Research Article

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Molecular identification of morphologically similar microcercous cercariae of two trematode families, Paragonimidae and Troglotremitidae, concurrently found in the same snail species of the subfamily Triculinae

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Abstract: Microcercous cercariae possess a very short tail and are produced by digenean species of several families including medically important species, such as members of the genera *Paragonimus* Braun, 1899, *Nanophyetus* Chapin, 1927 and *Troglotremita* Odhner, 1914. During our survey of cercariae of *Paragonimus* spp. in Vietnam, we found microcercous cercariae from ten (0.29%) out of 3,400 snails of Triculinae gen. sp. 2. They were morphologically and molecularly analysed for species identification. The molecular analysis, based on ITS2 sequences, revealed two distinct species: four specimens were identical to *Paragonimus proliferus* Hsia et Chen, 1964 (Paragonimidae Dollfus, 1939), and the other six specimens were closest to members of the family Troglotremitidae Odhner, 1914 and were temporarily named Troglotremitidae gen. sp. Morphologically, cercariae of the two species found in this study are similar to each other in their gross characteristics but can be distinguished from one another by subtle morphological details. The cercaria of *P. proliferus* has an I-shaped excretory bladder and does not have mucous gland cells. In contrast, that of Troglotremitidae gen. sp. has a Y-shaped excretory bladder and mucous gland cells. Besides, the redia of *P. proliferus* is elongate with a short intestine and contains 5–6 cercariae whereas that of Troglotremitidae gen. sp. is more round with a longer intestine and harbours 3–4 cercariae. Our results have shown the importance of the shape of the excretory bladder and the presence/absence of mucous gland cells of the cercaria as well as the shape and size of the redia, and its intestinal length as valuable taxonomic characters of intramolluscan trematode larvae. In addition, the finding of similar microcercous cercariae of different species in the same snail species suggests that careful attention to morphological details is required in the differentiation of *Paragonimus* cercariae and those of closely related species.

Keywords: morphology, ITS2, digenean larvae, microcercariae, Vietnam.

Digenean trematodes have a complex life cycle with the involvement of at least two hosts. Of which, the obligatory first intermediate hosts are usually snails, where the development of larval stages, such as sporocyst, redia, and cercaria, takes place (Adams 2006). Thus, studies on trematode larvae in snail hosts allow understanding the life cycles of trematodes and also studying the evolutionary relationships between snail hosts and parasites (Wilke et al. 2000, Lockyer et al. 2004).

The traditional identification of trematode larvae is mainly based on the morphological characteristics of the cercaria (Schell 1970). Generally, it is possible to identify cercarial specimens to the family level, and occasionally to the genus level, using morphological criteria, but it is not easy to differentiate cercariae of related genera/species based on their morphology alone (Frandsen and Christensen 1984). Alternatively, molecular techniques have been used to support the morphological identification (e.g.,

Bartoli et al. 2000, Pina et al. 2007, Chuboon and Wong-sawad 2009, Kudlai et al. 2015, Blasco-Costa et al. 2016, Chontanarith et al. 2017, Doanh et al. 2018).

Among cercarial groups, microcercous cercariae possess a short ‘knoblike’ tail (Cable 1938, Schell 1970) and are produced by digenean species of several families including medically important species, such as members of the genera *Paragonimus* Braun, 1899 (causing paragonimiasis for animals and humans), *Nanophyetus* Chapin, 1927 (a common parasite of humans in East Siberia and a vector for a rickettsial disease of canids referred to as ‘salmon poisoning disease’ in North America), and *Troglotremita* Odhner, 1914 (associated with skull lesion in mustelids) (Adams 2006, Blair et al. 2008).

During our previous survey of snail hosts of *Paragonimus* spp. in a highly endemic area in central Vietnam (Doanh et al. 2013, 2016), we identified the cercaria of *Paragonimus proliferus* Hsia et Chen, 1964 in an individ-

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Fig. 1. Microcercous cercariae from freshwater snails of Triculinae gen. sp. 2 collected in Huong Son Commune, Huong Hoa District, Quang Tri Province, Vietnam. **A, B** – cercaria and redia of *Paragonimus proliferus* Hsia et Chen, 1964; **C, D** – cercaria and redia of Troglotreumatidae gen. sp.

ual snail of Triculinae gen. sp. 2 (Doanh et al. 2018). In the present study, we examined a large number of snails of Triculinae gen. sp. 2 and found the cercaria of *P. proliferus* of the family Paragonimidae Dollfus, 1939 and an unidentified species of the family Troglotreumatidae Odhner, 1914. The morphological characteristics and molecular data on these cercariae were analysed and discussed herein.

