphium limosorum* n. sp. from the charadriiform bird *Limosa limosa* is described on basis of morphometrical study of museum material. The new species is characterized by medium-sized body up to 4.9 mm long, reniform head collar up to 511 wide, armed with 48 – 51 collar spines up to 91 μm, arranged in double row. The new species is largely similar to *Echinoparyphium recurvatum*, however, the most remarkable difference lays in the higher number of collar spines which are 48 – 51 in *E. limosorum* n. sp. but 45 in *E. recurvatum*. The authors discuss relative impact of numerical generic characters and propose an amending of the diagnosis of the genus *Echinoparyphium* given by Kostadinova (2005) as follows: Collar spines up to 51, sharply pointed, all in double row.

Keywords: *Echinoparyphium limosorum*; Trematoda; *Limosa limosa*; Charadriiformes; Slovakia; new species

Introduction

The research of helminths parasitic in migratory charadriiform birds which had been conducted in Slovakia from fifties to seventies of the last century, often revealed new information on alien parasites imported by their bird hosts from southern wintering habitats to Central Europe (e.g. Macko *et al.*, 2008a, b). Black-tailed Godwit *Limosa limosa*, population breeding in eastern Europe, i.e. in Ukraine, Belarus, Russia and, according to Ferianc (1977) very probably also in eastern Slovakia, mainly winters in East Africa as reported in the "Management Plan for Black-tailed Godwit *Limosa limosa* 2007 – 2009" under the program Natura 2000 of The European Commision (ec.europa.eu/environment/nature/conservation/wildbirds/hunting/docs/black_tailed_godwit.pdf). Nowadays, the Black-tailed Godwit is regarded a threatened species and the mentioned EC Management Plan is aimed to restoration of its populations in Europe. Therefore, recent collections of intestinal parasites of the bird are impracticable; however, re-evaluations of museum material often lead to a discovery of new non-described taxa.

The recent paper describes a new echinostomatid trematode species *Echinoparyphium limosorum* n. sp. on basis of 5 specimens from a *L. limosa* host. Since the year 1962, the material has been deposited as permanent slides in the personal collection of the first author. The paper proposes an amending of the generic diagnosis of *Echinoparyphium*.

Material and Methods

Five trematodes were recovered from a single host bird Black-tailed Godwit shot at the Dvor Keresztúr near the villages Pavlovo and Svätá Mária at the River Bodrog, southeastern Slovakia, in May 1962. The isolated trematodes were compressed, fixed in alcohol-formol-acetic acid (AFA), stained in Semichon's carmine and mounted in Canada balsam. Morphological traits were studied and measured using the microscope Olympus equiped with digital camera. Measurements are in micrometres (μm) except where otherwise indicated.

Except of detecting traditionally used morphological features, following indices were calculated according to Kostadinova (2005): BW% - the shape of body assessed by the maximum body-width as a proportion of the body-length; FO% - the relative length of the forebody (i.e. the distance from the anterior extremity to the centre of ventral sucker) expressed as a proportion of the body-length; U% – the relative uterine length (i.e. distance between the ovary and the posterior margin of the ventral sucker) expressed as a proportion of body-length; T% - relative length of the post-testicular field (i.e. distance from the posterior testis margin to the posterior extremity) estimated as a proportion of body-length.

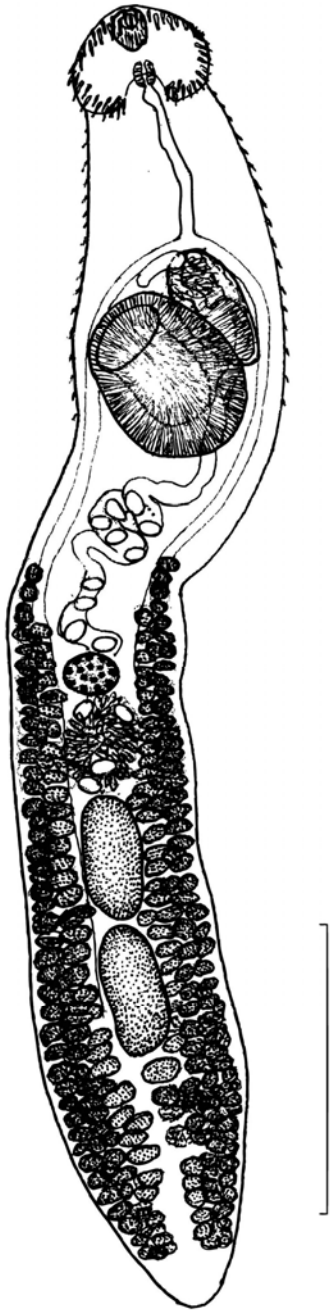

Fig. 1 *Echinoparyphium limosorum* n. sp. Holotype. Scale-bar 1 mm.

