Overview of renicolid digeneans (Digenea, Renicolidae) from marine gulls of northern Holarctic, with remarks on their species statuses, phylogeny and phylogeography

Kirill V. Galaktionov, Anna I. Solovyeva, April M. H. Blakeslee and Karl Skirnisson

Cite this article: Galaktionov KV, Solovyeva AI, Blakeslee AMH, Skirnisson K (2023). Overview of renicolid digeneans (Digenea, Renicolidae) from marine gulls of northern Holarctic with remarks on their species statuses, phylogeny and phylogeography. Parasitology 150, 55–77. https://doi.org/10.1017/S0031182022001500

Received: 28 July 2022
Revised: 2 October 2022
Accepted: 14 October 2022
First published online: 2 November 2022

Key words:
Life cycle; Littorina; marine gulls; molecular phylogeny; Renicola keimahuri; Renicola parvicaudatus; Renicolidae; Rhodometopa cercariae

Author for correspondence:
Kirill V. Galaktionov,
E-mail: kirill.galaktionov@gmail.com

Abstract

Renicolid digeneans parasitize aquatic birds. Their intramolluscan stages develop in marine and brackish-water gastropods, while metacercariae develop in molluscs and fishes. The systematics of renicolidas is poorly developed, their life cycles are mostly unknown, and the statuses of many species require revision. Here, we establish based on integrated morphological and molecular data that adult renicolidas from gulls Larus argentatus and Larus schistisagus and sporocysts and cercariae of Cercaria parvicaudata from marine snails Littorina spp. are life-cycle stages of the same species. We name it Renicola parvicaudatus and synonymized with it Renicola roscovitus. An analysis of the cox1 gene of R. parvicaudatus from Europe, North America and North Asia demonstrates a low genetic divergence, suggesting that this species has formed quite recently (perhaps during last glacial maximum) and that interregional gene flow is high. In Littorina saxatilis and L. obtusata from the Barents Sea, molecular analysis has revealed intramolluscan stages of Cercaria littorinae saxatilis VIII, a cryptic species relative to R. parvicaudatus. In the molecular trees, Renicola keimahuri from L. schistisagus belongs to another clade than R. parvicaudatus. We show that the species of this clade have cercariae of Rhodometopa group and outline morphological and behavioural transformations leading from xiphidiocercariae to these larvae. Molecular analysis has revealed 3 main phylogenetic branches of renicolidas, differing in structure of adults, type of cercariae and host range. Our results elucidate the patterns of host colonization and geographical expansion of renicolidas and pave the way to the solution of some long-standing problems of their classification.

Introduction

Renicolidas is a small family of digeneans (Trematoda, Digenea), currently comprising fewer than a hundred species, taking into account the descriptions of the larvae (Sudarikov and Stenko, 1984; Munyer and Holloway, 1990; Kharoo, 2013). Their transmission is implemented in marine and estuarine ecosystems. In the complex life cycle of renicolidas, the role of the first intermediate host is played by marine and brackish-water gastropods, while the role of the second intermediate host is mostly played by molluscs and fish. Adult renicolidas parasitize kidneys and ureters of marine or aquatic birds, exhibiting a strong pathogenic effect on their hosts (Campbell and Sloan, 1943; Hill, 1952, 1954; Riley and Owen, 1972; Mahdy and Shaheed, 2001; Jerdy et al., 2016; Matos et al., 2021). As they grow, their body becomes densely packed with eggs. It is next to impossible to discern diagnostic characters in such a worm. Considering, in addition, that adult worms of closely related species are similar morphologically, differentiating among them is a challenge. It is therefore unsurprising that the systematics of renicolidas is poorly developed. Only 2 genera are recognized within the family: Renicola Cohn, 1904 and Nephromonorcha Leonov, 1958. Their adults differ in the number of testes: 2 separate testes in the former and 1 testis (resulting from merging of the 2) in the latter genus (Sudarikov and Stenko, 1984; Gibson, 2008). Attempts to elaborate the classification of renicolidas (e.g. Wright, 1957; Odening, 1962; Riley and Owen, 1972) have not gained general recognition (reviewed in Gibson, 2008; Kharoo, 2013).

Intramolluscan stages of renicolidas are represented by mother sporocysts, which look like small membrane-enveloped aggregations of cells, and cercariae-producing sac-like daughter sporocysts, parasitizing the molluscan gonad and digestive gland (Wright, 1956; James, 1969). Life cycles of only a few renicolid species have been elucidated (Stunkard, 1964; Werding, 1969; Prevot and Bartoli, 1978). At the same time, several cercariae whose descriptions are present in the literature are considered as renicolid larvae (e.g. Martin and Gregory, 1951; Cable, 1956, 1963; James, 1968a, 1969; Martin, 1971; Sannia and James, 1977; Cannon,
1978, 1979; Hechinger, 2007, 2019; Martorelli et al., 2008; Flores et al., 2019). There are among them cercariae with contrasting morphotypes: from typical xiphidiocercariae (small styled cercariae with a simple tail) to large non-styled larvae of the Rhodometopa group with tail fins (Wright, 1953, 1956; Odening, 1962; Cable, 1963; Stunkard, 1971; Prevot and Bartoli, 1978). Such a high diversity of cercarial morphotypes within a small family is unusual for trematodes (Galaktionov and Dobrovolskij, 2003). This matter apparently requires clarification, all the more so, as the results of molecular studies are ambiguous: some molecular data confirm that the larvae of the Rhodometopa group belong to renicolids (Matos et al., 2019), while other data indicate the opposite (Heneberg et al., 2016).

Despite the contrasting differences in the morphotype, species identification of cercariae is problematic because they are morphologically very similar in closely related species. This is the case, in particular, of renicolid intramolluscan stages from intertidal snails Littorina spp. in the North Atlantic (NA). Stunkard and Shaw (1931) and Stunkard (1932) described cercariae Cercaria parvicaudata Stunkard and Shaw, 1931 and Cercaria roscovitai Stunkard, 1932 from these molluscs, but they are extremely difficult to differentiate (Stunkard, 1950; Galaktionov and Skirniss, 2000). After the life cycle of, presumably, C. roscovitai was elucidated and the species was named Renicola roscovita (Stunkard, 1932) Werding, 1969, it has been generally assumed that this is the dominant renicolid species using periwinkles as the first intermediate hosts in NA (Lauckner, 1980, 1983). In a study of cercariae from intertidal molluscs in Iceland, Galaktionov and Skirniss (2000) recorded only larvae corresponding to C. parvicaudata described by Stunkard and Shaw (1931) and Stunkard (1950). No cercariae matching the description of C. roscovitai have been found during long-term studies of the fauna of digenean intramolluscan stages associated with Littorina spp. at the coasts of NA and the North Pacific (NP) (K. V. Galaktionov, personal observation). All these observations indicate that the question of the species composition of renicolids in NA and NP should be revisited.

The aim of this study was to ascertain the species composition of renicolids using periwinkles as the first intermediate hosts and to determine their transmission routes into NA and NP. We used an integrative approach, combining the analysis of morphological and molecular data, which has been shown to be the most effective in addressing taxonomy, phylogeny and elucidation of digenean life cycles (Blasco-Costa and Poulin, 2017). Relatively few studies on renicolids have employed this approach (Skirniss et al., 2002–2003; Hechinger and Miura, 2014; O’Dwyer et al., 2014, 2015; Pattucci et al., 2015; Heneberg et al., 2016; Flores et al., 2019; Matos et al., 2019), and our study is an addition to their number. In the course of our research on trematodes from the nearshore areas of NA and NP seas, we have collected and analysed extensive material on both intramolluscan stages and adults of renicolids from coastal birds, including gulls. Based on this material, we ascertained the species composition of renicolids from gulls in NA and NP and outlined the ways towards the elucidation of some aspects of their classification, evolution and ways of host colonization and geographical expansion. In addition, we confirmed that the larvae of Rhodometopa group belonged to renicolids and suggested how the cercariae of this type could have originated during the evolution of the taxon.

**Material and methods**

**Material collection and treatment**

The material presented in this study was collected from definitive and intermediate hosts (birds and molluscs) in 2002–2021 on the Atlantic coasts of Europe and North America and Pacific coast of North Asia (Table 1). Gastropod molluscs Littorina saxatilis (Olivi, 1792) and Littorina obtusata (Linnaeus, 1758) were collected in the intertidal zone of the White Sea, Barents Sea (Eastern Murman and Finnmark) and Iceland, Littorina sikana Philippi, 1846, in the Sea of Okhotsk (Magadan region) and Littorina littorea (Linnaeus, 1758), in the White Sea and the North Sea (Texel, the Netherlands) (Table 1). We also included in the molecular analysis C. parvicaudata isolates from L. littorea collected at the coasts of North East Atlantic (NEA) and North West Atlantic (NWA) during the study by Blakeslee and Byers (2008), with the sequence data reported in Blakeslee and Fowler (2012), in the summer months between 2002 and 2005 (Table 1). Herring gull Larus argentatus Pontoppidan, 1763 and slaty-backed gull Larus schistisagus Stejneger, 1884 were obtained by shooting in accordance with local regulations in South-West Iceland (Reykjavik region) and the Sea of Okhotsk (Magadan region), correspondingly.

The molluscs were dissected under a stereomicroscope to identify those infected with renicolid intramolluscan stages. Some snails were placed in plastic jars filled with seawater (1 snail per jar) and exposed to sunlight or direct artificial light for 1 h. The jars were examined under a stereomicroscope and the individuals that had shed cercariae of Renicola spp. were selected. These snails, kept in the refrigerator under 4°C, were used as a source of cercariae, which were obtained when required following the same procedure as in case of freshly collected snails.

The species of renicolid intramolluscan stages was identified on the basis of the original descriptions by Stunkard and Shaw (1931) and Stunkard (1932, 1950). Live sporocysts and cercariae were observed, measured and photographed using Olympus CH40 compound microscope equipped with an Olympus XC-30 digital camera at the ‘Kartesh’ White Sea Biological Station of the Zoological Institute of the Russian Academy of Science (ZIN RAN); Leica compound microscope in the Institute of Pathology (Keldur, Iceland) and Leitz Dialux 20B compound microscope in the Institute of Biological Problems of the North (Magadan, Russia). Only newly shed cercariae were used for morphometric studies and scanning electron microscopy (SEM). Cercariae to be measured were fixed by heating in a drop of seawater on the object slide (until the water started to evaporate), and then gently pressed with a coverslip. Sporocysts and encysted metacercariae were measured in vivo. For SEM, we used cercariae C. parvicaudata newly shed from the White Sea L. littorea. Cercariae fixation procedure and treatment before SEM examination were done as described in Galaktionov et al. (2021). The treated cercariae were viewed under a FEI Quanta 250 scanning electron microscope in ‘Taxon’ Research Resource Center (http://www.ckp-rf.ru/ckp/3038/) of ZIN RAS. For molecular studies, we used renicolid intramolluscan stages whose species had been tentatively identified based on morphological criteria. This material was fixed in 95% ethanol.