MATERIALS AND METHODS

Collection and examination of snails

Freshwater snails, which were molecularly identified as Triculinae gen. sp. 2 of the subfamily Triculinae Annandale, were collected in the stream where crabs were heavily infected with metacercariae of *Paragonimus* spp., in Huong Son Commune, Huong Hoa District, Quang Tri Province (Doanh et al. 2016, 2018). Snails were examined for microcercous cercariae. In the laboratory, snails were washed with water and placed in 20 mm wide-mouth plastic containers with tap water for shedding cercariae.

The containers were examined under a stereomicroscope for the presence of emerged cercariae at night and the next morning. If any microcercous cercariae were found, they were used for further study. Afterward, all molluscs were separately compressed between two glass slides and examined under a microscope to find cercariae that did not emerge, and other intra-molluscan stages, such as rediae.

Morphological data

Live cercariae and rediae were observed under an Axio Lab A1 microscope (Carl Zeiss, Oberkochen, Germany). Then, live specimens were fixed in a hot 4% formaldehyde solution for mor-

phological study and 96% ethanol for molecular study. Morphological characteristics of cercariae and rediae were studied from both live and fixed specimens. Measurements of cercariae and rediae were taken from fixed specimens. All measurements are in micrometers.

Molecular analyses

A fragment of the internal transcribed spacer 2 region (ITS2) was chosen for analyses because this region has been proven to be a reliable marker and has been commonly used to identify digenean species (Esteban et al. 2014, Blasco-Costa et al. 2016, Chai 2019). Total genomic DNA was extracted from cercariae isolated from infected snails using the Dneasy Blood and Tissue Kit (QIAGEN, Hilden, Germany). The ITS2 region was amplified using forward primer 3S and reverse primer A28 (Bowles et al. 1995), following the amplification protocol described in Doanh et al. (2018). PCR products were purified and directly sequenced by an Ab3730 sequencer using a Big-Dye Terminator Cycle Sequencing Kit.

We obtained ten ITS2 sequences of ten cercarial specimens from ten infected snails. The new sequences were deposited in the DNA Data Bank of Japan (DDBJ) with the accession No. LC506420-LC506429 (Table 1). In addition, 20 sequences of the Paragonimidae and Troglotreumatidae, and an outgroup sequence (MK450527) of *Clonorchis sinensis* (Cobbold, 1875) (Table 1) were downloaded from GenBank and included in the phylogenetic analysis. These sequences were aligned using ClustalW in MEGA7.0 software (Kumar et al. 2016). The maximum likelihood phylogenetic tree was obtained using the best-fit Kimura 2-parameter model tested in MEGA 7.0. Branch support was estimated by bootstrap analyses with 1,000 replicates.

Fig. 2. Molecular phylogenetic analysis inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model. A discrete Gamma distribution (+G) was used to model evolutionary rate differences among sites. All positions containing gaps and missing data were eliminated. The bootstrap values are shown next to the branches. Evolutionary analyses were conducted in MEGA7. The nucleotide sequences obtained in this study (printed in bold) and others from the GenBank database are shown with Accession No., species name, and three letter country code of their geographical origin (CHN = China, IND = India, JPN = Japan, RUS = Russia, THA = Thailand, SVK = Slovakia, VNM = Vietnam).

