Institute of Parasitology, Biology Centre CAS
Folia Parasitologica 2023, 70: 009
doi: 10.14411/fp.2023.009

Folia Parasitologica
http://folia.paru.cas.cz

Research Article OPEN ACCESS

A global survey of tapeworms (Cestoda: Proteocephalidae) of 'true' frogs (Amphibia: Ranidae), including a tabulated list of all proteocephalids parasitising amphibians

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Abstract: Proteocephalid tapeworms of frogs of the family Ranidae ('true' frogs) are reviewed with emphasis on their species diversity, host specificity and geographical distribution. New molecular data (nuclear *lsr*DNA and mitochondrial COI sequences) are presented for tapeworms of four species of ranid frogs in North America, including the poorly known *Ophiotaenia saphena* Osler, 1931 of *Rana clamitans* Latreille and *R. catesbeiana* (Shaw), which is redescribed using new material from Arkansas, USA. Tapeworms of *R. sphenocephala* (Cope) and *R. pipiens* Schreber, the latter previously identified as *O. saphena*, represent another, putative new species, but are not formally described due to insufficient available material. *Proteocephalus papuensis* Bursey, Goldberg et Kraus, 2008 from *Sylvirana supragrisea* (Menzies) is transferred to *Ophiotaenia* La Rue, 1911 as a new combination. After a critical review of the literature, only nine nominal species of *Ophiotaenia* are recognised as valid, which is in contrast to the large number of ranid frogs (> 440 spp.). The reasons for this striking disparity are briefly discussed, and a key based on morphology is presented for the identification of all species of *Ophiotaenia* from the Ranidae. Molecular data are available for only two taxa from North America that form a monophyletic group. The relationships among tapeworms of ranid frogs occurring in other zoogeographical regions are not yet known. The taxonomic status of *Batrachotaenia* Rudin, 1917, which was erected to accommodate proteocephalids from amphibians, is also discussed. To facilitate future studies, a tabulated summary of all 32 species of proteocephalids belonging to three genera reported from amphibians (frogs and salamanders) is presented, with information on their hosts, distribution, and taxonomically important characters, including key measurements.

Key words: *Ophiotaenia*, Onchoproteocephalidea, global diversity, taxonomy, morphology, redescription, molecular data, Anura, Amphibia, Nearctic region

This article contains supporting tables (Tables 1–2) online at http://folia.paru.cas.cz/suppl/2023-70-009.pdf

Tapeworms (Cestoda) are intestinal parasites of all major groups of vertebrates, but their diversity is uneven across vertebrate groups: while birds and mammals harbour 1,639 and 1,540 species of adult tapeworms, respectively, only 24 species of tapeworms were reported from amphibians by Caira et al. (2017). In fact, more than 50 nominal tapeworm taxa have been described from amphibians: 25 species of the proteocephalid genus *Ophiotaenia* La Rue, 1911 (Onchoproteocephalidea: Proteocephalidae) reported by de Chambrier et al. (2017), three species of the proteocephalid genera *Australotaenia* de Chambrier et de Chambrier, 2010 and *Nomimoscolex* Woodland, 1934, and 18 species of the cyclophyllidean family Nematotaeniidae Lühe, 1910 listed by Jones (1987). Nevertheless, the cestode fauna of amphibians is undoubtedly depauperate, especially compared to the high species diversity of trematodes and nematodes found in these hosts (Ryzhikov et al. 1980, Prudhoe and Bray 1982, Baker 1987).

An updated list of species of *Ophiotaenia* currently includes 28 species that parasitise amphibians, because de Chambrier et al. (2017) inadvertently omitted *O. bonneti* de Chambrier, Coquille et Brooks, 2006 from *Rana vaillanti* Brocchi in Costa Rica, *O. calamensis* Puga et Formas, 2005 from *Telmatobius dankoi* Formas, Northland, Capetillo, Núñez, Cuevas et Brieva in Chile, and *Ophiotaenia papuensis* (Bursey, Goldberg et Kraus, 2008) comb. n. from *Sylvirana supragrisea* (Menzies), currently recognised as *Papurana supragrisea* (Menzies), in Papua New

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Guinea. Of these 28 species, no fewer than 21 cestodes occur in anurans, including nine species that parasitise frogs of the family Ranidae ('true' frogs).

Molecular data on tapeworms in amphibians are scarce, and this lack of information also affects species of *Ophiotaenia*, as *lsr*DNA sequences of only two species have been published, those of *Ophiotaenia filaroides* (La Rue, 1909) from the tiger salamander, *Ambystoma tigrinum* (Green) (GenBank accession number KP729416) and *Ophiotaenia saphena* Osler, 1931 from the northern leopard frog, *Rana pipiens* Schreber (KP729402), both from North America (de Chambrier et al. 2015). Both species appeared in a large clade (clade D of de Chambrier et al. 2015) with unresolved relationships that includes mainly proteocephalids of pimelodid catfishes and other Neotropical teleosts, but also includes the Nearctic *Ophiotaenia perspicua* La Rue, 1911 (type species of the genus), the Palaearctic *Ophiotaenia europaea* Odening, 1963, and the Neotropical *Ophiotaenia paraguayensis* Rudin, 1917 and *Ophiotaenia sanbernardinensis* Rudin, 1917, all from colubrid snakes (de Chambrier et al. 2015).