Gulls were dissected and the renicolid individuals were extracted from the kidney. These adults were fixed in 70% ethanol under a slight pressure of a coverslip. Samples of adults were stored in 70 and 95% ethanol for further morphological and molecular analysis, correspondingly. Carmine-stained whole mounts were used for morphological studies, to make drawings and photographs using Leica DM2500 compound microscope with camera lucida and ToupCam UCMOS14000 digital camera. All measurements presented in the paper are in micrometres, with the mean in parentheses. Drawings were made with the aid of camera lucida.
Table 1. List of samples used in this study and corresponding GenBank accession numbers

| Sample ID | Host species | Place | Region | Coordinates | GenBank accession numbers |
|-----------|--------------|-------|--------|-------------|--------------------------|
| 1stOP     | *L. sitkana* | Veselaya Bay | Sea of Okhotsk, Russia | 59°29.701′ N 150°55.176′ W | ON650718 ON652703 – |
| 4IMR      | *L. argentatus* | Akkrakot | NE Atlantic, SW Iceland | 64°18.270′ N 22°2.349′ W | – ON652704 – |
| 7saxIP    | *L. saxatilis* | Akkrakot | NE Atlantic, SW Iceland | 64°18.315′ N 22°2.319′ W | ON650719 ON652705 ON667890 |
| 8OnR      | *L. schistosagus* | Cape Njukiya | Sea of Okhotsk, Russia | 59°29.700′ N 151°4.282′ E | ON650720 ON652706 – |
| 10nR      | *N. lapillus* | Grotta | NE Atlantic, SW Iceland | 64°9.506′ N 22°1.018′ E | ON650721 ON652707 ON667891 |
| 13saxWSP  | *L. saxatilis* | Kerm-Ludy archipelago | White Sea, Russia | 66°25.107′ N 33°48.530′ E | – ON652708 ON667892 |
| 14obTWP   | *L. obtusata* | Korga Islet | White Sea, Russia | 66°18.061′ N 33°27.473′ E | – ON652709 ON667893 |
| 26saxBP   | *L. saxatilis* | Yarnyshnaya Bay | Barents Sea, Russia | 69°5.232′ N 36°3.303′ E | ON650722 ON652710 ON667894 |
| 27ilHR    | *L. littorea* | Texel | Wadden Sea, Netherlands | 53°0.115′ N 4°47.359′ E | ON650723 ON652711 ON667895 |
| 31lRWSR   | *L. littorea* | Cape Krasnyi | White Sea, Russia | 66°24.664′ N 33°42.911′ E | – ON652712 – |
| 32saxIC   | *L. saxatilis* | Grindavik | NE Atlantic, SW Iceland | 63°50.494′ N 22°25.194′ W | ON650724 ON652713 – |
| 41saxBP   | *L. saxatilis* | Yarnyshnaya Bay | Barents Sea, Russia | 69°5.283′ N 36°3.374′ E | – ON652714 – |
| 42saxBP   | *L. saxatilis* | Yarnyshnaya Bay | Barents Sea, Russia | 69°5.169′ N 36°3.142′ E | – ON652715 – |
| 43saxBP   | *L. saxatilis* | Yarnyshnaya Bay | Barents Sea, Russia, | 69°5.161′ N 36°3.120′ E | – ON652716 – |
| 57obtBP   | *L. obtusata* | Dalnevezelenetskaya Bay | Barents Sea, Russia, | 69°07.414′ N 36°5.892′ E | ON650725 ON652717 – |
| 58sIOp    | *L. sitkana* | Veselaya Bay | Sea of Okhotsk, Russia | 59°29.701′ N 150°55.176′ E | ON650726 ON652718 – |
| EUCPESBE1 | *L. littorea* | Esbjerg | Wadden sea, Denmark | 55°28.859′ N 08°24.625′ E | – ON652636 – |
| EUCPESBE2 | *L. littorea* | Esbjerg | Wadden sea, Denmark | 55°28.859′ N 08°24.625′ E | – ON652637 – |
| EUCPDU1R1 | *L. littorea* | Dublin | Irish sea, Ireland | 53°19.10′ N 06°06.58′ W | – ON652638 – |
| EUCPDU1R2 | *L. littorea* | Dublin | Irish sea, Ireland | 53°19.10′ N 06°06.58′ W | – ON652639 – |
| EUCPDU1R3 | *L. littorea* | Dublin | Irish sea, Ireland | 53°19.10′ N 06°06.58′ W | – ON652640 – |
| EUCPDU1R4 | *L. littorea* | Dublin | Irish sea, Ireland | 53°19.10′ N 06°06.58′ W | – ON652641 – |
| EUCPMIND1 | *L. littorea* | Mindin | NE Atlantic, France | 47°16.112′ N 02°10.262′ W | – ON652642 – |
| EUCPMIND2 | *L. littorea* | Mindin | NE Atlantic, France | 47°16.112′ N 02°10.262′ W | – ON652643 – |
| EUCPMIND3 | *L. littorea* | Mindin | NE Atlantic, France | 47°16.112′ N 02°10.262′ W | – ON652644 – |
| EUCPMIND4 | *L. littorea* | Mindin | NE Atlantic, France | 47°16.112′ N 02°10.262′ W | – ON652645 – |
| EUCPMIND5 | *L. littorea* | Mindin | NE Atlantic, France | 47°16.112′ N 02°10.262′ W | – ON652646 – |
| EUCPMOSSN1 | *L. littorea* | Moss | Oslofjord, Norway | 59°25.861′ N 10°39.148′ E | – ON652647 – |
| EUCPMOSSN2 | *L. littorea* | Moss | Oslofjord, Norway | 59°25.861′ N 10°39.148′ E | – ON652648 – |
| EUCPMOSSN3 | *L. littorea* | Moss | Oslofjord, Norway | 59°25.861′ N 10°39.148′ E | – ON652649 – |
| EUCPMOSSN4 | *L. littorea* | Moss | Oslofjord, Norway | 59°25.861′ N 10°39.148′ E | – ON652650 – |
| EUCPMOSSN5 | *L. littorea* | Moss | Oslofjord, Norway | 59°25.861′ N 10°39.148′ E | – ON652651 – |
| EUCPMOSSN6 | *L. littorea* | Moss | Oslofjord, Norway | 59°25.861′ N 10°39.148′ E | – ON652652 – |
| EUCPMOSSN7 | *L. littorea* | Moss | Oslofjord, Norway | 59°25.861′ N 10°39.148′ E | – ON652653 – |
| EUCPMOSSN8 | *L. littorea* | Moss | Oslofjord, Norway | 59°25.861′ N 10°39.148′ E | – ON652654 – |
| EUCPPOSTN1 | *L. littorea* | Ostende | Nothern sea, Belgium | 51°13.593′ N 02°56.596′ E | ON652655 – |
| EUCPPOSTN2 | *L. littorea* | Ostende | Nothern sea, Belgium | 51°13.593′ N 02°56.596′ E | – ON652656 – |
| EUCPPOSTN3 | *L. littorea* | Ostende | Nothern sea, Belgium | 51°13.593′ N 02°56.596′ E | – ON652657 – |
| EUCPTJRN1 | *L. littorea* | Tjarno | Skagerrak, Sweden | 58°53.107′ N 11°07.117′ E | – ON652658 – |
| EUCPTJRN2 | *L. littorea* | Tjarno | Skagerrak, Sweden | 58°53.107′ N 11°07.117′ E | – ON652659 – |
| EUCPTJRN3 | *L. littorea* | Tjarno | Skagerrak, Sweden | 58°53.107′ N 11°07.117′ E | – ON652660 – |

(Continued)
Table 1. (Continued.)

| Sample ID | Host species | Place | Region | Coordinates | GenBank accession numbers |
|-----------|--------------|-------|--------|-------------|--------------------------|
| EUCPTJARM4 | L. littorea | Tjarno | Skagerrak, Sweden | 58°53.107’ N 11°07.117’ E | – | ON652661 – |
| EUCPTRJRV1 | L. littorea | Trouville | English Channel, France | 49°21.851’ N 00°04.871’ E | – | ON652662 – |
| EUCPTRJRV2 | L. littorea | Trouville | English Channel, France | 49°21.851’ N 00°04.871’ E | – | ON652663 – |
| EUCPTRJRV3 | L. littorea | Trouville | English Channel, France | 49°21.851’ N 00°04.871’ E | – | ON652664 – |
| EUCPUBHD1 | L. littorea | Udbyhoj | Kattegat, Denmark | 56°36.565’ N 10°17.986’ E | – | ON652665 – |
| EUCPUBHD2 | L. littorea | Udbyhoj | Kattegat, Denmark | 56°36.565’ N 10°17.986’ E | – | ON652666 – |
| EUCPUBHD3 | L. littorea | Udbyhoj | Kattegat, Denmark | 56°36.565’ N 10°17.986’ E | – | ON652667 – |
| EUCPUBHD4 | L. littorea | Udbyhoj | Kattegat, Denmark | 56°36.565’ N 10°17.986’ E | – | ON652668 – |
| EUCPUBHD5 | L. littorea | Udbyhoj | Kattegat, Denmark | 56°36.565’ N 10°17.986’ E | – | ON652669 – |
| EUCPWBRE1 | L. littorea | Varberg | Kattegat, Sweden | 56°36.565’ N 10°17.986’ E | – | ON652670 – |
| EUCPWBRE2 | L. littorea | Varberg | Kattegat, Sweden | 56°36.565’ N 10°17.986’ E | – | ON652671 – |
| NACPBTH2 | L. littorea | Boothbay | NW Atlantic, USA | 43°50.55’ N 69°37.55’ W | – | ON652672 – |
| NACPBTH5 | L. littorea | Boothbay | NW Atlantic, USA | 43°50.55’ N 69°37.55’ W | – | ON652673 – |
| NACPBTH6 | L. littorea | Boothbay | NW Atlantic, USA | 43°50.55’ N 69°37.55’ W | – | ON652674 – |
| NACCCPMAY1 | L. littorea | Cape May | NW Atlantic, USA | 38°57.349’ N 74°52.568’ W | – | ON652675 – |
| NACCCPMAY2 | L. littorea | Cape May | NW Atlantic, USA | 38°57.349’ N 74°52.568’ W | – | ON652676 – |
| NACCCPMAY3 | L. littorea | Cape May | NW Atlantic, USA | 38°57.349’ N 74°52.568’ W | – | ON652677 – |
| NACCCPMAY4 | L. littorea | Cape May | NW Atlantic, USA | 38°57.349’ N 74°52.568’ W | – | ON652678 – |
| NACPHALIF1 | L. littorea | Halifax | NW Atlantic, USA | 44°37.479’ N 63°33.850’ W | – | ON652679 – |
| NACPHALIF2 | L. littorea | Halifax | NW Atlantic, USA | 44°37.479’ N 63°33.850’ W | – | ON652680 – |
| NACPHALIF3 | L. littorea | Halifax | NW Atlantic, USA | 44°37.479’ N 63°33.850’ W | – | ON652681 – |
| NACPMONTA1 | L. littorea | Montauk | NW Atlantic, USA | 41°04.309’ N 71°51.501’ W | – | ON652682 – |
| NACPMONTA2 | L. littorea | Montauk | NW Atlantic, USA | 41°04.309’ N 71°51.501’ W | – | ON652683 – |
| NACPMONTA3 | L. littorea | Montauk | NW Atlantic, USA | 41°04.309’ N 71°51.501’ W | – | ON652684 – |
| NACPMONTA4 | L. littorea | Montauk | NW Atlantic, USA | 41°04.309’ N 71°51.501’ W | – | ON652685 – |
| NACPMONTA5 | L. littorea | Montauk | NW Atlantic, USA | 41°04.309’ N 71°51.501’ W | – | ON652686 – |
| NACPODIOR1 | L. littorea | Odiorne, Rye | Gulf of Maine, USA | 43°00.215’ N 70°44.986’ W | – | ON652687 – |
| NACPTJRT1 | L. littorea | Point Judith | NW Atlantic, USA | 41°21.767’ N 71°28.828’ W | – | ON652688 – |
| NACPTJRT2 | L. littorea | Point Judith | NW Atlantic, USA | 41°21.767’ N 71°28.828’ W | – | ON652689 – |
| NACPTJRT3 | L. littorea | Point Judith | NW Atlantic, USA | 41°21.767’ N 71°28.828’ W | – | ON652690 – |
| NACPSPOD1 | L. littorea | Vineyard Haven | NW Atlantic, USA | 41°27.520’ N 70°35.164’ W | – | ON652691 – |
| NACPSPOD2 | L. littorea | Vineyard Haven | NW Atlantic, USA | 41°27.520’ N 70°35.164’ W | – | ON652692 – |
| NACPSPOD3 | L. littorea | Vineyard Haven | NW Atlantic, USA | 41°27.520’ N 70°35.164’ W | – | ON652693 – |
| NACPSPOD4 | L. littorea | Vineyard Haven | NW Atlantic, USA | 41°27.520’ N 70°35.164’ W | – | ON652694 – |
| NACPWELL1 | L. littorea | Wells | Gulf of Maine, USA | 43°20.067’ N 70°32.554’ W | – | ON652695 – |
| NACPWELL2 | L. littorea | Wells | Gulf of Maine, USA | 43°20.067’ N 70°32.554’ W | – | ON652696 – |
| NACPWELL3 | L. littorea | Wells | Gulf of Maine, USA | 43°20.067’ N 70°32.554’ W | – | ON652697 – |
| NACPYORKM1 | L. littorea | York | Gulf of Maine, USA | 43°20.067’ N 70°32.554’ W | – | ON652698 – |
| NACPYORKM2 | L. littorea | York | Gulf of Maine, USA | 43°20.067’ N 70°32.554’ W | – | ON652699 – |
| NACPYORKM3 | L. littorea | York | Gulf of Maine, USA | 43°20.067’ N 70°32.554’ W | – | ON652700 – |
| 11 Ersh | L. littorea | Woods Hole, MA | Cape Cod, USA | 41°31.502’ N; 70°40.403’ W | – | ON652701 – |
| 12 Ersh | L. littorea | Woods Hole, MA | Cape Cod, USA | 41°31.502’ N; 70°40.403’ W | – | ON652702 – |

L. littorea, Littorina littorea, L. saxatilis, Littorina saxatilis, L. argentatus, Larus argentatus, L. scitiiisagus, Larus scitiiisagus, N. lapillus, Nucello lapillus
DNA extraction, amplification and sequencing