Results

Echinoparyphium limosorum n. sp.
Type-host: *Linosa limosa* (L.) (Aves, Charadriiformes, Scolopacidae).
Type-locality: Dvor Keresztúr, River Bodrog (eastern Slovakia), 48°42´, 21°87´.
Site of infection: Small intestine.
Prevalence: 1 bird host.
Material studied: Holotype No. 380/62c; Paratypes: 380/62i - 4 specimens. All 5 specimens complete, gravid, paratypes with a few collar spines falling off. The material is deposited in the Helminthological collection at the Parasitological Institute SAS, Hlinkova 3, 040 01 Košice, Slovakia.
Etymology: Specific name of the trematode has been derived from the name of the bird type host.

Description (Figures 1 – 3). Data in brackets represent holotype characteristics.
Body elongate, with maximum width at level of ventral sucker. Body length 3 862 – 4 913 (4 684); maximum body width at the level of ventral sucker 628 – 739 (785), BW% = 15.2 – 16.8 (16.8). Body width at level of testes 549 – 706 (699). Forebody 989 – 1 350 (1 311), FO% = 23.1 – 28.8 (28.0), ventrally slightly bent. Tegument armed with small spines reaching level of posterior margin of ventral sucker, maximum length 15 at level of intestinal bifurcation (Fig. 1).
Head collar reniform, 363 – 511 (511) wide, with ventral ridge. Collar spines 48 – 51 (51, Fig. 2), in double row. Ventral spines 6 in number, 67 – 91 x 12-17 (67 – 83 x 15.5 – 17), lateral spines 49.4 – 69 x 11-14 (56 – 67 x 10.4 – 13), dorsal oral spines 39 – 68 x 9 – 12 (39 – 65 x 10.4 – 13), dorsal aboral spines longer, 64 – 75 x 11 – 14.3 (68 – 73 x 13 – 14). A single specimen show extremely small spines probably due to their vertical orientation.
Oral sucker spherical or subspherical (in 1 specimen pear-shaped), 111 – 164 x 109 – 146 (164 x 140). Ventral sucker muscular, 484 – 640 x 421 – 628 (640 x 523), its middle located 956 – 1350 (1313) from anterior body margin. Prepharynx of variable length, up to 78 (26) long. Pharynx muscular, elongate-oval, 91 – 111 x 60 – 90 (106 x 87). Oesophagus not very distinct, about 530 long. Intestinal bifurcation anterior to ventral sucker, intestinal branches uncertain.

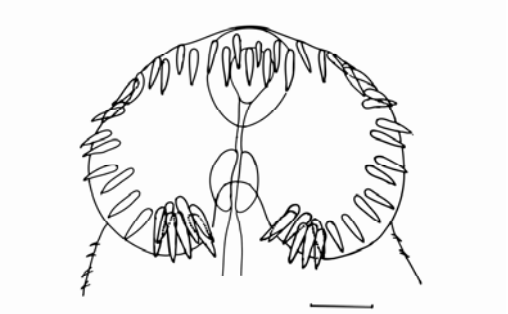

Fig. 2 *Echinoparyphium limosorum* n. sp. Holotype. Head collar with 51 collar spines. Scale-bar 100 µm.

Testes tandem, elongate-oval, smooth, contiguous or nearly contiguous, located in second body half; anterior testis 444 – 543 x 234 – 273 (468 x 249), posterior testis 475 – 577 x 203 – 271(483 x 241). Post-testicular region 706 – 957 (909.5) long, T% = 16.5 – 20.3 (19.4) of body length. Cirrus-sac muscular, elongate-oval, 367 – 413 x 156 – 280 (413 x 280), antero-dorsal to ventral sucker, reaching from intestinal bifurcation to middle of ventral sucker. Internal seminal vesicle saccular, simple. Pars prostatica developed, distinct in anterior third of cirrus-sac. Cirrus tubular, unarmed, of unclear length. In region of

genital pore, funnel-like opening resembling more or less muscular sphincter, is located.
Ovary spherical or nearly oval, comact, median, pre-equatorial in 2 specimens and equatorial in 3 specimens, 156 – 218 x 191 – 226 (156 x 204) in diameter. Distance from ventral sucker to ovary 659 – 989 (769), U% = 15.5 – 18.7 (16.4) in 4 specimens and U% = 23.1 in 1 specimen. Mehlis´ gland large, median, immediately pretesticular, spherical or elongate-oval, 273 – 468 x 226 – 351 (390 x 304). Uterus moderately developed, with few intercaecal loops between ovary and ventral sucker. Eggs not numerous, 14 – 42 (14). Egg size depending on their number: 88 – 114 x 52 – 69 (104 – 113 x 61 – 69) in 4 specimens with 14 – 19 eggs, 68 – 86 x 53 – 60 in a specimen with 42 mostly deformed eggs. Vitellarium follicular with follicules up to 78 – 86 x 133 – 152, distributed in 2 lateral fields between level of middle uterus and posterior extremity, in post-testicular region fields not confluent in 3 specimen, nearly confluent in 1 specimen and confluent in 1 specimen.
Excretory vesicles not observed, excretory pore terminal.