Recently, new material of *Ophiotaenia* tapeworms was collected from four species of 'true' frogs (Ranidae) in North America, allowing molecular data to be obtained and their relationships and intraspecific morphological variability to be evaluated. In addition, a little-known species is redescribed and a global survey of *Ophiotaenia* tapeworms of ranids is presented. A key to identifying all species of *Ophiotaenia* from ranid frogs is also included. In addition, a tabulated list of all proteocephalids reported from amphibians worldwide has been compiled, with information on their hosts, distribution and taxonomically important characteristics, including key measurements, to facilitate future research on this interesting, but rather neglected group of tapeworms.

Despite the lack of molecular data on tapeworms of amphibians (see de Chambrier et al. 2015, 2017), we also sought to answer, at least tentatively, the following questions: (1) Do the proteocephalid tapeworms of ranid frogs form a monophyletic group? (2) Are these tapeworms closely related to the type species of the genus, *O. perspicua*, i.e., do they belong to the 'true' *Ophiotaenia* (= *Ophiotaenia* sensu stricto)? (3) Do the available data support the concept of Freze (1965) for *Batrachotaenia* Rudin, 1917, i.e., for the inclusion of proteocephalids from frogs and salamanders?

MATERIALS AND METHODS

Morphological study

The morphological study is mainly based on the evaluation of tapeworms collected by the present authors between 1999 and 2019 in the USA. Due to restrictions, which included the closure that also included lockdowns of museum collections during the COVID-19 pandemic in 2020 and 2021, it was not possible to examine all material deposited in parasite collections. Newly collected tapeworms were processed as described in detail in previous reports (de Chambrier 2001, Scholz et al. 2013). Tapeworms isolated from fresh hosts were carefully rinsed in saline and immediately fixed with hot, near-boiling 4% formaldehyde solution (formalin). For scanning electron microscopic (SEM) observations, scoleces of a formalin-fixed specimen of *Ophiotaenia gracilis* Jones, Cheng et Gillespie, 1958 from *Rana catesbeiana* (Shaw), Arkansas, USA (host code US 800b), and a specimen of *O. saphena* Osler, 1931 from *Rana clamitans* Latreille (US 928), Oklahoma, USA, were dehydrated through a graded ethanol series, dried in hexamethyldisilazane, coated with gold and examined in a JEOL JSM-740 1F scanning electron microscope at the Institute of Parasitology in České Budějovice. All measurements in the descriptions are in micrometres unless otherwise stated. The abbreviations used in the descriptions are: x = mean; n = number of measurements; US = host field number. Host names follow Frost (2022) except that we follow Yuan et al. (2016) in using the genus *Rana* Linnaeus instead of *Lithobates* Fitzinger for North American leopard frogs.

Acronyms of parasitological collections are as follows: CNHE – Colección Nacional de Helmintos, Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City, Mexico; IPCAS – Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic; MHNG-PLAT – Natural History Museum, Geneva, Switzerland; USNM – U.S. National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA. Because of the absence of comparative material, some measurements were taken directly from the original drawings, which are sometimes somewhat schematic. Where appropriate, mean (x) and (n) the number of measurements are provided in parentheses.

Molecular study

New sequences of nuclear *lsr*DNA (1,415 nucleotides (nt)) and mitochondrial COI (1,608 nt) genes were generated for nine isolates of *Ophiotaenia* spp. from *R. catesbeiana*, *R. clamitans*, *R. pipiens*, and *R. sphenocephala* (Cope). Genomic DNA was isolated using the Monarch Genomic DNA Purification Kit (New England Biolabs, Inc., Ipswich, USA) according to the manufacturer's instructions. PCR amplification of partial *lsr*DNA (D1–D3) was performed according to the protocol described by Brabec et al. (2012) or Scholz et al. (2013). PCR amplification of partial COI was performed using primers B2-TrpF and B2-16SR following the protocol described by Alves et al. (2020). PCR amplicons were purified using the ExoSAP-IT PCR Cleanup enzyme kit from Thermo Fisher Scientific, Inc. (Waltham, MA, USA) according to the manufacturer's protocol. PCR amplicons were then sequenced from both strands using the PCR primers and additional internal sequencing primers 300F and ECD2 for *lsr*DNA (Littlewood et al. 2000) and NeoP760R or Gang 970R for COI (Scholz et al. 2020). The newly generated sequences were assembled and edited using Geneious v. 11 (Biomatters, Auckland, New Zealand).