We determined the sequences of 28S ribosomal RNA (rRNA) and cox1 mitochondrial genes for rediae and cercariae of Renicola spp. from infected periwinkles and birds (Table 1). Genomic DNA was extracted with cetrimonium bromide (CTAB) detergent according to the published protocol with modifications (Winnepenninckx et al., 1993) from ethanol-fixed isolates. Fixed specimens were rinsed in 1X phosphate-buffered saline for 15 min before extraction. The D1–D3 fragment of 28S rRNA gene was amplified with primers ZY-1 (5′-ACCCGCTGAATTTAAGCATAT-3′) (Palm et al., 2009) and 1500R (5′-GCTATCTGAGGGA AACTTG-3′) (Olson et al., 2003) according to the following temperature profile: initial DNA denaturation at 95°C for 5 min, then 30 cycles (95°C for 1 min; 55°C for 30 s; 72°C for 1 min) and a final elongation step at 72°C for 5 min. The cox1 gene fragments were amplified with primers JB3 (5′-TTTTTTTGCGATCCTGAGGTTTAT-3′) and JB4-5 (5′-TAAAGAAGAACATAATGAAAATG-3′) (Bouwers et al., 1992) with the following conditions: initial DNA denaturation at 95°C for 5 min, then 30 cycles (95°C for 1 min; 53°C for 30 s; 72°C for 45 s) and a final elongation step at 72°C for 5 min. PCR reactions were run on the Mastercycler personal 5332 (Eppendorf, USA) thermal cycler. ITS2 fragment was amplified with NC13(ITS2)/F (5′-ATCGATGAA GAA CGC AGC-3′) and DD28SR1′ (5′-ACA AAC AAC CCG ACT CCA AG-3′) primers according to Heneberg et al. (2016). PCR products were purified following a modified protocol (Dychenko et al., 2008; Galaktionov et al., 2021). DNA sequencing was performed at the Development of Molecular and Cellular Technologies Resource Centre at St. Petersburg State University and the University of New Hampshire (Durham, New Hampshire, USA). Two cox1 gene sequences of samples from NWA L. littorea (5′-TAAGAAAAGAACATAATGAAAATG-3′) and DD28SR1′ (5′-ACA AAC AAC CCG ACT CCA AG-3′) primers were kindly provided by Natalia Ershova (University of Chicago). All the sequences obtained in this study were deposited in GenBank (Table 1).

Alignments and phylogenetic analyses

We performed alignment, trimming and basic analyses in Geneious 7.1.4 http://www.geneious.com (Kearse et al., 2012) of the newly generated sequences together with 28S rRNA gene and cox1 partial sequences retrieved from GenBank for other Renicola spp. Genetic divergences among taxa were calculated as uncorrected p-distances for each gene region using MEGA v. X (Tamura et al., 2013). Phylogenetic relationships were reconstructed using Bayesian inference (BI) on MrBayes v. 3.2.6 (Ronquist et al., 2012) and maximum likelihood (ML) on MEGA X (Kumar et al., 2018). The most suitable evolutionary models were determined by the corrected Akaike information criterion in the PartitionFinder program (https://github.com/biHCI/partitionfinder). The Hasegawa–Kishino–Yano model with estimates of gamma-distributed among-site rate variation (HKY + G) was chosen as best fitted for cox1 gene. Kimura 2-parameter model with estimates of gamma-distributed among-site rate variation was chosen for fragments of 28S rRNA genes. Genetic divergences among taxa were calculated as uncorrected p-distances for each gene region using MEGA X (Kumar et al., 2018). Mismatch distribution and Tajima’s D neutrality test were calculated in DNASP 6 program (Rozas et al., 2017). We also performed the species partitioning with clustering algorithm implemented in ASAP tool (Puillandre et al., 2020). Haploptype network was reconstructed with PopArt tool (Leigh and Bryant, 2015).

Results

Molecular results showed that renicolid intramolluscan stages from L. littorea and L. sitkana and most isolates from L. obtusata and L. saxatilis, identified as C. parvicauda based on morphological criteria, belonged to one and the same species. Their sequences also matched that of the adult from the Icelandic hering gull, which made it possible to complete the life cycle of this species. We named it Renicola parvicauda (Stunkard and Shaw, 1931) nov. comb. (see Molecular results and Remarks for details).

Among the isolates from L. obtusata and L. saxatilis, initially identified as C. parvicauda, the analysis of molecular markers made it possible to differentiate intramolluscan stages of the cryptic species, which we named Cercaria littorinae saxatilis VIII larva nov. In slaty-backed gulls of the Sea of Okhotsk, besides R. parvicaudatus, we found the adults of one more Renicola species, which we identified as Renicola keimahuri Yamaguti, 1939.

Description

Family Renicolidae Dollfus, 1939

Renicola parvicauda (Stunkard and Shaw, 1931) nov. comb. [syn. C. parvicauda Stunkard and Shaw, 1931, R. rosovittus (Stunkard, 1932) Werding, 1969; sexual adults of Renicola thaidus Stunkard, 1964].

Zoobank LSID: urn:lsid:zoobank.org:pub:86EDD019-DF69-487C-A6C9-DF790F4966D

Type host (definitive): herring gull L. argentatus Poppeptidand, 1763, slaty-backed gull L. schistisagus Stejneger, 1884 (Laridae). Site in definitive host: kidney.

Type locality: South-West Iceland.

Other localities (in definitive host): Nagaeva Bay, Sea of Okhotsk.

Type material: 11 syntypes (on slides 3732-1, 3732-2, 3733-1, 3733-2, 3734-1 and 3734-2), deposited in the Collection in Helminths, section Trematoda, of the Zoological Institute of the Academy of Sciences, St. Petersburg, Russia. This material represents paragenophores.

First intermediate host: L. littorea (Linnaeus, 1758), L. saxatilis (Olivi, 1792), L. obtusata (Linnaeus, 1758) and L. sitkana Philippi, 1846 (Caenogastropoda: Littorinimorph: Littorinidae) (natural). Site in first intermediate host: gizzard.

Localities (in first intermediate host): NEA, NWA, NP. Secondary intermediate host: Mytilus edulis (Linnaeus, 1758), Cerastoderma edule (Linnaeus, 1758), Argopecten irradians irradians (Lamarck, 1819), occasionally L. littorea, L. saxatilis and L. obtusata.

Representative DNA sequences: 28S rDNA (ON650718, ON650721, ON650723, ON650724, ON650726), cox1 (ON652703, ON652704, ON652707–ON652709, ON652711–ON652713, ON652718, ON652636–ON652702) and ITS2 rDNA (ON667891–ON667893, ON667895) (according to Table 1).

Sexual adults (Table 2, Figs 1 and 2)

The description is based on morphologically identical adults from herring gull obtained in South-West Iceland. One of the adult worms matched intramolluscan stages of C. parvicaudata in the marker DNA sequences.

Body ovoid, rounded anteriorly and attenuated posteriorly. Size of worms varying greatly depending on number of eggs in uterus. Oral sucker subterminal to terminal, transversely elongated-oval. Ventral sucker 3–5 times smaller than oral sucker, in posterior third of body. Ventral sucker poorly discernible in large worms with numerous eggs. Prepharynx absent; pharynx small, often deeply embedded in wall of oral sucker. Oesophagus short, caeca 2, extending into posterior third of body. Testes oval, lying laterally of the ventral sucker, more or less opposite to each other. Testes somewhat larger than right testes. Seminal vesicle lying anteriorly of ventral sucker approximately at level of middle to anterior part of ovary, median or lightly dextral of body midline. Ovary dextral (rarely sinistral), pretesticular, larger than testes, variously lobed. Seminal receptacle median or lightly dextral, just anterior to ventral sucker.
Table 2. Morphometric parameters of adults of Renicola spp. parasitizing gulls

|                      | R. parvicaudatus | R. parvicaudatus | R. rascovitus | R. murmanicus | R. thaidus | R. keimahuri | R. keimahuri | R. sternae | R. lari |
|----------------------|-----------------|-----------------|--------------|--------------|-----------|-------------|-------------|-----------|--------|
|                      | Our data (from | Our data (from | After Werding | After Belopolskaya | After Stunkard | data (from | After Yamaguti | data (from | data (from | data (from |
|                      | Lorus argentatus, | Lorus schistisagus, | (1969) (from | (1952) (from | (1964) | Lorus | (1939) | Okhotsk, | Lius carbo | Lius hirundo |
|                      | Iceland, N = 5) | Sea of Okhotsk, | Lorus | Lorus | Lorus | Schistisagus, | (1939) | N = 18) | (from | (1978) |
|                      |                 | N = 3)          |            |            |          | Sea of |             |           |           |        |
| Body length          | 850–1680 (1173 ± 156) | 1005–1550 (1321 ± 163) | 960–1340 | 528–1143 | 700–1160 | 593–1218 | 1150–2100 | 571–1629 | 1225–1945 |
| Body width           | 429–1062 (705 ± 107) | 556–975 (793 ± 123) | 575–805 | 530–580 | 400–600 | 363–868 | 470–1000 | 514–1057 | 560–1039 |
| Pharynx length       | 100–218 (165 ± 24) | 268–346 (312 ± 40) | 210–240 | 159–185* | 260–300* | 130–367 | 150–200* | 145–285 | 158–227 |
| Pharynx width        | 111–323 (218 ± 40) | 292–387 (338 ± 27) | 250–295 | 105–360 | 239 ± 20 | 174–368 | 195–270 | 193 |
| Pharynx vesicle length | 50–83 (70 ± 5) | 68–83 (76 ± 4) | 42–67* | 29–31 | 52–60* | 64–107 | 40–60* | 48–87 | 65–86 |
| Pharynx vesicle width | 50–73 (63 ± 4) | 60–73 (65 ± 4) | 35–36 | 60–120 (79 ± 4) | 36–87 (65 ± 11) | 54–80 (65) |
| Oesophagus length    | 63–130 (95 ± 19) | 82–130 (106 ± 24) | 34–41* | 20* | 82–109 (92 ± 3) | 60–70* | 84–108 (96 ± 6) | 76–101 (85) |
| Ventral sucker length | 32–75 (49 ± 8) | – | 34–41* | 20* | 82–109 (92 ± 3) | 60–70* | 84–108 (96 ± 6) | 76–101 (85) |
| Ventral sucker width | 30–75 (45 ± 8) | – | 74–104 (91 ± 3) | 84–110 (96 ± 6) | 72–102 (87) |
| Left testes length   | 79–136 (108 ± 14) | 83–107 (97 ± 7) | 35–50* | 49–50 | 60–90* | 58–150 (94 ± 9) | 110–120 | 80–116 (97 ± 14) | 130–220 (165) |
| Left testes width    | 53–126 (81 ± 17) | 60–69 (64 ± 3) | 47–49 | 36–82 (51 ± 4) | 90 | 58–74 (66 ± 11) | 32–117 (75) |
| Right testes length  | 72–170 (125 ± 23) | 81–100 (90 ± 5) | 35–50* | 49–50 | 60–90* | 50–148 (95 ± 8) | 110–120 | 80–116 (95 ± 13) | 100–217 (163) |
| Right testes width   | 50–134 (91 ± 23) | 45–69 (59 ± 7) | 47–49 | 26–78 (53 ± 5) | 90 | 58–74 (66 ± 11) | 48–135 (177) |
| Seminal vesicle length | 43–77 (56 ± 11) | 50–71 (59 ± 6) | 22–37 (31 ± 5) | 35* |
| Seminal vesicle width | 27–60 (40 ± 10) | 24–50 (35 ± 8) | 26–35 (31 ± 3) |
| Ovary length         | 118–214 (162 ± 23) | 125–180 (149 ± 16) | 150–210 | 139 | 160–240 | 91–280 (172 ± 13) | 260–290 | 87–261 (199 ± 55) | 256–435 (357) |
| Ovary width          | 103–180 (137 ± 17) | 130–214 (158 ± 28) | 115–180 | 90 | 120–160 | 30–152 (107 ± 10) | 110–130 | 87–145 (112 ± 14) | 80–238 (146) |
| Seminal receptacle length | 53 | 36–50 (43 ± 7) | 38* |
Uterus strongly developed, occupying most of body. Eggs numerous, elongated (length about 3 times greater than width), operculate, with thin eggshell. Vitellarium follicular; follicles in 2 lateral fields in posterior third of body extended from the base of attenuated posterior part of body to level of middle or anterior border of ovary; consisting of 10–18 large follicles on ovarian side of body and 13–18 on opposite side. follicles most often fusing together. Excretory bladder Y-shaped, with distinct lateral diverticula; bifurcates just posterior to the ventral sucker, arms extending into forebody up to level of oral sucker.