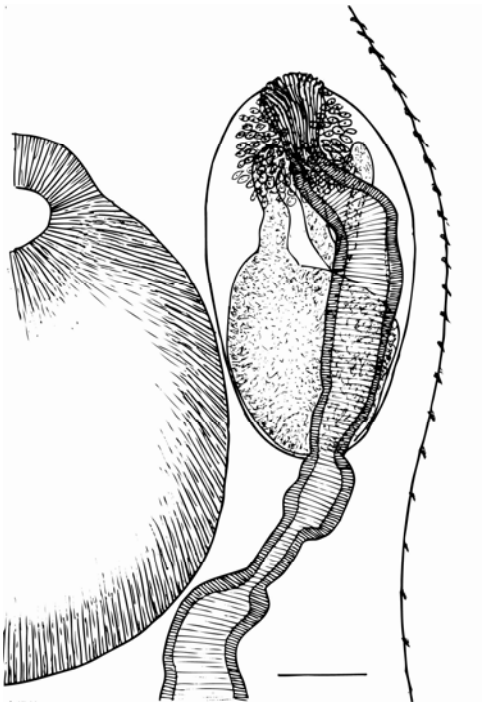

Fig. 3 *Echinoparyphium limosorum* n. sp. Paratype. Terminal part of genital ducts with not protuberant cirrus, lateral view. Scale-bar 100 μm.

Remarks
The above characteristics correspond with the generic diagnosis of the *Echinoparyphium* Dietz, 1909, as stated by Kostadinova (2005), including the proposed indices BW%, FO% and T%. The exceptions lie in 1) higher number of collar spines (48 – 51) in the new species, versus 29 – 45 in the generic diagnosis, 2) slightly longer uterus in a single specimen (U% = 23,1 versus 3 – 20 in generic diagnosis) due to an apparent damage of the anterior part of the specimen, 3) discrepancy in number and size of eggs between 4 specimens containing 14 – 19 larger eggs and 1 specimen with 42 much smaller eggs; this might be easily interpreted in terms of "crowding effect" (see the similar feature described in *Dicrocoelium dendriticum* by Macková *et al.* (1997), and 4) higher level of variation in the distribution of vitelline fields in post-testicular region which either are separated or are confluent.

Differential diagnosis
As the number of collar spines of echinostomatids is regarded as one of the weightiest differential generic features, we have compared the new species not only with the *Echinoparyphium* congeners which according to the latest generic diagnosis possess maximum of 45 spines (Kostadinova, 2005), but also with additional species of the relative genera having from 43 to 55 collar spines.
In Black-tailed Godwit, several *Echinoparyphium* species were found in Central Europe (Hudec, Šťastný *et al.*, 2005). They are *E. aconiatum* (Dietz, 1904) (having 37 collar spines), *E. cinctum* (Rudolphi, 1802) (43 spines), *E. clerci* Skrjabin, 1915 (41 spines), *E. mordwilkoi* Skrjabin, 1915 (45 spines), *E. paracinctum* Bykhovskaya-Pavlovskaya, 1953 (43 spines) and *E. recurvatum* (Linstow, 1873) (45 spines).
Out of the above mentioned *Echinoparyphium* parasites of *L. limosa*, only the species *E. recurvatum* and *E. mordwilkoi* possess the higher number of 45 collar spines. The new species *E. limosorum* is relatively very similar to *E. recurvatum*. They differ each other mainly in 1) the number and size of collar spines (48 – 51 in the number, up to 91 μm long in *E. limosorum* vesus 45 and up to 76 μm in *E. recurvatum* (see Kanev *et al.*, 2008); 2) morphology of vitelline fieds in the post-testicular region which may be confluent in *A. limosorum* but not in *E. recurvatum*.
Regarding *E. mordwilkoi,* Kanev *et al.* (2008) considered this species belonging to the *E. recurvatum* species complex. Except of the difference in the number of spines (45 versus 48 – 51 in the new species), *E. mordwilkoi* has the smaller body size (up to 2.8 x 0.54 mm versus 3.8 – 4.9 x 0.6 – 0.7 mm in the new species) and the relatively long post-testicular part T % = 31.4, as counted from the drawing of *E. mordwilkoi* published by Skrjabin and Bashkirova (1956), versus T % = 20.3 in the new species.
The congeners *E. aconiatum*, *E. cinctum*, *E. clerci* and *E. paracinctum* have not only the lower number of collar spines but also different size of the body and internal organs.
Skrjabin and Bashkirova (1956) enumerated also the species *Echinoparyphium petrowi* Nevostrueva, 1953 having 49 collar spines. However, the species has been later designated as the type species of the newly established genus *Neoacanthoparyphium* Yamaguti, 1958 (Yamaguti, 1958) and it differed from *E. limosorum* n. sp. aparently in the minute size of the body up to 0.8 mm, small organs and collar spines of very distinct size.
Considering *Echinostoma* Rudolphi, 1809 species, only *E. academica* Skrjabin, 1915 was found even in Back-tailed Godwit in various regions (Bychovskaya-Pavlovskaya, 1962; Hudec and Šťastný, 2005). It differs from *L. limo-*