RESULTS

Prevalence of microcercous cercariae and molecular identification

A total of 3,400 snails of Triculinae gen. sp. 2 were examined for cercariae. Microcercous cercariae were found from ten snails (0.29%). The molecular analyses indicated two distinct sequences: four ITS2 sequences of four specimens were completely identical with each other and 464 bp in length. The other six ITS2 sequences from six specimens were also completely identical with each other and 481 bp in length. The BLAST search for highly similar sequences in GenBank revealed that four ITS2 sequences showed 100% identity to sequence (LC360503) of *Paragonimus proliferus*, while the other six sequences showed

the highest similarity 89–93% to members of the family Troglotrematidae. They are temporarily named Troglotrematidae gen. sp. The result of the pairwise comparison showed that the ITS2 sequences of Troglotrematidae gen. sp. were 15–20% distant from those of *Paragonimus* spp. and different from those of the Troglotrematidae at 10.9–16.3%. In the phylogenetic tree (Fig. 2), all species of *Paragonimus* formed a common clade with a high bootstrap value and members of the Troglotrematidae also formed a common clade with a moderate bootstrap value. Four sequences of *P. proliferus* obtained in this study were clustered with that of *P. proliferus* (LC360503) in the clade of the Paragonimidae, while all six sequences of Troglotrematidae gen. sp. formed a distinct group in the clade of the Troglotrematidae.

Table 1. ITS2 sequences used in the present study with GenBank accession numbers, name of species and their geographical origin.

Species	Location origin	GenBank No.	Reference
<i>Clonorchis sinensis</i> (Cobbold, 1875) (out-group)	China	MK450527	Qiu et al. 2019
Nanophyetidae gen. sp.	Slovakia	KM594165	Heneberg et al. 2015
Nanophyetidae gen. sp.	Slovakia	KM594150	Heneberg et al. 2015
<i>Nanophyetus japonensis</i> Saito, Saito, Yamashita, Watanabe et Sekikawa, 1982	Japan	LT796186	Unpublished
<i>Nanophyetus japonensis</i>	Japan	LT796185	Unpublished
<i>Nanophyetus salmincola</i> (Chapin, 1926)	Russia	LN871823	Unpublished
<i>Nanophyetus schikhobalowi</i> Skrjabin et Podyapolskaya, 1931	Russia	LT745961	Voronova et al. 2017
<i>Nanophyetus schikhobalowi</i>	Russia	LT745962	Voronova et al. 2017
<i>Paragonimus bangkokensis</i> Miyazaki et Vajrasthira, 1967	Vietnam	FJ615213	Doanh et al. 2009
<i>Paragonimus harinasutai</i> Miyazaki et Vajrasthira, 1968	Thailand	AF159609	Blair et al. 1999a
<i>Paragonimus heterotremus</i> Chen et Hsia, 1964	Vietnam	LC360505	Doanh et al. 2018
<i>Paragonimus macrorchis</i> Chen, 1962	Thailand	AF159608	Blair et al. 1999a
<i>Paragonimus miyazakii</i> Kamo, Nishida, Hatsushika et Tomimura, 1961	Japan	AB629937	Unpublished
<i>Paragonimus ohirai</i> Miyazaki, 1939	Japan	U96911	Blair et al. 1997
<i>Paragonimus proliferus</i> Hsia et Chen, 1964	Vietnam	LC506420	Present study
<i>Paragonimus proliferus</i>	Vietnam	LC506421	Present study
<i>Paragonimus proliferus</i>	Vietnam	LC506422	Present study
<i>Paragonimus proliferus</i>	Vietnam	LC506423	Present study
<i>Paragonimus proliferus</i>	Vietnam	LC360503	Doanh et al. 2018
<i>Paragonimus skrjabini</i> Chen, 1959	China	AY618729	Blair et al. 2005
<i>Paragonimus westermanni</i> (Kerbert, 1878)	India	JN656198	Devi et al. 2013
<i>Paragonimus westermanni</i>	Vietnam	LC360504	Doanh et al. 2018
<i>Paragonimus vietnamensis</i> Doanh, Shinohara, Horii, Habe, Nawa et Le, 2007	Vietnam	AB270689	Doanh et al. 2007
Troglotrematidae gen. sp.	Vietnam	LC506424	Present study
Troglotrematidae gen. sp.	Vietnam	LC506425	Present study
Troglotrematidae gen. sp.	Vietnam	LC506426	Present study
Troglotrematidae gen. sp.	Vietnam	LC506427	Present study
Troglotrematidae gen. sp.	Vietnam	LC506428	Present study
Troglotrematidae gen. sp.	Vietnam	LC506429	Present study
Troglotrematidae gen. sp.	Japan	AB521803	Sato et al. 2010
Troglotrematidae gen. sp.	Slovakia	KM594158	Heneberg et al. 2015