The phylogenetic relationships of the studied species of *Ophiotaenia* were evaluated based on partial *lsr*DNA sequences. The sequences generated in the present study were aligned with published sequences that formed a clade with *Ophiotaenia* cf. *saphena* in the phylogenetic analysis of the Proteocephalidae by Alves et al. (2020; their Fig. 2) using ClustalW implemented in Geneious ver. 11. *Nomimoscolex sudobim* Woodland, 1935, *Choanoscolex abscisus* (Riggenbach, 1895) and *Regoella brevis* Arredondo, de Chambrier et Gil de Pertierra, 2013 were used as outgroups based on the topology in the phylogenetic tree of Alves et al. (2020). The alignment was trimmed to the length of the shortest sequence in-

cluded in the dataset (991 nt); 26 nucleotide positions with ambiguous homology were excluded from the analyses. Phylogenetic relationships were reconstructed using maximum likelihood (ML) and Bayesian inference (BI). Analyses were performed using the GTR + I + G model, which was predicted to be the best model by the Akaike Information Criterion in jModelTest 2.1.2 (Darriba et al. 2012). The ML analysis was performed using PhyML ver. 3.0 (Guindon et al. 2010) with nonparametric bootstrap validation based on 100 pseudoreplicates. The BI analysis was performed using MrBayes software ver. 3.2.3 (Ronquist et al. 2012). Markov chain Monte Carlo chains were run for 10,000,000 generations, log-likelihood scores were recorded to estimate burn-in, and only the last 75% of trees were used to build the consensus tree. COI data were not used for the phylogenetic analyses because of the limited availability of COI sequences of *Ophiotaenia* spp. in 'true' frogs. Pairwise genetic distances (uncorrected p-distance) between *lsr*DNA and COI sequences were calculated using MEGA ver. 11 (Tamura et al. 2021). In addition, species delimitation analysis was performed with the Bayesian version of the Poisson Tree Processes model (bPTP) (Zhang et al. 2013) using the tree estimated via BI analysis. This was performed using the bPTP web server (Zhang et al. 2013; available at http://species.h-its.org/ptp) with default parameters.

RESULTS

Annotated list of species of *Ophiotaenia* La Rue, 1911 in the Ranidae of the world

1. *Ophiotaenia bonneti* de Chambrier, Coquille et Brooks, 2006

Type and only host: Vaillanti's frog, *Rana vaillanti* Brocchi.

Type locality: San Gerardo, Guanacaste Province, Costa Rica.

Distribution: Costa Rica.

Type material: Holotype (MHNG-PLAT-0037237) and six paratypes (MHNG-PLAT-0037238–0037241; IPCAS C-400; USNM 1392301).

Material studied: de Chambrier et al. (2006).

Morphological description: de Chambrier et al. (2006).

Representative DNA sequences and phylogenetic relationships: No molecular data are available, because attempts to amplify DNA from ethanol-fixed samples by PCR failed, probably due to the poor quality of the fixative used.

Reference: de Chambrier et al. (2006).

Comments. This species was found as part of the project 'Inventory of the Eukaryotic Parasites of Vertebrates of the Area de Conservacion Guanacaste' in Costa Rica (P.I. Daniel R. Brooks). In total 147 *R. vaillanti* were examined for parasites, and 33 of them, or a prevalence of 22%, were found to harbour *O. bonneti* (see de Chambrier et al. 2006). To date, this is one of only two species of *Ophiotaenia* described in ranids in the Neotropics (the other is *Ophiotaenia hernandezi* Flores-Barroeta, 1955– see below); both occur in Central America.

2. *Ophiotaenia gracilis* Jones, Cheng et Gillespie, 1958

Syns *Batrachotaenia gracilis* (Jones, Cheng et Gillespie, 1958) Freze, 1965; *Proteocephalus gracilis* (Jones, Cheng et Gillespie, 1958) Brooks, 1978

Type host: American bullfrog, *Rana catesbeiana* (Shaw) (misspelled as *R. catesbiana* in the original description).

Additional host (to be confirmed): *Rana clamitans* Latreille.

Type locality: Mountain Lake, Giles County, Virginia, USA.

Distribution: USA (Colorado, Virginia).

Type material: Likely does not exist (reported to have been deposited in the U.S. National Museum but not present there).

Material studied: None.

Morphological description: Jones et al. (1958).

Representative DNA sequences and phylogenetic relationships: No molecular data are available.

References: Jones et al. (1958), Buhler (1970), Andrews et al. (1992), Bursey and DeWolff (1998).

Comments. Jones et al. (1958) described this tapeworm as the second species of *Ophiotaenia* found in *R. catesbeiana*, based on specimens found in 3 of 46 (7%) bullfrogs from Mountain Lake, Virginia. The species differs from *Ophiotaenia magna* Hannum, 1925, another parasite of *R. catesbeiana* in North America (see below), in its smaller size (total length 100–200 mm vs. 600 mm), more anterior position of the gonopore (at 1/5–1/6 of the proglottid length in *O. gracilis* vs. 1/3 in *O. magna*), greater number of testes (135–155 vs. 100–125), and more elongate, rather than quadrate, shape of the gravid proglottids (Jones et al. 1958). In addition, the two species differ strikingly in the position of the vagina relative to the cirrus-sac (see below in comments on *O. magna*).