sorum n. sp. by 1) much larger body up to 8.5 x 1.2 mm; 2) lower number of 43 collar spines; 3) characteristic scale-like type of tegumental armament and 4) well developed uterus with numerous eggs.
There are another bird *Echinostoma* species described from Europe and Asia, possessing similar number of collar spines as the new species: *E. chloropodis* Zeder, 1800 (47 spines), *E. coecale* Murashkinzev, 1937 (49 spines), *E. corvi* Yamaguti, 1935 (47), *E. sarcinum* Dietz, 1909 (47) and *E. travassosi* Skrjabin, 1924 (47) (Skrjabin & Bashkirova, 1956). However, all of them are characterized by rather large body size, well developed uterus, numerous eggs which together with other features differentiate them clearly from *E. limosorum* n. sp. Within the genera *Echinostoma* and *Euparyphium*, there are some other species of medium-sized body having around 50 collar spines, parasitic various bird and mammal hosts from South America, Australia and the Phillippines. However, all the species differ from *E. limosorum* n. sp. by many diagnostic markers.

Discussion

An identification of many echinostomatid species and genera remains still problematic due to the fact that distinct differential markers still do not exist on the morphological level. For instance, Yamaguti (1958) differentiated two groups of genera, one comprising *Echinostoma* and the other *Echinoparyphium*, using uncertain characteristics: "Uterus more or less long, with numerous eggs" versus "Uterus rather short with not numerous eggs". Therefore, Kostadinova (2005) tried to find more accurate and relevant criteria which would help to distinguish echinostomatid genera. Apart from the traditionally used features like the degree of development and the morphology of the collar, the number, shape, arrangement and relarive size of the collar spines, the morphology of the male terminal genitalia, etc., Kostadinova (2005) proposed four additional indices, each assessed as a proportion of the character to the body length (see Material and Methods). On one hand, such relative criteria decrease the variation in real size of morphological features, however, the boundary values of indices might overlap when comparing extremely varying populations or species including those of discussed genera *Echinoparyphium*, *Echinostoma*, and *Euparyphium*. Moreover, newly described species may be characterized by morphological markers which in general fit with some generic diagnosis but a single feature surpasses it.
The recently described *E. limosorum* n. sp. demonstrates the above mentioned problems. All but one characteristic correspond with the generic diagnosis of *Echinoparyphium*; the exception is represented by the higher number of collar spines than 45, until now the maximum number within the genus *Echinoparyphium* (Kostadinova, 2005). It is evident, that the strict numerical characters of individual echinostomatid genera should be modified in some cases. Therefore, we suggest amending the generic diagnosis as follows: Collar spines up to 51, sharply pointed, all in double row.
Similar situation might appear already when a new species are described and their generic affiliation seems to be unclear. It is apparent that some room for subjective decision should be left to specialists experienced in alfha taxonomy, especially in a case that the museum material is evaluating without any opportunity to use additional, e.g. molecular approaches (see Kostadinova *et al.*, 2003). Besides, especially valuable are recent re-descriptions of rarely found species even though they were only on morphological basis and omit molecular analyses (e.g. Santoro *et al.*, 2008).
Many problems lie in an uncertainty of the generic category as discussed by Macko *et al.* (2008a, b). In any case, it is inevitable to assemble and publish papers summarizing current species spectrum of individual genera which supplement the current keys directed only to lists of genera and higher taxa (Gibson *et al.*, 2002, Jones *et al.*, 2005, Bray *et al.*, 2008). Fortunately, such revisions are currently appearing also within the traditionally very problematic family Echinostomatidae (Kostadinova & Gibson, 2002, Faltýnková et. al., 2008a, b).

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