Morphological description of microcercous cercariae in snails of Triculinae gen. sp. 2

Cercaria of *Paragonimus proliferus* Fig. 1A,B

Description (measurements based on 30 specimens)

Mature cercaria has a stylet and a short tail (Fig. 1A). Cercaria body elongate oval, measuring 285–320 × 115–155 (305 × 121). Oral sucker rounded, 60–75 × 60–70 (72 × 67), larger than ventral one, located at anterior extremity and accompanied with stylet, 35–50 × 7.5–8.7 (39 × 7.7), with rounded, enlarged base and tapering to sharp point. Ventral sucker rounded, situated slightly posterior to middle of body, 35–50 × 35–50 (43 × 43). Prepharynx relatively long, pharynx about midway between suckers. Intestinal bifurcation slightly anterior to ventral sucker; caeca terminate at the posterior end of body. Penetration gland cells 14 in total, arranged as submedian group of three anterior to ventral sucker and group of four anterolateral to ventral sucker on each side. Gland ducts extend forward along lateral margins of body, open on anterior sides of stylet. Excretory bladder I-shaped, situated between posterior end of ventral sucker and the end of body. Tail ‘knob-shaped’, spinous, 25–30 × 25–27.5 (27 × 26).

Redia elongate, 900–1,100 × 200–300, intestine short, 180–220, about 20% of body length, containing 5–6 cercariae (Fig. 1B). Cercariae of *P. proliferus* were found in four (0.12%) snails.

Cercaria of Troglotrematidae gen. sp. Fig. 1C,D

Description (measurements based on 30 specimens)

Mature cercaria with a stylet and short tail (Fig. 1C). Cercaria body elongate oval, measuring 210–240 × 90–98 (227 × 93). Oral sucker rounded, 65–75 × 60–70 (71 × 67), larger than ventral one, located at the anterior extremity and accompanied with a stylet of 35–42.5 × 7.5–8.7 (38 × 7.7) with rounded, enlarged base and tapering to a sharp point. Pharynx located just posterior to oral sucker, but esophagus and caeca not observed. Ventral sucker rounded, situated slightly posterior to middle of body, 40–47.5 × 40–47.5 (43 × 43). Penetration gland cells 16 in total, arranged in 4 clusters of 4 cells each at anterolateral to ventral sucker. Gland ducts extended forward along lateral margins of body and opening on anterior sides of stylet. Excretory bladder Y-shaped, situated between posterior end of ventral sucker and end of body. Adhesive (mucous) gland cells in ventral part of posterior end of body. Tail ‘knob-shaped’, 15–22.5 × 15–17.5 (18.5 × 16).

Redia oval, 440–500 × 360–410, intestine terminating at near posterior end of body, 240–260, about 50% of body length, containing 3–4 cercariae (Fig. 1D).

Cercariae of Troglotrematidae gen. sp. were found in six (0.18%) snails.

Remarks

Microcercous cercariae of two species found in this study are similar to each other in their gross morpho-

gy with a stylet at the anterior end and a short tail (Fig. 1A,C). However, they are different from each other in the presence/absence of adhesive (mucous) gland cells and the shape of the excretory bladder. In addition, their redial stage is also distinguishable from each other in the shape and size of the body, the ratio of intestinal length and body length (20% vs. 50%), and the number of cercariae per redia (Fig. 1B,D). The morphological characters and measurements of cercariae from four infected snails are similar to those of *P. proliferus* previously described by Doanh et al. (2018), while microcercous cercariae from the other six infected snails do not correspond to any reported species.

DISCUSSION

As mentioned above, microcercous cercariae are produced by members of some digenean families (Cable 1938), including the Troglotrematidae from which the Paragonimidae Dollfus, 1939 and Nanophyetidae Dollfus, 1939 were erected as distinct families. However, Blair et al. (2008) did not recognise the Nanophyetidae as distinct, and still placed them within the Troglotrematidae. The molecular analyses in the present study supported this view, with sequences classified into the Nanophyetidae by Hengberg et al. (2015) being intermingled with those of Troglotrematidae in the phylogenetic tree.