**Intramolluscan stages**

The description is based on examination of intramolluscan stages from *L. littorea* collected in Texel (the Netherlands) and in the White Sea, from *L. saxatilis* and *L. obtusata* collected in Iceland (Reykjavik region) and in the White Sea, and from *L. sitkana* collected in the Sea of Okhotsk (Nagaeva Bay).

Intramolluscan stages isolated from each snail were conspecific, as confirmed by the analysis of the molecular markers.

**Sporocyst**

| Measurements based on 30 live specimens |
|-----------------------------------------|
| Sporocyst (Fig. 3A) elongate oval, 437–876 × 213–444 (641 ± 25 × 345 ± 9), containing 1–12 (4) motile cercariae and numerous embryos. Sporocysts occupy the molluscan gonad tissue forming a tumour-like structure. The pseudo-tumour, milky white in case of early infection, becomes lemon-yellow or orange as cercariae mature in the sporocysts. The pigment responsible for the colour of the tumour is mostly concentrated in the surrounding host tissue, not in the sporocyst wall. |

**Cercaria**

| Table 3, Figs 3B and 4 |
|------------------------|
| Seminal receptacle width | 44 |
| Left vitellaria length | 142–548 (356 ± 8.2) |
| Right vitellaria length | 212–394 (293 ± 3.3) |
| Egg length (EL) | 38–48 (43 ± 0.8) |
| Egg width (EW) | 13–19 (16 ± 0.5) |
| EL/EW | 2.2–3.5 (2.7 ± 0.1) |

Host species is indicated in brackets in the column heads; N, number of measured individuals; * diameter of organs; measurements of live worms are given in square brackets.

![300 µm](https://doi.org/10.1017/S0031182022001500)
Fig. 2. Representative microphotographs of sexual adults of *Renicola* spp. analysed in this study (ventral view): *R. parvicaudatus* from the Icelandic *Larus argentatus* (A); *R. parvicaudatus* (specimen heavily pressed by cover glass) from *L. schistisagus* of the Sea of Okhotsk (B); *R. keimahuri* from *L. schistisagus* of the Sea of Okhotsk (C).

Fig. 3. Microphotographs of the intramolluscan stages and cercaria of *R. parvicaudatus*: daughter sporocysts in the gonad of *Littorina littorea* (A); cercaria (B) and metacercariae encysted in the same molluscan host where daughter sporocysts develop (C). *mt*, Molluscan tissue; *sp*, daughter sporocysts.
Table 3. Morphometric parameters of cercariae of *R. parvicaudatus* and closely related species (‘Parvicaudata’ group)

| Parameter          | *R. parvicaudatus* | C. littorinae saxatilis VIII | C. parvicaudata After Stunkard (1950) | C. roscovita After Werding (1969) | C. roscovita After James (1969) | Renicola sp. NZ After O’Dwyer et al. (2014) | Renicola sp. 1 Aus After O’Dwyer et al. (2015) | Renicola sp. 2 Aus After O’Dwyer et al. (2015) |
|--------------------|--------------------|-----------------------------|----------------------------------------|-----------------------------------|----------------------------------|---------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Body length        | 189–333 (262 ± 6.2) | 218–333 (280 ± 6.8)         | 140–360                                | 129–330 (240)                    | 280–350                          | 205–264 (240)                              | 239–307 (268)                                | 226–310 (263)                                |
| Body width         | 73–143 (101 ± 3.5)  | 86–120 (101 ± 2.3)          | 60–120                                 | 45–135 (89)                      | 80–100                           | 77–101 (86)                                | 71–90 (82)                                   | 77–130 (107)                                 |
| Tail length        | 155–197 (175 ± 2)  | 135–203 (181 ± 3.4)         | 60–300                                 | 44–240 (146)                     | 50–300                           | 150–207 (166)                              | 168–222 (193)                                | 124–189 (154)                                |
| Tail width         | 16–26 (21 ± 0.4)   | 16–21 (20 ± 0.6)            | –                                     | –                                 | max. 33 (19)                     | 16–24 (19)                                  | 15–21 (18)                                   | 16–19 (18)                                   |
| Oral sucker length | 45–60 (51 ± 1.2)   | 38–50 (44 ± 0.9)            | 35–60*                                 | 42–50*                           | 36–39* (42)                      | 35–50*                                     | 33–40 (37)                                   | 33–44 (36)                                   |
| Oral sucker width  | 40–58 (46 ± 1.3)   | 33–45 (41 ± 0.8)            | –                                     | –                                 | [33]                             | –                                          | 29–37 (33)                                   | 28–35 (32)                                   |
| Pharynx length     | 15–25 (20 ± 0.7)   | 13–20 (15 ± 0.4)            | 12–14*                                 | 14–18*                           | 14                               | 10–18*                                     | 12                                            | 15                                            |
| Pharynx width      | 15–23 (19 ± 0.5)   | 10–20 (15 ± 0.5)            | –                                     | –                                 | 14                               | –                                          | 12                                            | –                                             |
| Ventral sucker length | 43–55 (48 ± 1)   | 35–45 (41 ± 0.9)            | 34–50*                                 | 34–50*                           | 33–36 (35)                       | 30–45*                                     | 30–36 (33)                                   | 29–47 (38)                                   |
| Ventral sucker width | 43–58 (48 ± 1.4)  | 33–53 (44 ± 1.1)            | –                                     | –                                 | [38]                             | –                                          | 26–36 (32)                                   | 30–41 (34)                                   |
| Stylet length      | 12–15 (13 ± 0.3)   | 12–17 (14 ± 0.4)            | 15                                    | 16–18                            | 14                               | 13–18                                      | 10–12                                        | 11                                            |
| Stylet width 1     | 4–6 (5 ± 0.2)      | –                            | –                                     | –                                 | –                                | 1                                          | 1                                            | 2                                             |
| Stylet width 2     | 3–4 (3 ± 0.1)      | 3–5 (4 ± 0.2)               | 3.2                                    | 2–3                              | 3                                | –                                          | 1                                            | 2                                             |

N, number of measured individuals; * diameter; measurements of live worms are given in square brackets. Stylet width 1 – width in the broad part of the spearhead; stylet width 2 – width of the handle.
Cercariae small, body oval, highly contractile, body length more than 1.5 times greater than tail length. Oral sucker ventro-subterminal, muscular, approximately the same size as ventral sucker. Oral sucker armed with a single row of 38–43 spines (Fig. 4C). Stylet spear-shaped with a weakly expressed light-refracting spearhead, dorsal to mouth opening (Fig. 4B). Ventral sucker equatorial, armed with 2 alternating rows of spines of 38–40 (Fig. 4D). Anteriorly to external row of spines, ventral sucker bears 6 characteristic short sensory papillae surrounded by wide convex tegumental collars (2 anterior and 4 posterior) (Fig. 4D).

Penetration gland cells numbering 6 pairs. Their nucleated bodies arranged symmetrically on either side of oesophagus approximately at level of its middle and posteriorly. Ducts skirting oral sucker dorsally and opening at each side with common bundle near external opening of stylet pocket. Anterior parts of ducts forming pronounced curve near anterior end of oral sucker (Fig. 4A). Contents of penetration gland cells finely granular, stained with neutral red.

Entire body of larva densely packed with tegumental cystogenous gland cells. Two types of these cells distinctly seen: cells with coarsely granular contents staining with neutral red and cells with granular unstaining contents. Cells of first type with distinct nuclei, nuclei in cells of second type indistinguishable. At final stages of larva formation gland cells apparently discharging some of contents into tegument, granular material being visible throughout body and not only in cells.

Prepharynx not pronounced, pharynx rounded, intestine short, bifurcating anteriorly of ventral sucker. Excretory bladder Y-shaped, its arms skirting the ventral sucker posteriorly. Main collecting tubes opening at either side into unpaired part of bladder close to its bifurcation. Excretory formula $2[(3 + 3 + 3) + (3 + 3 + 3)] = 36$.

Metacercaria (Fig. 3C)
Metacercariae are enclosed in a spherical cyst 150–180 μm in diameter; cyst wall is 10–20 μm thick. The preferred second intermediate host is the mussel *M. edulis*. In mussels, the cysts with metacercariae are located in the hepatopancreas and, more rarely, in the tentacles at the mantle edge. The cercariae may also encyst in the same individuals of *Littorina* spp. that harbour daughter sporocysts. In this case they are located in the host tissues between the sporocysts. Encystment in periwinkles is more common during the cold season, after the arrest of cercarial emergence.

*Cercaria littorinae saxatilis* VIII larva nov. (Table 3)
First intermediate host: *L. saxatilis* and *L. obtusata* (Caenogastropoda: Littorinimorpha: Littorinidae) (natural).
Site in first intermediate host: Dalniye Zelentsy, Barents Sea, Grindavik, South-West Iceland.
Representative DNA sequences: 28S rDNA (ON650719, ON650722, ON650725), cox1 (ON652705, ON652710, ON652714–ON652717) and ITS2 rDNA (ON667894) (according to Table 1).
Etymology: the name of the intramolluscan stages continues the tradition of the classification of cercariae and parthenitae developing in molluscs Littorina spp., introduced by Lebour (1911) and continued by James (1968b, 1969), Sannia and James (1977) and Newell (1986).

The species was identified based on the analysis of molecular markers of intramolluscan stages from snails L. saxatilis collected in Iceland (Reykjavik region) and the Barents Sea (coast of the Kola Peninsula) (see molecular results). Daughter sporocysts and cercariae of C. littorina saxatilis VIII are morphologically and morphometrically identical to the intramolluscan stages of R. parvicaudatus described above (Table 3).

Renicola keimahuri Yamaguti, 1939 (Table 2, Figs 2C and 5)
Representative slides: 47 individuals on slides 3735-1–3735-10, deposited in the Collection of Helminths, section Trematoda, of the Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia. This material represents paragenophores.

Representative DNA sequences: 28S rDNA (ON650720) and cox1 (ON652706) (according to Table 1).

This species has been described by Yamaguti (1939) based on individuals from spectacled guillemot (Cepphus carbo Pallas, 1811) obtained in Japan. In our material, R. keimahuri was represented by adults from slaty-backed gull from the northern part of the Sea of Okhotsk. Considering the differences in the hosts and the geographic sites, we provide the description of the adult worms found in our study.

Worms small, drop-shaped. Oral sucker rounded, subterminal. Ventral sucker subequatorial, approximately 2–3 times smaller than the oral sucker. Prepharynx absent; pharynx small, overlapped anteriorly by oral sucker. Oesophagus short, caeca 2, extending into posterior third of body. Testes longitudinally oval, close together, sometimes partly overlapping, dorsal from ventral sucker. Vasa efferentia start from the anterior part of each testis, pass anteriorly and fuse to form a short vas deferens just before opening into the seminal vesicle (Fig. 5B). Seminal vesicle anterior to ventral sucker; median or slightly dextral of body midline. Ejaculatory duct short, opening into genital atrium. Seminal vesicle, a few prostatic gland cells and ejaculatory duct enveloped by fine membranous structure. Genital atrium slightly sinistral of seminal vesicle, opens ventrally with genital pore.

Ovary dextral (rarely sinistral), pretesticular, deeply lobbed. Oviduct starting from posterior part of ovary, receiving first seminal receptacle and then duct of vitelline reservoir. Ootype weakly developed, tubular, surrounded by Mehlis’ gland cells. All ducts of female reproductive system mentioned above as well as seminal receptacle located dorsally of ventral sucker, at level of its anterior part or somewhat anteriorly. Laurer’s canal absent. Ootype passing into uterus, which forms numerous ascending and descending loops and opens into genital atrium from behind. In mature worms uterus loops are densely packed with eggs and occupy almost all body volume except caudal end. Eggs operculate, elongate, their length approximately twice greater than width. Vitellarium lateral to caeca in middle third of body, consisting of 6–8 large follicles on ovarian side of body and 7–10 on opposite side. Transverse yolk ducts originating on each side as pair of ducts filled with yolk, fusing into single duct before joining with each other to form vitelline reservoir. Vitelline reservoir dorsal at the level of anterior part of ventral sucker or pre-acetabular. Excretory bladder Y-shaped with short stem in caudal end of body and 2 arms extending to level of pharynx. Stem and branches with distinct lateral diverticula.

Molecular results
Our study generated 9 partial D1–D3 fragments of 28S rDNA (1160 bp) and 82 new mitochondrial DNA cox1 gene sequences (313 bp) for Renicola spp. (Table 1). Both ML and BI analyses resulted in consensus trees with similar topologies (Figs 6–8).

In addition, we obtained 6 ITS2 sequences (354–374 bp) for several isolates: 7saxIP, 10nIR, 13saxWSP, 14obtWSP, 26saxBP and 27litHR (Table 1).