Buhler (1970) studied the life cycle of *O. gracilis* from *R. catesbeiana* in Colorado (Brisco Lake in Weld County) and found the copepod *Eucyclops agilis* (Koch) to be a suitable experimental host for *O. gracilis*. Fully developed metacestodes were found in copepods after 16 days of development. Bursey and DeWolf (1998) reported *O. gracilis* from *R. clamitans* in Ohio but did not provide morphological data. Therefore, this report needs to be verified; specimen is deposited in the USNM no. 1382719.

3. *Ophiotaenia hernandezi* (Flores-Barroeta, 1955) de Chambrier, Coquille et Brooks, 2006

Syns *Proteocephalus hernandezi* Flores-Barroeta, 1955; *Batrachotaenia hernandezi* (Flores-Barroeta, 1955) Freze, 1965

Type and only host: *Rana* sp. (species not known).

Type locality: Finca Monte de Oro, Departamento de Sololá, Guatemala.

Distribution: Guatemala.

Type material: Reportedly deposited in the National Helminthological Collection of Mexico under accession number CNHE 181-6, but the only reported specimen has never been deposited there and has apparently been lost (Luis García-Prieto, curator of CNHE, pers. comm. on 30 August 2021).

Material studied: None.

Morphological description: Flores-Barroeta (1955).

Representative DNA sequences and phylogenetic relationships: No molecular data are available.
Reference: Flores-Barroeta (1955).

Comments. The species was described by Flores-Barroeta (1955) from a single tapeworm found in an unidentified frog of the genus *Rana* collected by Eduardo Caballero y Caballero near Lake Atitlán, Guatemala, and relatively few measurements were given. There are six species of *Rana* that occur in Guatemala: *Rana forreri* Boulenger, *Rana juliani* Hillis et de Sá, *Rana macroglossa* Brocchi, *Rana maculata* Brocchi, and *Rana vaillanti* Brocchi – AmphibiaWeb (2023). The author did not make an explicit differential diagnosis of the new species, but simply placed it in Group 1 of *Proteocephalus* based on the classification of Meggitt (1927). The most characteristic feature of the species is the posterior position of the vagina in relation to the cirrus-sac. The species also has a relatively broad scolex (conspicuously wider than the neck) with a distinct apical cone (see Fig. 1 in Flores-Barroeta 1955). The above morphological features, which are not common in species of *Ophiotaenia* of amphibians, as well as the geographical origin of this species (Central America, i.e., the northern part of the Neotropical region), support the validity of this species, which has never been found since its original description.

This species also has testes in one field that are not interrupted medially in the region of the uterine stem, which is unusual in species of *Ophiotaenia*. Among proteocephalids of frogs, this feature is also found in *Ophiotaenia niuginii* (Schmidt, 1975), *O. papuensis* and *Ophiotaenia tigrina* (Woodland, 1925) (see below). The presence of one or two testicular fields was used by Freze (1965) and Rego (1994) as one of the key characters to distinguish tapeworms of the genera *Proteocephalus* Weinland, 1858 and *Ophiotaenia*.

4. *Ophiotaenia magna* Hannum, 1925

Syns *Ichthyotaenia magna* (Hannum, 1925) Meggitt, 1927; *Batrachotaenia magna* (Hannum, 1925) Freze, 1965

Type host: American bullfrog, *Rana catesbeiana* Shaw.
Additional hosts (to be confirmed): Plains leopard frog, *Rana blairi* Mecham, Littlejohn, Oldham, Brown et Brown; green frog, *Rana clamitans*; Tarahumara frog, *Rana tarahumare* Boulanger.
Type locality: Stillwater, Oklahoma, USA.
Distribution: USA (California, Michigan, Oklahoma, Texas), Mexico (Sonora – to be confirmed; see Comments).
Type material: Not known to exist.
Material studied: None.
Morphological description: Hannum (1925).
Representative DNA sequences and phylogenetic relationships: No molecular data are available.
References: Hannum (1925), Harwood (1932), Andrews et al. (1992), Goldberg et al. (2000).

Comments. The species was described by Hannum (1925) from *R. catesbeiana*, from Oklahoma. The morphological description was detailed and the illustrations of the new species were of good quality. Hannum (1925) described that the vaginal canal is anterior to the cirrus-sac in its proximal (median) part and then turns posteriorly on the ventral side of the cirrus-sac to open posterior to the cirrus-sac into the genital atrium. This feature distinguishes *O. magna* from other species of *Ophiotaenia* found in ranid frogs, including *O. gracilis* from the same frog host (*R. catesbeiana* – see above).