In the cercarial stage, the presence/absence of a cluster of mucous gland cells arranged radially around the posteroventral end of the body is a unique feature to distinguish *Paragonimus*, the sole genus in the Paragonimidae, from genera of the Troglotrematidae (see Blair et al. 2008). In agreement with this, we observed mucous gland cells in the cercaria of Troglotrematidae gen. sp., but not in *Paragonimus proliferus*. In addition, we found that the differentiation between them in the shape of the excretory bladder, I-shaped in *P. proliferus* and Y-shaped in Troglotrematidae gen. sp., is more easily recognised. Regarding this feature, I-shaped excretory bladder was reported in members of the Paragonimidae (e.g. Yamaguti 1943, Komiya and Ito 1950, Tomimura et al. 1989, Iwagami et al. 2009, Doanh et al. 2018), Y-shaped form was described from *Troglotrema acutum* (Leuckart, 1842) (see Vogel and Voelker 1978), and U- or V-shaped forms were recorded from species of *Nanophyetus* (see Bennington 1960, Filimonova 1963, Saito 1985) of the Troglotrematidae.

Thus, we suppose that the shape of the excretory bladder can be used as a useful character for the differentiation of

cercariae at the genus/family level. Although the numbers of penetration gland cells of the two types were different from each other, it is not easy to observe all gland cells in every specimen. This might explain reported differences in the numbers of gland cells between cercariae of the same *Paragonimus* species from the same locality (Blair et al. 1999b).

In contrast to similarities between the two cercarial types, their redial stages are easily distinguishable from each other in the shape and the size of the body, in the ratio of intestinal length to body length (20% vs. 50%), and in the number of cercariae per redia (Fig. 1B,D). These differences were also found between *Nanophyetus salmincola* (Chapin, 1926) and *Nanophyetus schikhobalowi* Skrjabin et Podyapolskaya, 1931 (see Bennington 1960, Filimonova 1963), and between three species of *Paragonimus*, *P. heterotremus* Chen et Hsia, 1964, *P. westermanni* (Kerbert, 1878) and *P. proliferus* (see Doanh et al. 2018). This suggests that more data on redial stage should be collected to clarify if its characters can support the differentiation between digenean species at intramolluscan stages. Based on the above-mentioned characters, the redia of Troglotrematidae gen. sp. found in this study is significantly different from those of the previously known species, namely *N. salmincola*, *N. schikhobalowi*, *N. japonensis* Saito, Saito, Yamashita, Watanabe et Sekikawa, 1982, and *T. acutum* (see Bennington 1960, Filimonova 1963, Vogel and Voelker 1978, Saito 1985). Further studies are necessary to collect adult worms to assign this microcercous cercaria to a species.

To our best knowledge, this is the first record of the concurrence of cercariae of *Paragonimus* and a closely related species of the Troglotrematidae in the same snail species, suggesting that the detailed examination of morphological characteristics and molecular analyses should be combined for accurate identification. The shape of the excretory bladder in addition to the presence/absence of the adhesive gland cells can be used as valuable taxonomic features for distinguishing these cercariae of two closely related families. Also, morphometrics (shape, size, ratio of length of intestine and body, and number of cercariae) of the redial stage provide additional traits for differentiation between genera/species.

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REFERENCES

- ADAMS A.M. 2006: Foodborne trematodes. In: Y.R. Ortega (Ed.), Foodborne Parasites. Springer Science+Business Media, New York, pp. 161–196.
- BARTOLI P., JOUSSON O., RUSSELL-PINTO F. 2000: The life cycle of *Monorchis parvus* (Digenea: Monorchidae) demonstrated by developmental and molecular data. J. Parasitol. 86: 479–489.
- BENNINGTON E. 1960: The life history of the salmon poisoning fluke, *Nanophyetus salmincola* (Chapin). J. Parasitol. 46: 91–100.
- BLAIR D., AGATSUMA T., WATANOBE T., OKAMOTO M., ITO A. 1997: Geographical genetic structure within the human lung fluke, *Paragonimus westermanni*, detected from DNA sequences. Parasitology 115: 411–417.
- BLAIR D., CHANG Z., CHEN M., CUI A., WU B., AGATSUMA T., IWAGAMI M., CORLIS D., FU C., ZHAN X. 2005: *Paragonimus skrjabini* Chen, 1959 (Digenea: Paragonimidae) and related species in eastern Asia: a combined molecular and morphological approach to identification and taxonomy. Syst. Parasitol. 60: 1–21.
- BLAIR D., TKACH V.V., BARTON D.P. 2008: Family Troglotrematidae Oehner, 1914. In: D.I. Gibson, R.A. Bray and A. Jones (Eds),