In all our trees, Renicola spp. involved in the analysis were mostly distributed across 2 large clades (I and II). Renicola somatertiae Belopol’skaya, 1952 (10nIR) formed a separate branch (Figs 6 and 7), which was sister to clade I in the tree based on D1–D3 fragment of 28S rRNA (Fig. 6) and sister to clade I + II in the cox1 tree (Fig. 7). In clade I, isolates morphologically identified as C. parvicaudata grouped with Australian isolates of Renicola sp. 1 Aus O’Dwyer et al., 2015 and Renicola sp. 2 Aus O’Dwyer et al., 2015 into one and the same cluster, which we will refer to as the ‘Parvicaudata’ group (Figs 6 and 7). In this group,
isolates tentatively identified as *C. parvicaudata* were distributed across 2 separate branches. One of the branches comprised isolates of *R. parvicaudatus sensu stricto*, and the other comprised several isolates from Iceland (7saxIP) and the Barents Sea (26saxBP, 41–43saxBP and 57obtBP), which we referred to as *C. littorinae saxatilis* VIII (see above) (Figs 6 and 7). Isolates 1siOP and 58siOP in the tree based on partial D1–D3 fragments of 28S rDNA were separate from the samples of *R. parvicaudatus* from the Netherlands (27litHR) and Iceland (32saxIC). However, genetic distances between the latter 2 samples on the one hand and 1siOP and 58siOP on the other made up 0.003 ± 0.002 and 0.004 ± 0.002, respectively, and were indistinguishable from the distance within the pooled group of these isolates, 0.003 ± 0.001 (Table S1b and S1d). The average interspecific genetic divergence

Fig. 6. Phylogenetic relationships between *Renicola* spp. based on maximum-likelihood and Bayesian inference (BI) analyses of the D1–D3 fragment of 28S rRNA genes dataset: phylogenetic tree reconstructed with D1–D3 fragments of 28S rRNA genes (A); phylogenetic tree reconstructed with D3 fragment of 28S rRNA genes (B). Maximum-likelihood bootstrap support values inferred from 1000 replicates are followed by posterior probabilities from BI analysis. Bootstrap values followed by posterior probabilities are shown in nodes. Asterisk indicates posterior probabilities. Coloured circles indicate groups detected by ASAP tool. Yellow circles indicate *R. parvicaudatus*; yellow/black circles indicate *C. littorinae saxatilis* VIII. Light-blue ellipses indicate 'Parvicaudata' group.
amongst *Renicola* spp. ranged from 0.015 ± 0.005 (*C. littorinae saxatilis* VIII/*Renicola* sp. 2 Aus) to 0.166 ± 0.011 (*R. keima-huri*/*Renicola* sp. 2 Aus) (Table S1a). 

*Renicola* sp. 2 Aus was a sister species to *C. littorinae saxatilis* VIII, while *Renicola* sp. 1 Aus was closer to *R. parvicaudatus* (Fig. 6). The genetic distance between the group of isolates of *R. parvicaudatus* and *C. littorinae saxatilis* VIII, calculated based on partial D1–D3 fragments of 28S rDNA, made up 0.028 ± 0.005, which corresponds to the interspecific level for *Renicola* (Table S1a). An analysis in ASAP also confirmed that the differences between *R. parvicaudatus* and *C. littorinae saxatilis* VIII corresponded to the interspecies level (Fig. 6, coloured circles). Thus, *C. littorinae saxatilis* VIII should be considered as a cryptic species relative to *R. parvicaudatus*. Our analysis also confirmed that *Renicola* sp. 1 Aus and *Renicola* sp. 2 Aus were independent species. Isolates of intramolluscan stage *Renicola* sp. *Huston* et al., 2018 (MH257770.1) found in the cerithiid gastropod *Clypeomorus batillariaeformis* Habe and Kosuge, 1966 (see *Huston* et al., 2018) and *Renicola* sp. VT-2002 (AY116871.1) from Eurasian curlew *Numenius arquata* (Linnaeus, 1758)
Renicola keimahuri (8OmR) was placed in clade II. Within this clade, it was a sister taxon to Renicola thapari Caballero, 1953 and Renicola sterna (Heneberg et al., 2016), the only member of the genus Nephromonorcha represented in GenBank.

We involved in the analysis of a short fragment of 28S rRNA gene obtained from the isolate identified by Litvaitis and Rohde (1999) as *R. roscovitus* (AF023113), as it was the only marker available in GenBank for this species (Fig. 6B). The support of the branches decreased, and ASAP analysis became impossible because the branches were too short and the programme sorted all the samples into 2 groups only. However, the main clades remained unchanged in the resulting tree. The genetic distance between *R. roscovitus* (AF023113) and isolates of *R. parvicaudatus* by the shortened fragment of 28S rRNA gene made up 0.018 ± 0.007, which is equivalent to the intraspecific level (0.011 ± 0.004) (Table S1c and S1e).

In contrast to 28S rRNA gene, there are numerous nucleotide sequences of renicolds for *cox1* in GenBank. In our *cox1* phylogenetic tree, the species of the ‘Parvicaudata’ group formed a separate branch within clade I. A sister branch was represented by renicolid xiphidiocercaria species from New Zealand (*Renicola* sp. Martorelli et al., 2008 (FJ765490–FJ765493)) and North America (*Renicola* sp. ‘martini’ Hechinger and Miura, 2014 and *Renicola* sp. ‘polychaetophila’ Hechinger and Miura, 2014) (Fig. 7).

The phylogenetic reconstruction and the analysis in ASAP showed that groups of isolates of *R. parvicaudatus* and *C. littoralis saxatilis* VIII diverged (Fig. 7). Intragroup p-distances in these 2 groups varied from 0.003 ± 0.003 to 0.016 ± 0.007, while the intergroup distance made up 0.106 ± 0.016. This corresponds to the interspecific genetic divergence, which, as estimated by *cox1*, ranged amongst *Renicola* spp. from 0.094 ± 0.016 (*R. parvicaudatus/Renicola* sp. 1 Aus) to 0.291 ± 0.025 (*R. somateriae/Renicola sternae* Heneberg et al., 2016) (Table S2a). The group of *R. parvicaudatus* contained all samples from NEA, NWA and NP, including those tentatively identified (based on the colour of sporocysts) as *R. roscovitus* (11 Ersh and 12 Ersh).
Similarly to the tree based on D1–D3 fragment of 28S rRNA, the Australian species Renicola sp. 1 Aus in the cox1 tree appeared as a sister to R. parvicaudatus, while C. littorinae saxatilis VIII together with Renicola sp. 2 Aus and Renicola sp. NZ O’Dwyer et al., 2014 formed a sister clade to them. P-distances between Renicola sp. 2 Aus and Renicola sp. NZ (0.035 ± 0.01) corresponded to intraspecific genetic diversity (Table S3, pair distances, Table S2b), and an analysis in ASAP did not show them to be separate species, either. Within clade II, R. keimahuri (80mR) was closest to R. sternae and Renicola lari Timon-David, 1933, but p-distance between the former species and the latter 2 species (0.121 ± 0.018 and 0.125 ± 0.018, respectively, Table S2a) corresponded to the interspecific level. These 3 species were also distinct based on ASAP (Fig. N2, coloured circles).

In the tree based on ITS2 fragment of 28S rRNA (Fig. 8), Cercaria doricha Rothschild, 1935 and Cercaria pythionike Rothschild, 1935 belonged to the Renicolidae, grouping with representatives of clade II according to D1–D3 28S rDNA and cox1 phylogenetic trees. The analysis in ASAP showed that C. doricha and C. pythionike were separate species, closest to Renicola sloanei but distinct from it. Genetic distances between C. doricha and C. pythionike also corresponded to the interspecific level (0.026 ± 0.009, Table S4). Renicula parvicaudata and C. littorinae saxatilis VIII diverged in the ITS2 tree, while the genetic distance between them based on this rDNA fragment made up 0.044 ± 0.010, which corresponds to the interspecific level (Table S4).

To study the history and the structure of R. parvicaudatus population, we calculated the mismatch distribution and constructed a haplotype network (Fig. 9). Mismatch distribution showed low pairwise differences and was skewed unimodal (Fig. 9A). We detected 10 haplotypes, which were arranged in a ‘star’ network (Fig. 9B). Most isolates represented the main haplotype, except the isolates from the White Sea and one of the Sea of Okhotsk (0.026 ± 0.009, Table S4). Renicula parvicaudata and C. littorinae saxatilis VIII shared a common origin from the ITS2 tree, while the genetic distance between them based on this rDNA fragment made up 0.044 ± 0.010, which corresponds to the interspecific level (Table S4).

To study the history and the structure of R. parvicaudatus population, we calculated the mismatch distribution and constructed a haplotype network (Fig. 9). Mismatch distribution showed low pairwise differences and was skewed unimodal (Fig. 9A). We detected 10 haplotypes, which were arranged in a ‘star’ network (Fig. 9B). Most isolates represented the main haplotype, except the isolates from the White Sea and one of the Sea of Okhotsk (0.026 ± 0.009, Table S4). Renicula parvicaudata and C. littorinae saxatilis VIII shared a common origin from the ITS2 tree, while the genetic distance between them based on this rDNA fragment made up 0.044 ± 0.010, which corresponds to the interspecific level (Table S4).

To study the history and the structure of R. parvicaudatus population, we calculated the mismatch distribution and constructed a haplotype network (Fig. 9). Mismatch distribution showed low pairwise differences and was skewed unimodal (Fig. 9A). We detected 10 haplotypes, which were arranged in a ‘star’ network (Fig. 9B). Most isolates represented the main haplotype, except the isolates from the White Sea and one of the Sea of Okhotsk (0.026 ± 0.009, Table S4). Renicula parvicaudata and C. littorinae saxatilis VIII shared a common origin from the ITS2 tree, while the genetic distance between them based on this rDNA fragment made up 0.044 ± 0.010, which corresponds to the interspecific level (Table S4).

To study the history and the structure of R. parvicaudatus population, we calculated the mismatch distribution and constructed a haplotype network (Fig. 9). Mismatch distribution showed low pairwise differences and was skewed unimodal (Fig. 9A). We detected 10 haplotypes, which were arranged in a ‘star’ network (Fig. 9B). Most isolates represented the main haplotype, except the isolates from the White Sea and one of the Sea of Okhotsk (0.026 ± 0.009, Table S4). Renicula parvicaudata and C. littorinae saxatilis VIII shared a common origin from the ITS2 tree, while the genetic distance between them based on this rDNA fragment made up 0.044 ± 0.010, which corresponds to the interspecific level (Table S4).

To study the history and the structure of R. parvicaudatus population, we calculated the mismatch distribution and constructed a haplotype network (Fig. 9). Mismatch distribution showed low pairwise differences and was skewed unimodal (Fig. 9A). We detected 10 haplotypes, which were arranged in a ‘star’ network (Fig. 9B). Most isolates represented the main haplotype, except the isolates from the White Sea and one of the Sea of Okhotsk (0.026 ± 0.009, Table S4). Renicula parvicaudata and C. littorinae saxatilis VIII shared a common origin from the ITS2 tree, while the genetic distance between them based on this rDNA fragment made up 0.044 ± 0.010, which corresponds to the interspecific level (Table S4).

To study the history and the structure of R. parvicaudatus population, we calculated the mismatch distribution and constructed a haplotype network (Fig. 9). Mismatch distribution showed low pairwise differences and was skewed unimodal (Fig. 9A). We detected 10 haplotypes, which were arranged in a ‘star’ network (Fig. 9B). Most isolates represented the main haplotype, except the isolates from the White Sea and one of the Sea of Okhotsk (0.026 ± 0.009, Table S4). Renicula parvicaudata and C. littorinae saxatilis VIII shared a common origin from the ITS2 tree, while the genetic distance between them based on this rDNA fragment made up 0.044 ± 0.010, which corresponds to the interspecific level (Table S4).

To study the history and the structure of R. parvicaudatus population, we calculated the mismatch distribution and constructed a haplotype network (Fig. 9). Mismatch distribution showed low pairwise differences and was skewed unimodal (Fig. 9A). We detected 10 haplotypes, which were arranged in a ‘star’ network (Fig. 9B). Most isolates represented the main haplotype, except the isolates from the White Sea and one of the Sea of Okhotsk (0.026 ± 0.009, Table S4). Renicula parvicaudata and C. littorinae saxatilis VIII shared a common origin from the ITS2 tree, while the genetic distance between them based on this rDNA fragment made up 0.044 ± 0.010, which corresponds to the interspecific level (Table S4).