Type material is not known and not available in the USNM, where only voucher specimens of *O. magna* are deposited, namely specimens from *R. catesbeiana* from Calero Reservoir, Santa Clara County, California, collected by P. Johnson on 10 April 1998 (USNM 1386418), and from *R. tarahumare* from Sonora, Mexico, collected by M.D. Robinson on 1 August 1989 (USNM 1384378). The latter specimens were identified as *O. magna* by C.R. Bursey, but no morphological data were provided. This record should be confirmed by molecular data because the frog host occurs in a relatively distant region. Bursey and Goldberg (2001) found 21 tapeworms identified as *O. magna* in *Rana tarahumarae* (Boulenger) from Sonora, Mexico, with a prevalence of 24% and a mean intensity of 2.1 specimens. The record of *O. magna* in *R. clamitans* in Texax by Harwood (1932) should also be confirmed.

Goldberg et al. (2000) reported *O. magna* in *R. blairi* in Texas. The authors found three tapeworms in three of 21 frogs examined in Carson County, Texas, while another 128 *R. blairi* from Colorado, Iowa, Kansas, and Nebraska were negative. However, no morphological description of these tapeworms was provided to support species identification. Therefore, this host record should also be confirmed.

5. *Ophiotaenia niuginii* (Schmidt, 1975) de Chambrier, Scholz, Mariaux et Kuchta, 2017

Syn. *Proteocephalus niuginii* Schmidt, 1975

Type and only host: Arfak Mountains frog, *Rana arfarki* (= *Papurana arfaki* (Meyer)).
Type locality: Mount Suckley, New Guinea.
Distribution: Papua New Guinea.
Type material: USNM 1368544 (holotype), 1368545 (paratype).
Material studied: None (type material could not be borrowed by the present authors due to restrictions related to the COVID-19 pandemic in 2020 and 2021).
Morphological description: Schmidt (1975).
Representative DNA sequences and phylogenetic relationships: No molecular data are available.
Reference: Schmidt (1975).

Comments. This species was briefly described and simply illustrated by Schmidt (1975) based on tapeworms found by W. H. Ewers in New Guinea. *Ophiotaenia niuginii* is unique, except for two proteocephalids, in that its eggs develop in pairs (see Fig. 15 in Schmidt 1975). The only proteocephalids with paired eggs are *Proteocephalus platystomi* Lynsdale, 1959, a poorly known parasite of a pimelodid catfish in Amazonia (Lynsdale 1959) that has never been found since its original description (Alves et al. 2017), and *Ophiotaenia papuensis* (see below). In addition, *O. niuginii* can be distinguished by the presence of a single testicular field (see Fig. 13 in Schmidt 1975), the ex-

doi: 10.14411/fp.2023.009

Table 1. Measurements of *Ophiotaenia* tapeworms from *Rana* spp. in North America redescribed in the present paper.

Species	***Ophiotaenia saphena*** – original description	***Ophiotaenia saphena*** – present redescription	***Ophiotaenia saphena*** – immature	***Ophiotaenia saphena*** – present redescription	***Ophiotaenia* sp.** – immature
Host (host field code)	*Rana clamitans* (type host)	*Rana clamitans* (US786a, 793a, 795a)	*Rana clamitans* (USA 3, 4, 14)	*Rana catesbeiana* (US 800a–c)	*Rana pipiens* (USA 5, 23, 40)
Country	Michigan, USA	Arkansas, USA	Wisconsin, USA	Arkansas, USA	Wisconsin, Mississipi, USA
Collection No.	USNM 1367067	MHNG-PLAT-00129871, 00129872	MHNG-PLAT-0032849, 0032850, 0035547	IPCAS C-917/1	MHNG-PLAT-0032851, 0034675, 0035299
Reference	Osler (1931)	present study	present study	present study	present study
Total length (mm)	up to 280 (mean 153)	70–120	108	72–119	39–42
Maximum width (mm)	1.59	1.53	0.90	0.89	0.70–0.86
Proglottid length/width ratio mature	[1.07]	0.75–1.0	N/A	0.84–1.22	N/A
Proglottid length/width ratio pregravid-gravid	[1.19]	0.86–1.59	N/A	1.61–2.46	N/A
Scolex width	270–320	340–405	390–470	340–425	295–370
Scolex length	200–250	240–325	290–410	245–310	185–275
Sucker width	120–150	160–230	175–200	155–175	115–180
Apical organ (presence)	present (degenerate)	present (degenerate)	present	absent	present
Apical organ width	23	55–65	95–130	not applicable	75–130
Testis number	88–120	94–136 (x = 108; n = 5)	104	115–140 (x = 123; n = 7)	84
Testis size	50–80	30–55 × 20–50	45–65	45–60 × 35–60	40–55 × 15–25
Testis fields	2	2	2	2	2
Cirrus-sac – relative length[1]	about 17%	17–26% (x = 21%; n = 18)	22%	19–23% (x = 21%; n = 12)	19–27%
Genital pore – position[2]	about 33% [23–26%]	15–23% (x = 20%; n = 14)	28%	16–22% (x=20%; n = 11)	17–21%
Ovary – relative width[3]	[70–76%]	71–82% (x = 77%; n = 14)	72%	69–80% (x = 74%; n = 13)	72–80%
Ovary – surface ratio[4]	[8.4–8.9%]	8.9–15.4%	N/A	8.7–9.0%	N/A
Mehlis' gland – width	N/A	70–100	60–70	70–100	60–70
Mehlis' gland – relative size[5]	N/A	12.2–18.4% (x = 15.3%; n = 10)	9.7–12.0%	8.2–12.8% (x = 10.2%; n = 12)	10.5–12.0%
Position of vagina[6]	N/A	mainly anterior (96%)	anterior or posterior (59–41%)	anterior	not observable
Vaginal sphincter	N/A	absent	N/A	absent	not observable
Aporal vitelline follicles – relative length[7]	[91–92%]	94–99%	N/A	93–98%	81–90%
Poral vitelline follicles – relative length[8]	[94–95%]	92–98%	N/A	93–96%	82–93%
Uterine diverticula	14–18	9–15	N/A	40–45	N/A
Embryophore diameter	N/A	17–20	N/A	N/A	N/A
Oncosphere diameter	N/A	10–12	N/A	N/A	N/A
Supplementary layer of embryophore	N/A	absent	N/A	N/A	N/A
Type of uterus formation[9]	2	2	2	2	2