- Keys to the Trematoda, Vol. 3. CAB International and Natural History Museum, Wallingford, pp. 277–290.
- BLAIR D., WU B., CHANG Z.S., GONG X., AGATSUMA T., ZHANG Y.N., CHEN S.H., LIN J.X., CHEN M.G., WAIKAGUL J., GUEVARA A.G., FENG Z., DAVIS G.M.A. 1999a: Molecular perspective on the genera *Paragonimus* Braun, *Euparagonimus* Chen and *Pagumogonimus* Chen. J. Helminthol. 73: 295–299.
- BLAIR D., XU Z.B., AGATSUMA T. 1999b: Paragonimiasis and the genus *Paragonimus*. Adv. Parasitol. 42: 113–222.
- BLASCO-COSTA I., CUTMORE S.C., MILLER T.L., NOLAN M.J. 2016: Molecular approaches to trematode systematics: ‘best practice’ and implications for future study. Syst. Parasitol. 93: 295–306.
- BOWLES J., BLAIR D., MCMANUS D.P. 1995: A molecular phylogeny of the human schistosomes. Mol. Phyl. Evol. 4: 103–109.
- CABLE R.M. 1938: Studies on larval trematodes from Kentucky with a summary of known related species. Am. Midl. Nat. 19: 440–404.
- CHAI J.Y. 2019: Human Intestinal Flukes from Discovery, Treatment and Control. Springer Nature B.V., Dordrecht, 549 pp.
- CHONTANANARTH T., TEJANGKURA T., WETCHSART N., CHIMBURUT C. 2017: Morphological characteristics and phylogenetic trends of trematode cercariae in freshwater snails from Nakhon Nayok province, Thailand. Kor. J. Parasitol. 55: 47–54.
- CHUBOON A., WONGSAWAD C. 2009: Molecular identification of larval trematode in intermediate hosts from Chiang Mai, Thailand. Southeast Asian J. Trop. Med. Public Health 40: 1216–20.
- DEVI K.R., NARAIN K., MAHANTA J., NIRMOLIA T., BLAIR D., SAIKIA S.P., AGATSUMA T. 2013: Presence of three distinct genotypes within the *Paragonimus westermani* complex in north-eastern India. Parasitol. 140: 76–86.
- DOANH P.N., HORII Y., NAWA Y. 2013: *Paragonimus* and paragonimiasis in Vietnam: an update. Korean J. Parasitol. 51: 621–27.
- DOANH P.N., SHINOHARA A., HORII Y., HABE S., NAWA Y., THE D.T., LE N.T. 2007: Morphological and molecular identification of two *Paragonimus* spp., of which metacercariae concurrently found in a land crab, *Potamiscus tannanti*, collected in Yenbai Province, Vietnam. Parasitol. Res. 100: 1075–1082.
- DOANH P.N., SHINOHARA A., HORII Y., YAHIRO S., HABE S., VANNAVONG N., STROBEL M., NAKAMURA S., NAWA Y. 2009: Morphological differences and molecular similarities between *Paragonimus bangkokensis* and *P. harinasutai*. Parasitol. Res. 105: 429–439.
- DOANH P.N., TU L.A., DUNG B.T., LOAN H.T., NONAKA N., HORII Y., BLAIR D., NAWA Y. 2016: Molecular and morphological variation of *Paragonimus westermani* in Vietnam with records of new second intermediate crab hosts and a new locality in a northern province. Parasitology 143: 1639–46.
- DOANH P.N., TU L.A., HIEN H.V., DUC N.V., HORII Y., BLAIR D., NAWA Y. 2018: First intermediate hosts of *Paragonimus* spp. in Vietnam and identification of intramolluscan stages of different *Paragonimus* species. Parasit. Vectors 11: 328.
- ESTEBAN J.G., MUNOZ-ANTOLI C., TOLEDO R., ASH L.R. 2014: Diagnosis of human trematode infections. In: R. Toledo, B. Fried (Eds), Digenetic Trematodes. Springer Science + Business Media, New York, pp. 293–330.
- FILIMONOVA L.V. 1963: [Life cycle of trematode *Nanophyetus schikhobalowi*.] Tr. Gel'mintol. Lab. Akad. Nauk SSSR. 13: 347. (In Russian.).