To study the history and the structure of R. parvicaudatus population, we calculated the mismatch distribution and constructed a haplotype network (Fig. 9). Mismatch distribution showed low pairwise differences and was skewed unimodal (Fig. 9A). We detected 10 haplotypes, which were arranged in a ‘star’ network (Fig. 9B). Most isolates represented the main haplotype, except the isolates from the White Sea and one of the Sea of Okhotsk (0.026 ± 0.009, Table S4). Renicula parvicaudata and C. littorinae saxatilis VIII shared a common origin from the ITS2 tree, while the genetic distance between them based on this rDNA fragment made up 0.044 ± 0.010, which corresponds to the interspecific level (Table S4).

To study the history and the structure of R. parvicaudatus population, we calculated the mismatch distribution and constructed a haplotype network (Fig. 9). Mismatch distribution showed low pairwise differences and was skewed unimodal (Fig. 9A). We detected 10 haplotypes, which were arranged in a ‘star’ network (Fig. 9B). Most isolates represented the main haplotype, except the isolates from the White Sea and one of the Sea of Okhotsk (0.026 ± 0.009, Table S4). Renicula parvicaudata and C. littorinae saxatilis VIII shared a common origin from the ITS2 tree, while the genetic distance between them based on this rDNA fragment made up 0.044 ± 0.010, which corresponds to the interspecific level (Table S4).

To study the history and the structure of R. parvicaudatus population, we calculated the mismatch distribution and constructed a haplotype network (Fig. 9). Mismatch distribution showed low pairwise differences and was skewed unimodal (Fig. 9A). We detected 10 haplotypes, which were arranged in a ‘star’ network (Fig. 9B). Most isolates represented the main haplotype, except the isolates from the White Sea and one of the Sea of Okhotsk (0.026 ± 0.009, Table S4). Renicula parvicaudata and C. littorinae saxatilis VIII shared a common origin from the ITS2 tree, while the genetic distance between them based on this rDNA fragment made up 0.044 ± 0.010, which corresponds to the interspecific level (Table S4).
also from the Woods Hole region, to the list of the first intermedi-ate hosts. *Cercaria roscovita* has been described by Stunkard (1932) from *L. saxatilis* from Roscoff (France, Atlantic coast). Intramolluscan stages of *C. parvicaudata* and *C. roscovita* are barely distinguishable from each other. Stunkard (1950), when differentiating between these 2 species, noted that ‘except for the difference in colour of the daughter sporocysts, the 2 species are almost identical’. However, the colour of the parthenitae cannot be considered as a reliable character for species differentiation (Werdinger, 1969; Galaktionov and Skirniess, 2000). It depends on the infection age: young groups of sporocysts of *R. parvicauda-tus* (infection of the current year) in periwinkles at the White Sea are white, while old groups that have overwintered in the molluscan host are lemon yellow (Nikolaev et al., 2021). Nadakal (1960) has shown that daughter sporocyst and redial colour is determined by the presence of β-carotene accumulated both in the molluscan tissues and in the parasites. The source of carotenoids in the molluscan organism is the alga the molluscs feed on. In case of the renicolid in our material, it was not so much the sporocysts that were coloured but the layers of molluscan tissues between them. Our analysis of *cox1* sequences of the sporocysts from lemon-coloured pseudo-tumours (*C. roscovita* in accordance with Stunkard (1950)) and from orange-coloured ones (*C. parvi-caudata* in accordance with Stunkard (1950)) showed that they belonged to the same species, which we refer to as *R. parvicauda-tus*. To conclude, differences in the colour of the sporocysts (or, rather, in the colour of the surrounding host tissues) cannot be considered as a diagnostic character.

There is 1 character that remains to be discussed, and it is the number of penetration gland cells. Stunkard (1950) indicated that the cercariae of *C. parvicaudata* had 6 pairs of penetration gland cells, while cercaria of *C. roscovita* had ‘several’ (Stunkard, 1932, 1950). This character was later used for differentiating the cercariae of these 2 species by James (1968a, 1969). It is difficult to count the penetration gland cells in renicolid cercariae, because the distal parts of their ducts are extremely narrow while the nuclei-containing cell bodies are obscured by numerous cystogenous gland cells. Werdinger (1969) noted that the number and the exact location of penetration gland cells in the cercariae described by him as *R. roscovitus* could not be determined. This may be the reason why Stunkard (1950) did not include this character into the list of characters differentiating the 2 species of cercariae under consideration.

However, the number of penetration gland cells is mentioned in the identification keys by James (1968a), who differentiated the cercariae of *C. parvicaudata* and those of *C. roscovita* based on sporocyst colour and the number of penetration gland cells. It is noteworthy that the cercaria of *C. roscovita* is said to have ‘numerous’ gland cells. In the drawing of a cercaria of this species from *L. saxatilis* in Cardigan Bay, Wales (UK), 15–17 pairs of penetration gland cells can be counted, whose external pores form 2 longitudinal rows on either side of the stylet (Fig. 77, p. 301, James, 1969). This drawing disagrees with our data and with the drawing of a cercaria of *R. roscovita* in Werdinger (1969), in which the ducts of the penetration gland cells open in 2 compact groups near the anterior edge of the oral sucker in the area of the stylet, that is, exactly as they do in *C. parvicaudata*.

In our opinion, it was *C. parvicaudata* that Werdinger (1969) studied, not *C. roscovita* described by James (1969). This opinion is supported by the fact that Werdinger (1969) worked with intramolluscan stages from *L. littorea*, while *C. roscovita* has been reported only from *L. saxatilis* and *Meleraphhe neritoideis* (Linnaeus, 1758) (Syn. *Littorina neritoides*) (Stunkard, 1932, 1971; James, 1968a, 1969). The region where Werdinger (1969) collected his material is the same as the region where Litvaitis and Rohde (1999) worked: the coast of Germany, including Isle of Sylt (Wadden Sea). Moreover, the sequence of short 28S DNA fragment of *R. roscovita* (AF023113) from Litvaitis and Rohde (1999) matched the sequences that we obtained for *C. parvicauda-tus* (Fig. 6B). Snails *L. littorea* from the North Sea coast (Texel Island, the Netherlands) surveyed in our study were infected only with intramolluscan stages of *C. parvicaudata*, as supported by molecular data (Figs 6A and 7).

In addition to *C. parvicaudata* and *C. roscovita*, cercariae of 3 other renicolid species are recorded in periwinkles in NA: *Cercaria emasculans* Pelseneer, 1906, *Cercaria brevicauda* Pelseneer, 1906 and *C. littorinae saxatilis* V an N Sanna and James, 1977 (James, 1968a; Sanna and James, 1977). They differ from *C. parvicaudata* and *C. roscovita* in morphometric characteristics, the shape of the stylet, the number of penetration gland cells and the position of their ducts in the larval body. *Cercaria littorinae saxatilis* VI, which has been described from *L. saxatilis* in the north of Iceland (Eyjafjordur) (Sanna and James, 1977), is strikingly different from the larvae of the other species, because it has only 1 pair of penetration gland cells. We did not find any cercariae of this species in the south-western Iceland though we dissected more than 10 000 individuals of *L. saxatilis* and *L. obtusata* in the course of our surveys; we registered only intramolluscan stages of *C. parvicaudata* (Galaktionov and Skirniess, 2000; Skirniess and Galaktionov, 2002; K. V. Galaktionov, personal observations) and, as molecular analysis showed, those of a cryptic species *C. littorinae saxatilis* VIII.

Cercariae of *Renicola* sp. NZ, *Renicola* sp. 1 *A* us and *Renicola* sp. 2 *Aus* from Australian and New Zealand *Australiotorina* spp., which make up the ‘Parvicaudata’ group together with *R. par-vicaudatus* and *C. littorinae saxatilis* VIII, differ from the latter 2 species genetically as well as in the number of penetration gland cells (5 pairs), number and position of large spines in the suckers and of sensory papillae on the body surface (chaetotaxy) (O’Dwyer et al., 2014, 2015; Denisova and Shchenkov, 2020).

Summing up, our molecular and morphological studies indicate that *R. parvicaudatus* is the most common species among the renicolid intramolluscan stages in snails *Littorina* spp. at the Atlantic coast of Europe and North America. There are no credible findings of *C. roscovita* in this area. Werdinger (1969) suggested to synonymize these 2 species under the name of *R. roscovitus* (as noted before, he dealt with *R. parvicaudatus*). Nevertheless, it is premature to synonymize *C. roscovita* Stunkard, 1932 with *R. par-vicaudatus* because: (1) a cercaria with numerous penetration gland cells, minutely described by James (1969), should be attributed to *C. roscovita* Stunkard, 1932 and (2) Denisova and Shchenkov (2020) found that the number and position of the sensory receptors on the body of cercariae *C. parvicaudata* from *L. littorea* at the White Sea were different from those of *C. roscovita* from *L. saxatilis* near Roscoff (Richard, 1971), that is, the same snail species and the same site from which this larva was first described by Stunkard (1932). It cannot be ruled out that the species *R. roscovitus* does exist, and its transmission is implemented further southwards in the Atlantic (e.g. British Isles, France). At the same time, intramolluscan stages of *R. roscovitus* parasite only snails *L. saxatilis* and *M. neritoideis*, while *R. parvicaudatus* is found in *L. littorea* and, more rarely, in *L. saxatilis* and *L. obtu-sata*. To note, the analysis of *cox1* sequences of the isolates from the Atlantic coast of France including the vicinity of Roscoff (EUCPTROUV1, EUCPTROUV2, EUCPTROUV3, EUCPMIND1, EUCPMIND2, EUCPMIND3, EUCPMIND4 and EUCPMIND5 – Table 1) did not reveal the presence of any species different from *R. parvicaudatus*. Whether or not *R. roscovitus* is a true species can only be established in integrative morphological and molecular studies of intramolluscan stages of renicolid in periwinkles from the British Isles and the Atlantic coast of France.
Renicola keimahuri

Adult worms of the second species isolated from gulls L. schistisagus from the Sea of Okhotsk in our study (isolate 8OmR) morphologically correspond to R. keimahuri described by Yamaguti (1939) from spectacled guillemot C. carbo in Japan. They are somewhat smaller than the worms described by Yamaguti (1939) (Table 2), which may be associated with the host-induced variability. Leonov et al. (1963) recorded R. keimahuri in larids in Kamchatka: slaty-backed gull (L. schistisagus), black-legged kittiwake [Rissa tridactyla (Linnaeus, 1758)], common tern [Sterna hirundo (Linnaeus, 1758)] and Aleutian tern (Onychoprion aleuticus) Baird, 1869. This broad range of hosts may indicate that we deal with a complex of close or cryptic species. Detailed morphological and molecular studies are needed to prove or disprove this hypothesis. To note, this hypothesis is also supported by some morphological differences of R. keimahuri in Leonov et al. (1963) from the first description by Yamaguti (1939) and the description given above: fewer vitelline follicles (4–5) and testes that do not touch each other but are spaced apart [Fig. 20, p. 151 from Leonov et al. (1963)]. At the same time, we examined mounted specimens of R. keimahuri from gulls L. schistisagus of Kamchatka (mounts ## 2439/Tr–2441/Tr, col. & det. Leonov) deposited in the collection of the Centre of Parasitology of the Russian Academy of Sciences and found that they fully corresponded to those described in this study.

Both by the molecular marker cox1 and by morphological criteria R. keimahuri is closest to the European species R. sterna described by Heneberg et al. (2016) from common tern (S. hirundo) and to R. lari from the herring gull (L. argentatus) and black-headed gull (L. ridibundus) (Linnaeus, 1766) (Prevot and Bartoli, 1978). These 3 species are similar in size and morphology (Table 2). Renicola sterna differs from R. keimahuri in having separate testes lying beside the ventral sucker (but see remark in Discussion) and in somewhat greater number of follicles in the separate testes lying beside the ventral sucker (but see remark in Discussion) and in somewhat greater number of follicles in the separate testes lying beside the ventral sucker (but see remark in Discussion) and in somewhat greater number of follicles in the separate testes lying beside the ventral sucker (but see remark in Discussion) and in somewhat greater number of follicles in the separate testes lying beside the ventral sucker (but see remark in Discussion). Despite their morphological similarity, R. keimahuri, R. sterna and R. lari are quite distinct genetically.

Discussion

It was shown for the first time that out of all Renicola spp. using snails Littorina spp. as the first intermediate hosts in the nearshore areas of NA seas the dominant species is R. parvicaudatus, as identified based on the combination of morphological characters. The analysis of molecular markers and morphology showed that the adults of this species are found in gulls from Iceland and the Sea of Okhotsk. This means that we successfully elucidated the life cycle of this species. According to the Code of Zoological Nomenclature (ICZN, 1999), we name it R. parvicaudatus (Stunkard and Shaw, 1931) nov. comb. The name R. roscovitus (Stunkard, 1932) Werding, 1969 and the name R. thaidus Stunkard, 1964 used by Stunkard (1964) for the adult worms should be considered as its synonyms.