[1] ratio of cirrus-sac length to proglottid width; [2] ratio of distance of genital pore from anterior margin of proglottid to proglottid length; [3] ratio of ovary width to proglottid width; [4] ratio of area occupied by ovary to whole proglottid area (see de Chambrier et al. 2012); [5] ratio of width of Mehlis' gland to proglottid width; [6] position of vagina to cirrus-sac; [7, 8] ratio of length of band of vitelline follicles to length of proglottid on aporal and poral sides, respectively; [9] see de Chambrier et al. (2004) for type of uterine development. Measurements taken by the present authors from illustrations in the original descriptions are in brackets; N/A – not available.

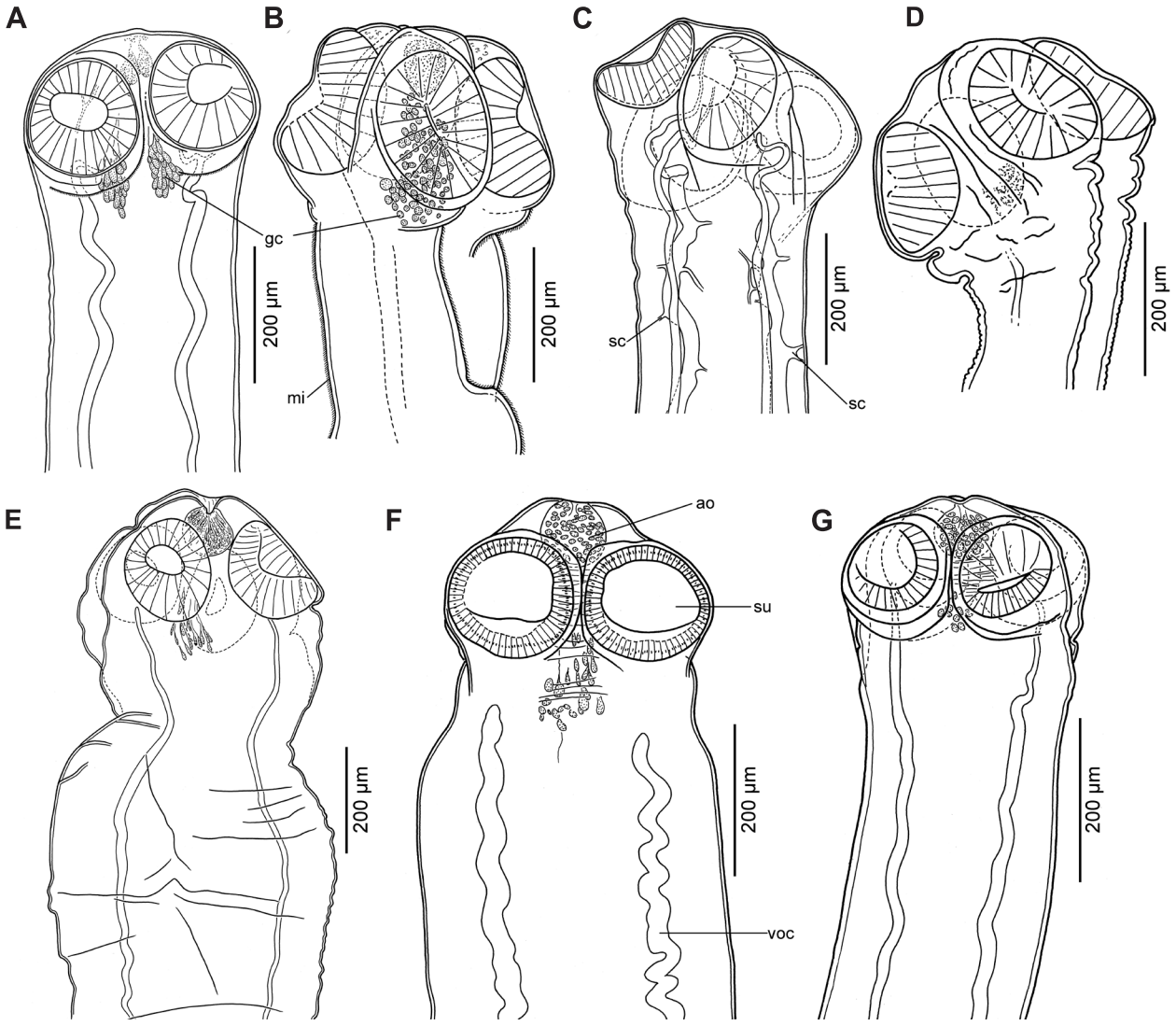

Fig. 1. Scoleces of *Ophiotaenia saphena* Osler, 1931 from *Rana clamitans* Latreille (US 793a, 795a), Arkansas, USA (MHNG-PLAT-0129872, 0129871) (A, B) and Wisconsin, USA (MHNG-PLAT-0035547) (E), and from *Rana catesbeiana* (Shaw) (host field No. US 800a, c), Arkansas, USA (IPCAS No C-917) (C, D); and *Ophiotaenia* sp. (not *O. saphena*) from *Rana pipiens* Schreber (USA 5 and 23), Wisconsin, USA (MHNG-PLAT-0032851, 0063342) (F, G). Adult tapeworms (A–D), immature tapeworms (E–G). *Abbreviations*: ao – apical organ; gc – gland cells; mi – microtriches; su – suckers; vo – ventral osmoregulatory canal.

tensive development of uterine diverticula that fill almost the entire gravid proglottids (Fig. 14), and the characteristic anterior position of the vagina relative to the cirrus-sac (Figs 12, 13 in Schmidt 1975).