- FRANDSEN F., CHRISTENSEN N.Ø. 1984: An introductory guide to the identification of cercariae from African freshwater snails with special reference to cercariae of trematode species of medical and veterinary importance. Acta Trop. 41: 181–202.
- HENEGER P., FALTÝNKOVÁ A., BIZOS J., MALÁ M., ŽIAK J., LITERÁK I. 2015: Intermediate hosts of the trematode *Collyriclum faba* (Plagiochiida: Collyriclidae) identified by an integrated morphological and genetic approach. Parasit. Vectors. 8: 85.
- IWAGAMI M., RAJAPAKSE R.P.V.J., YATAWARA L., KANO S., AGATSUMA T. 2009: The first intermediate host of *Paragonimus westermani* in Sri Lanka. Acta Trop. 109: 27–29.
- KOMIYA Y., ITO J. 1950: Contribution to the morphology of *Paragonimus westermani*. Jpn. J. Med. 3: 309–314.
- KUDLAI O., STUNZENAS V., TKACH V. 2015: The taxonomic identity and phylogenetic relationships of *Cercaria pugnax* and *C. helvetica* XII (Digenea: Lecithodendriidae) based on morphological and molecular data. Folia Parasitol. 62: 003.
- KUMAR S., STECHER G., TAMURA K. 2016: MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol. Phyl. Evol. 33: 1870–1874.
- LOCKYER A.E., JONES C.S., NOBLE L.R., ROLLINSON D. 2004: Trematodes and snails: an intimate association. Can. J. Zool. 82: 251–269.
- PINA S.M.R., RUSSELL-PINTO F., RODRIGUES P. 2007: Clarification of *Cercaria sevilla* (Digenea: Microphallidae) life cycle using morphological and molecular data. J. Parasitol. 93: 318–322.
- QIU Y.Y., GAO Y., LI Y., MA X.X., LV Q.B., HU Y., QIU H.Y., CHANG Q.C., WANG C.R. 2019: Comparative analyses of complete ribosomal DNA sequences of *Clonorchis sinensis* and *Metorchis orientalis*: IGS sequences may provide a novel genetic marker for intraspecific variation. Infect. Genet. Evol. 78: 104125.
- SAITO S. 1985: Cercaria of *Nanophyetus japonensis* from the freshwater snail, *Semisulcospira libertina*, in Japan and its experimental infection. Jpn. J. Parasitol. 34: 41–53.
- SATO H., IHARA S., INABA O., UNE Y. 2010: Identification of *Euryhelms costaricensis* metacercariae in the skin of *Tohoku hy-nobiid* salamanders (*Hynobius lichenatus*), northeastern Honsh, Japan. J. Wildl. Dis. 46: 832–42.
- SCHELL S.C. 1970: How to Know the Trematodes. Dubuque, W. C. Brown Company Publishers, 355 pp.
- TOMIMURA T., SUGIYAMA H., YOKOTA M. 1989: Parasitological survey of the first intermediate host of *Paragonimus westermani* in Iga area of Mie prefecture, Japan. Jpn. J. Vet. Sci. 51: 325–326.
- VOGEL H., VOELKER J. 1978: On the life cycle of *Trogloremata acutum*. Trop. Med. Parasitol. 29: 385–405.
- VORONOVA A.N., CHELOMINA G.N., BESPROZVANNYKH V.V., TKACH V.V. 2017: Genetic divergence of human pathogens *Nanophyetus* spp. (Trematoda: Troglorematidae) on the opposite sides of the Pacific Rim. Parasitology 144: 601–612.
- WILKE T., DAVIS G.M., GONG X., LIU H.X. 2000: *Erhaia* (Gastropoda: Risssooidea): phylogenetic relationships and the question of *Paragonimus* coevolution in Asia. Am. J. Trop. Med. 62: 453–459.
- YAMAGUTI S. 1943: On the morphology of the larval forms of *Paragonimus westermani*, with special reference to their excretory system. Jap. J. Zool. 10: 461–469.

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