The cercariae of R. parvicaudatus and those of R. roscovita are difficult to differentiate. This circumstance gave rise to a long-lasting confusion. It started with an experimental study of Werding (1969), who identified the cercaria of the renicolid species whose life cycle he studied as C. roscovita and named the species R. roscovitus. As explained in the Remarks section above, Werding (1969) actually studied the cercariae of R. parvicaudatus. This means that intramolluscan stages from Littorina spp. identified in numerous ecological and faunistic studies as R. roscovitus (e.g. Lauckner, 1987; Granovitch and Johannesson, 2000; Thieltges, 2006; Thieltges and Rick, 2006; Mouritsen and Elkjær, 2020) actually belong to R. parvicaudatus or to its cryptic species C. littorinae saxatilis VIII described in this study.

‘Parvicaudata’ species complex: composition and phylogeography

The species of the ‘Parvicaudata’ group form a separate clade on phylograms constructed on the basis of molecular markers used in our study. All these species use intertidal snails Littorina spp. and Austrotolithorina spp. (Littorinoidea, Littorinidae) as the first intermediate host. The definitive host is known only for R. parvicaudatus, but other species of the ‘Parvicaudata’ group probably also use gulls or other birds, such as sandpipers, that feed on nearshore invertebrates. A sister clade of the ‘Parvicaudata’ group is formed by species whose first intermediate hosts are various molluscs from the superfamily Cerithioidea, some of which belong to the family Cerithiidae (C. littorinae saxatilis VIII) (H. B. Sowerby II, 1855) (Leung et al., 2009) and some to the Potamidaceae (Cerithiodeopsis californica (Haldeman, 1840)) (Hechinger and Miura, 2014). This observation suggests that the formation of the ‘Parvicaudata’ group was associated with the colonization of periwinkles as the first intermediate host.

The only morphological differences between cercariae of R. parvicaudatus and C. littorinae saxatilis VIII on the one hand and the larvae of Australian-New Zealand species on the other are the number of penetration gland cells and the number and position of large spines in the suckers and the sensory papillae on the body surface (see Remarks). Renicola sp. NZ and Renicola sp. 2 Aus have some differences in the latter 2 characters and the size (O’Dwyer et al., 2015), but genetic divergence between them was within the species level (Tables S2b and S3, Figs 6A and 7), which means that they are likely to be morphs of the same species. At the same time, genetic differences between morphologically indistinguishable cercariae of R. parvicaudatus and C. littorinae saxatilis VIII corresponded to those between different species (Tables S1a, S2a and S4), which suggests that they are cryptic species. At the same time, C. littorinae saxatilis VIII is genetically closer to Australian-New Zealand species that to R. parvicaudatus (Tables S1a, S2a and S4, Figs 6 and 7).

To sum up, morphological differences between the renicolid cercariae may not necessarily mean that they belong to different species. By the same token, the absence of morphological differences does not prove that the cercariae are conspecific. The case of Renicola sp. NZ and Renicola sp. 2 Aus shows that subtle differences in cercarial morphology and chaetotaxy revealed with the use of SEM (O’Dwyer et al., 2014, 2015) are not always reliable criteria for species differentiation. These considerations strongly indicate that an integrative approach is the key to ascertaining the species status of digeneans. This approach should involve the analysis of morphological characters (preferably, of all life-cycle stages), molecular markers and the data on the larval and adult biology, host range, transmission pathways and geographic distribution (Blasco-Costa et al., 2016; Blasco-Costa and Poulin, 2017; Gonchar and Galaktionov, 2021, 2022).

Small genetic distances between the species of the ‘Parvicaudata’ group (Tables S1a, S2a and S4) indicate its relatively recent formation. The differentiation of R. parvicaudatus could be associated with the colonization of a new first intermediate host, the snail L. (Littorina) littorea, the only Atlantic species of the subgenus Littorina. Its ancestor split from its NP sister species L. (Littorina) squalida Broderip and G. B. Sowerby I, 1829 and colonized the NA via the Arctic route ca. 5.5–2.4 million
years ago (Reid, 1996; Reid et al., 1996, 2012). This assumption is supported by the facts that R. parvicaudatus is the only renicolid parasitizing L. (L.) littorea and that it occurs in the latter more frequently than in the Atlantic periwinkles of the subgenus Neritrema, i.e. L. (N.) saxatilis and L. (N.) obtusata (our data). Intramolluscan stages of R. parvicaudatus have never been registered in L. (L.) squalida in NP (Tsimaljakul et al., 1978; Rybakov, 1983; our data), and out of all the Pacific Neritrema, only L. (N.) sitkana serves as their host, and only rarely (Tsimaljakul et al., 1978; our data). Intramolluscan stages of the other species of the ‘Parvicaudata’ group develop in Atlantic periwinkles of the subgenus Neritrema (C. littorinae saxatilis VIII as well as C. emasculans, C. brevicauda and C. littorinae saxatilis VI, which most likely also belong to this group) or in Australian Austrolittorina spp. (James, 1968a, 1969; Sannia and James, 1977; O’Dwyer et al., 2014, 2015; our data).

The star-like patterns in coxl haplotype network for R. parvicaudatus suggest a low geographic structure (Fig. 9B). Thus, we may conclude that R. parvicaudatus is represented by a single population throughout its Holartic range (so far this species has not been detected at the Pacific coast of North America). The widespread haplotype positioned at the centre of the network can be considered as the ancestral one (Jenkins et al., 2018). The other haplotypes, which are linked to this dominant haplotype by a single mutational step or a few steps, are the result of recent mutation events. Unimodal mismatch distribution (Fig. 9A) and significant negative value of the Tajima’s D indicate a bottleneck event, possibly dating from the last glacial maximum (LGM).

During LGM, the transmission of event, possibly dating from the last glacial maximum (LGM). Neritrema suggest a low geographic structure (Fig. 9B). Thus, we likely also belong to this group) or in Australian Austrolittorina spp. (James, 1968a, 1969; Sannia and James, 1977; O’Dwyer et al., 2014, 2015; our data).

Two circumstances explain the fact that R. parvicaudatus has a broad geographic distribution and, at the same time, its coxl haplotypes are identical or very similar in different parts of its range. Firstly, the definitive hosts of this parasite are highly mobile migrating birds such as gulls, and secondly, the life span of adult worms in them is very long. Gulls that breed at high latitudes (e.g. L. argentatus, Larus fuscus Linnaeus, 1758, Larus canus Linnaeus, 1758, Larus glaucescens Naumann, 1840, Larus glaucoides Meyer, 1822, L. schistisagus) make long seasonal migrations along the coasts of Europe and North America (Helberg et al., 2009; Newton, 2010; Hallgrimson et al., 2012; Klaassen et al., 2012; Davis et al., 2016; Anderson et al., 2020). White-headed gulls associated with coastal habitats, such as L. argentatus and L. canus, which have a circumpolar distribution, were shown to have a limited population genetic subdivision among northern Arctic populations (Sonsthagen et al., 2012). This observation indicates that there is an intense genetic exchange between the populations of these birds owing to their migratory activity. Some individuals, apparently, are even capable of making trans-Arctic flights. Otherwise, the coincidence of R. parvicaudatus haplotypes in NA and NP would be difficult to explain. Trans-Arctic flights are known for Arctic-breeding seabirds (Clairbaux et al., 2019) and have recently been reported for a larid bird, the black-legged kittiwake (R. tridactyla) (Ezhov et al., 2021). Another option is the transfer of the parasite by birds from the Atlantic coast of North America to the Pacific coast, and from there to the coast of North Asia.

The snag of both hypothetical variants of the trans-Arctic transfer of R. parvicaudatus is the absence of its first intermediate hosts, the periwinkles, at the coasts of the Siberian seas and at the Arctic coast of North America (Arctic coast of Alaska and the Canadian Arctic Archipelago) (Reid, 1996). Only long-living helminths such as renicoli can endure such a long flight. According to Werding (1969), the lifespan of R. parvicaudatus (R. roscovisus in Werding’s article) in the final host is at least 7 months. The fact that one of the haplotypes from the Sea of Okhotsk coincides with the dominant one indicates that there is an ongoing exchange between NA and NP parts of the R. parvicaudatus population (Fig. 9B). It may be associated with the warming of the Arctic, which opens opportunities for trans-Arctic bird migrations (Clairbaux et al., 2019; Ezhov et al., 2021). Another haplotype of R. parvicaudatus from the Sea of Okhotsk is significantly different from the Atlantic one (Fig. 9B), possibly indicating some degree of isolation between the NP and the NA part of the parasite’s population. Another evidence of the possibility of some local differentiation within the population of R. parvicaudatus is the fact that all the haplotypes from the White Sea are different from the dominant one (Fig. 9B).

In our opinion, it is premature to hypothesize about the ways of geographical expansion of other species of the ‘Parvicaudata’ group since genetic data are limited and we do not know the actual number of species constituting the group, the array of their second intermediate and definitive hosts and their ranges. The establishment of the Australian-New Zealand species was probably associated with the colonization of the local Austrolittorina spp., the incursion of the ancestral species being ensured by migrating birds. Considering that in our phylograms the Australian species Renicola sp. 1 Aus is sister to the Holarctic R. parvicaudatus, while the group comprising Australian-New Zealand Renicola sp. 2 Aus and Renicola sp. NZ is sister to the NA C. littorinae saxatilis VIII, we can assume that Australia and New Zealand were colonized as a result of 2 putative independent events.

Notes on taxonomy and phylogeny of renicoliids

Two large clusters can be seen in the phylograms constructed on the basis of the molecular markers used in our study (Figs 6 and 7). Renicola parvicaudatus falls into cluster I, while R. keimahuri falls into cluster II. There are considerable morphological differences between species in cluster I and those in cluster II. Moreover, these differences are pronounced at all life-cycle stages. The adults differ in the position of vitellaria and testes, which is considered as an important taxonomical character in renicoliids (Wright, 1956, 1957; Odening, 1962; Sudarikov and Stenko, 1984; Gibson, 2008). The adults of R. parvicaudatus (the only species from cluster I for which adults have been described) have vitellaria in the posterior body part and separate, non-contiguous testes. At the same time, in all adults from cluster II described so far vitellaria are located lateral to the caeca in the middle third of
Structural differences between the species from the 2 clusters identified in our molecular phylogenies concern not only adults but also cercariae. Cercariae of all species from cluster I look like typical xiphidiocercariae: small size (body and tail each approximately 150–250 μm long), stylet, 1–6 pairs of penetration gland cells (rarely more), excretory formula 2[(3 + 3 + 3) + (3 + 3 + 3)] = 36, main collecting tubes join the stem of the excretory bladder is shifted for- ward and join the outgrown branches of the excretory bladder. Simple tail (Hechinger and Miura, 2014; O’Dwyer et al., 2014, 2015). Xiphidiocercariae are also known for some renicolid cercariae of which molecular data are lacking, e.g. Cercaria opaca Holliman, 1961, Cercaria caribbea XXXII Cable, 1956, C. caribbea XXXIII Cable, 1956 (Cable, 1956; Holliman, 1961). The cercaria of R. somatieres (isolate 10nIR, syn. R. thaidus Stunkard, 1964), which is sister to I + II in the phylogenetic trees (Figs 6 and 7), also looks like a typical xiphidiocercaria (Stunkard, 1964).

Cercariae of Renicola buchanani (Martin and Gregory, 1951) and R. cerithidicola Martin, 1971 in clade II have the same general appearance but lack the stylet (Martin and Gregory, 1951; Martin, 1971). In R. lari, which is similar to R. keimahuri, cercaria, besides lacking the stylet, also have a well-developed excretory bladder with a short stem and arms extending to the anterior end of the body and carrying numerous lateral diverticula (Prevot and Bartoli, 1978). In addition, though the excretory formula remains the same, the main collecting tubes join not the stem but the arms of the excretory bladder. Prevot and Bartoli (1978) considered cercariae R. lari and a similar C. caribbea VIII Cable, 1956 as a transitional morphotype to the typical cercariae of Rhodometopa group. The latter are large (body up to 2 mm), have a long tail with fin-folds, numerous penetration gland cells that form 1–3 groups in the anterior part of the body, a well-developed T-shaped excretory bladder with lateral diverticula in the stem and the arms and numerous flame cells (Stunkard, 1932; Rothschild, 1933; Wright, 1956).

Cable (1963) noted that excretory system of cercariae of Rhodometopa group was organized similarly to that in the adults. In the course of development of adult renicoids, the excretory bladder expands considerably and forms lateral diverticula, as it does in Rhodometopa cercariae. The number of flame cells also increases in the course of development, which is a characteristic of trematodes (Galaktionov and Dobrovolskij, 2003). On the basis of these observations, Cable (1963) suggested that Rhodometopa cercariae were more advanced than xiphidiocercariae and had certain traits of adult organization, particularly pronounced in the structure of their excretory system.