6. *Ophiotaenia olor* (Ingles, 1936) Yamaguti, 1938

Syns *Crepidobothrium olor* Ingles, 1936; *Batrachotaenia olor* (Ingles, 1936) Freze, 1965; *Proteocephalus olor* (Ingles, 1936) Brooks, 1978

Type and only host: Northern red-legged frog, *Rana aurora* Baird et Girard, but possibly California red-legged frog *Rana draytonii* Baird et Girard, because *R. aurora* does not occur in Alameda County (see http://www.californiaherps.com/frogs/pages/r.aurora.html).

Type locality: Boy Scout Creek, Oakland, Alameda County, California, USA.

Distribution: USA (California).

Type material: Holotype (USNM 1321671).

Material studied: Holotype – one two slide with anterior part of contracted specimens and one slides with nine gravid proglottids.

Morphological description: Ingles (1936).

Representative DNA sequences and phylogenetic relationships: No molecular data are available.

Reference: Ingles (1936).

Comments. This species was described by Ingles (1936) as *Crepidobothrium olor* from *Rana aurora*. It differed from other species described from amphibians in having a very long undifferentiated neck. According to Ingles (1936), the general shape of the mature and gravid proglottids resembles that of *O. magna* and *O. saphena*, but *O. olor* differs from these two in that its proglottids are rarely longer than wide, which is not the case in the first two species mentioned (Ingles 1936).