A series of transition forms from renicoid xiphidiocercariae to the cercariae of Rhodometopa group can be arranged. In cercariae of R. buchanani and R. cerithidicola, the site where the main collecting tube leaves the stem of the excretory bladder is shifted forwards; in R. buchanani it is located just before the bifurcation (Martin and Gregory, 1951; Martin, 1971). Cercariae of C. caribbea VII Cable, 1956, C. caribbea VIII, C. caribbea IX Cable, 1956 and R. lari not only lack the stylet, but also have a well-developed excretory bladder with lateral diverticula; the main collecting tube starts not from the stem but from the arms (Cable, 1956; Prevot and Bartoli, 1978). In drawings showing successive stages of embryogenesis in cercariae of C. caribbea VII (Cable, 1956; Plate 3, Fig. 16, p. 550) one can see that the site of the origin of main collecting tube, which in early embryos is located at the site of bifurcation of the excretory bladder, is shifted forwards together with the outgrown branches of the excretory bladder. This also seems to be the case during the ontogenesis of adults in renicoids with xiphidiocercariae, since their adults also have outgrown branches of the excretory bladder with numerous diverticula.

Another morphological character shared by the renicoid xiphidiocercariae and the Rhodometopa cercariae is the organization of surface structure in the oral and the ventral sucker. SEM studies of xiphidiocercariae of the ‘Parvicauda’ group have revealed 1–2 rows of large spines in the suckers and 6 large uniciliated sensory papillae (2 anterior and 4 posterior) with a wide convex tegumental collars in the ventral sucker (Fig. 4D) (O’Dwyer et al., 2014; Denisova and Shchenkov, 2020; this study and our unpublished data). Rothschild (1935) noted a circle of spines and 6 large cuticular tubercles outside of them in the ventral sucker of a typical Rhodometopa cercaria C. pythionike. These ‘cuticular tubercles’ are arranged in the same manner as the sensory papillae in renicoid xiphidiocercariae and are, undoubtedly, sensory papillae, too.
The final evidence that Rhodometopa cercariae belong to renicolid species came from the analysis of sequences of ITS2 rDNA of 2 typical larvae of Rhodometopa group: *C. pythionike* and *C. doricha* (Matos et al., 2019). This conclusion was supported by our analysis based on ITS2 rDNA sequences for a greater number of renicolid species (Fig. 8). To note, *C. pythionike* and *C. doricha* did not group with renicoids in the NCBI Blast analysis by Heneberg et al. (2016), which now seems to have been an error associated with the scarcity of the relevant sequences in the GenBank at the time of the analysis. In our phylogenetic tree both larvae of Rhodometopa group grouped with the species that belonged to clade II in cox1-based tree (Fig. 7). These 2 larvae clearly belong to different species. *Cercaria pythionike* is close to *R. sloanei*, but ASAP analysis convincingly shows that it is a distinct species.

It has been suggested that the formation of Rhodometopa cercariae in renicolid species was associated with the colonization of plankton-eating fish as the second intermediate host and through them, of fish-eating seabirds such as alcids, penguins, petrels, pelicans, and other piscivorous species. This hypothesis is supported by the fact that *C. doricha* and *C. pythionike* group in the phylogenetic tree together with *R. sloanei*, a parasite of several species of penguins and alcids (Matos et al., 2019, 2021).

Renicolidae belong to the superfamilly Microphalloidea Ward, 1901 (suborder Xiphidiata) (Cribb et al., 2003; Olson et al., 2003; Pérez-Ponce de León and Hernández-Mena, 2019), whose cercariae possess the stylet. Its origin is thought to be associated with the involvement of arthropods as the second intermediate host into the life cycle of the ancient microphalloideans (Cribb et al., 2003). The cercaria uses the stylet to penetrate the arthropod cuticle or arthrodial membranes. In renicolid cercariae the stylet is reduced to some degree or even absent, as in the larvae of Rhodometopa group and ‘transitional morphotypes’. The reduction of the stylet is associated with the transition to the use of organisms without rigid cuticular covers, such as molluscs and fish, as the second intermediate host. Only a few of the stylet-bearing renicolid cercariae penetrate polychaetes (Hechinger and Miura, 2014) and occasionally crabs (Robson and Williams, 1970) alongside with molluscs.

Metacercariae of species with xiphidiocercariae develop in invertebrates inhabiting nearshore areas, usually the intertidal zone. Therefore, the range of their definitive hosts is limited by the birds feeding on these invertebrates such as gulls, terns and sandpipers. Colonization of fish-eating seabirds became possible after renicolids began to use fish, especially planktonic fish, as the second intermediate host. This transition called for new adaptations of the definitive host, and their cercariae belong to the Rhodometopa group or to ‘transitional morphotype’. The third branch is represented for now by 1 species, *R. somateriae*, a typical parasite of sea ducks, with xiphidiocercaria in the life cycle.

In our opinion, it is premature to attempt a thorough taxonomic revision of the renicolids. This task would be meaningful after the accumulation of molecular data, especially on morphologically contrasting species, the elucidation of life cycles of a greater number of species and the determination of the range of their hosts. A detailed analysis of the morphological features of adults and cercariae is also necessary.

**Supplementary material.** The supplementary material for this article can be found at https://doi.org/10.1017/S0031182022001500.

**Data.** Data available on request from the authors.

**Acknowledgement.** The authors are grateful to the White Sea Biological Station of the Zoological Institute of the Russian Academy of Sciences (ZIN RAS) for providing fieldwork infrastructure. We thank Dr Gennady Atrashkevich, Dr Kira Regel, Dr Kirill Nikolaev and Dr Ivan Levakin for their help with sampling and primary treatment of the material, Dr A. Nikolayev for his help with SEM and Dr Natalia Ershova who provided us with 2 cox1 gene sequences of *Renicola cf. roscovita*. We also thank Dr D. I. Gibson (NHM, London) for his assistance in taxonomical questions. We wish to acknowledge the ‘Taxon’ Research Resource Center (http://www.ckp-rfu.ru/ckp/3038/) of ZIN RAS and the research resource centre ‘Molecular and Cell Technologies’ of St. Petersburg State University for granting access to their facilities. We are grateful to Natalia Lentsman for her help with the manuscript preparation. We thank the anonymous reviewers for their well-considered comments on an earlier draft of the manuscript.

**Author’s contributions.** Contributions are addressed as follows: conceptualization (K. V. G., A. I. S.); data collection (all authors); data analysis and interpretation (K. V. G., A. I. S. A. M. H. B.); writing (K. V. G., A. I. S.).

**Financial support.** The fieldwork at the White Sea Biological Station and at the ‘Taxon’ Research Resource Center was partly financed by the research programme of Zoological Institute RAS, project number 122031100260-0. The treatment and analysis of the accumulated data were supported by the Russian Science Foundation (Grant No. 18-14-00170-P).

**Conflict of interest.** None.

**Ethical standards.** Not applicable.
larval digenea in L. littorea on the North Yorkshire Coast. Journal of Helminthology 44, 163–168.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA and Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian Phylogenetic inference and model choice across a large model space. Systematic Biology 61, 539–542. doi: 10.1093/sysbio/sys029

Rothschild M (1935) The trematode parasites of Turritella communis Link. from Plymouth and Naples. Parasitology 27, 152–157.

Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE and Sánchez-Gracia A (2017) DnaSP 6: DNA sequence polymorphism analysis of large datasets. Molecular Biology and Evolution 34, 3299–3302.

Rubio-Godoy M, Pérez-Ponce de León G, Mendoza-Garfias B, Carmona-Isunzaf MC, la Moral N and Drummondt H (2012) Hybridization among Arctic white-headed gulls (Larus hyperboreus) obscures the genetic legacy of the Pleistocene. Ecology and Evolution 3, 98–102.

Sannia A and James BL (1977) The digenea in marine mussels from Eyjafjörður, North Iceland. Ophelia 16, 97–109.

Skirnison K and Galaktionov KV (2002) Life cycles and transmission patterns of seabird digeneans in SW Iceland. Sarsia 87, 144–151.

Skirnison K, Guðmundsdóttir B, Andrésdóttir V and Galaktionov KV (2002–2003) ITS1 nuclear rDNA sequences used to clear the life cycle of the morphologically different larvae and adult renicolid (Renicola, Digenea) parasites found in Iceland. Bulletin of the Scandinavian Society for Parasitology 12–13, 50.

Sonsthagen SA, Chesser RT, Bell DA and Dove CJ (2012) Hybridization among Arctic white-headed gulls (Larus spp.) obscures the genetic legacy of the Pleistocene. Ecology and Evolution 2, 1278–1295.

Stunkard HW (1932) Some larval trematodes from the coast in the region of Roscoff, Finistere. Parasitology 24, 321–343.

Stunkard HW (1930) Further observations on Cercaria parvicaudata Stunkard and Shaw, 1931. Biological Bulletin of the Marine Laboratory, Woods Hole 99, 136–142.

Stunkard HW (1964) Studies on the trematode genus Renicola: observations on the life-history, specificity, and systematic position. Biological Bulletin of the Marine Laboratory, Woods Hole 126, 468–489.

Stunkard HW (1971) Renicolid trematodes (Digenea) from the renal tubules of birds. Annales de Parasitologie (Paris) 46, 109–118.

Stunkard HW and Shaw CR (1931) The effect of dilution of sea water on the activity and longevity of certain marine cercariae, with description of two new species. Biological Bulletin of the Marine Laboratory, Woods Hole 61, 242–271.

Stunkard HW, Nigrelli RF and Gandal ChP (1958) The morphology of Renicola philippinensis, n. sp., a digenetic trematode from the pheasant-tailed Jacana, Hydrophasianus chirurgus (Scopoli). Zoologica: Scientific Contributions of the New York Zoological Society 43, 105–112.

Sudarikov YE and Stenko RP (1984) Trematodes of the family Renicolidae. In Sonin MD (ed.), Helminths of Farming and Hunting Animals. Moscow, USSR: Nauka, pp. 34–89 (in Russian).

Tamura K, Stecher G, Peterson D, Filipski A and Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30, 2725–2729.

Thielges DW (2006) Effect of infection by metacercarial trematode Renicola roscovita on growth in intertidal blue mussel Mytilus edulis. Marine Ecology Progress Series 319, 129–134.

Thielges DW and Rick J (2006) Effect of temperature on emergence, survival and infectivity of cercariae of the marine trematode Renicola roscovita (Digenea: Renicolidae). Diseases of Aquatic Organisms 73, 63–68.

Tkach VV, Pawlowski J, Mariaux J and Swiderski Z (2001) Molecular phylogeny of the suborder Plagiorchiata and its position in the system of Digenea. In Littlewood DTJ and Bray RA (eds), Interrelationships of Digenea. In Littlewood DTJ and Bray RA (eds), Systematic Biology 55, 264–287.

Tuzovic J and Ristic M (2013) Hybridization among office white-headed gulls (Larus spp.) obscures the genetic legacy of the Pleistocene. Ecology and Evolution 2, 1278–1295.

Ussar JP and Cunningham CW (2001) Phylogeography and historical ecology of the North Atlantic intertidal. Evolution 55, 2455–2469.

Wardrop B (1960) Morphology, development and ecology of Trematoda-Larven der Strandschnecke Littorina littorea. Marine Biology 3, 306–333.

Winneppeninckx B, Backeljau T and De Wachter R (1993) Extraction of high molecular weight DNA from molluscs. Trends in Genetics 9, 407.

Wright CA (1953) Probable relationship between the Rhodometopa group of cercariae and the trematode genus Renicola Cohn. Nature 171, 1072–1073.

Wright CA (1954) Trematodes of the genus Renicola from birds in British zoos, with descriptions of two new species. Proceedings of the Zoological Society of London 124, 51–61.

Wright CA (1956) Studies on the life history and ecology of the trematode genus Renicola Cohn, 1904. Proceedings of the Zoological Society of London 126, 1–49.

Wright CA (1957) Two kidney flukes from Sudanes birds with a description of a new species. Journal of Helminthology 31, 229–238.

Yamaguti S (1939) Studies on the helminth fauna of Japan. Part 25. Helminths of Farming and Hunting Animals. Moscow, USSR: Nauka, pp. 69–126.

Yamaguti S (1958) The morphology of Renicola philippinensis, n. sp., a digenetic trematode from the pheasant-tailed Jacana, Hydrophasianus chirurgus (Scopoli). Zoologica: Scientific Contributions of the New York Zoological Society 43, 105–112.

Sudarikov YE and Stenko RP (1984) Trematodes of the family Renicolidae. In Sonin MD (ed.), Helminths of Farming and Hunting Animals. Moscow, USSR: Nauka, pp. 34–89 (in Russian).