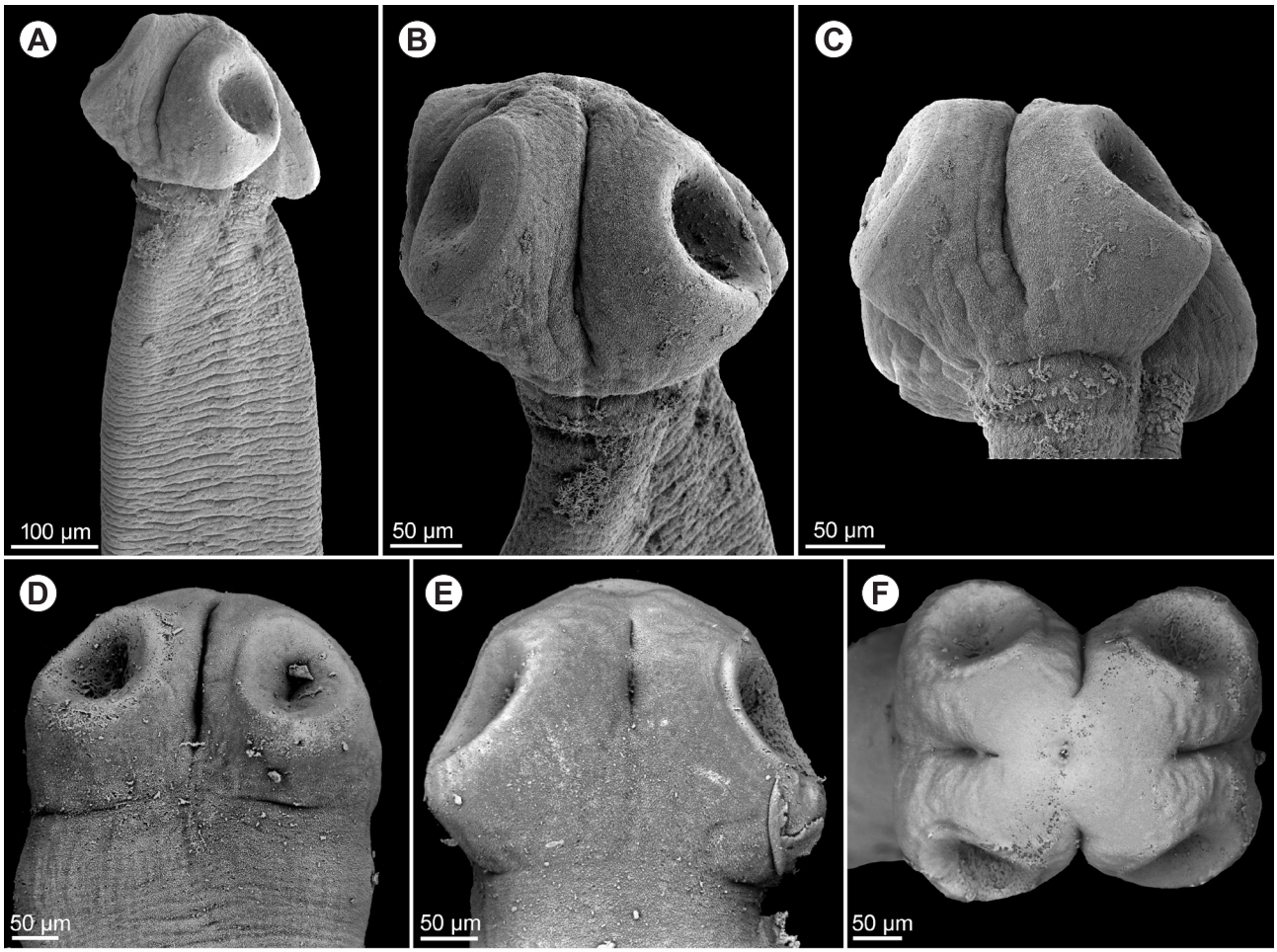

Fig. 2. Scanning electron micrographs of scoleces of *Ophiotaenia saphena* Osler, 1931 from *Rana catesbeiana* (Shaw) (US 800b), Arkansas, USA (IPCAS C-917) and *Rana clamitans* Latreille (USA 4), Wisconsin, USA (MHNG-PLAT-0032850) (D–F).

Examination of the holotype confirmed the presence of a long neck region (strobila without primordia of genital complexes). Immature proglottids are much wider than long, but this part of the strobila is strongly contracted. However, gravid proglottids of the holotype are wider than long to quadrate (length : width ratio for gravid proglottids 1.00–1.74; mean 1.36, n = 7).

Ophiotaenia olor can also be distinguished from the otherwise similar *O. saphena* by the relative size of the cirrus-sac, whose length is only 11% of the proglottid width (compared to 17–26% in *O. saphena*), and by the relative width of the ovary, whose width is only 61–71% of the proglottid width (compared to 73–82% in *O. saphena*) (Table 1). As far as the present authors are aware, *O. olor* has not been reported since its original description.

7. *Ophiotaenia papuensis* (Bursey, Goldberg et Kraus, 2008) comb. n.

Syn. *Proteocephalus papuensis* Bursey, Goldberg et Kraus, 2008

Type and only host: Papua gray frog, *Sylvirana supragrisia* (Menzies), currently recognised as *Papurana supragrisea* (Menzies).

Type locality: Southern slope of Oya Waka, Milne Bay Province, Papua New Guinea.

Distribution: Papua New Guinea.

Type material: Holotype (USNM 1394392), paratypes (USNM 1394393, 1394394).

Material studied: None (due to COVID-19 pandemic restrictions, it was not possible to request a loan of type specimens).

Morphological description: Bursey et al. (2008).

Representative DNA sequences and phylogenetic relationships: No molecular data are available.

References: Bursey et al. (2008).

Comments. The species was described by Bursey et al. (2008) as *Proteocephalus papuensis* from *Sylvirana supragrisea* (now *Papurana supragrisea*) in Papua New Guinea. The species is placed in *Ophiotaenia* because it has all the typical characteristics of this genus (see Rego 1994). *Ophiotaenia papuensis* resembles *O. niuginii* in the presence of minute microtriches (erroneously called cuticular spines in the original description) on the scolex and strobila, oriented so that the strobila appears to bear longitudinal stripes, gravid proglottids that are longer than wide, and paired eggs. The authors distinguished *O. papuensis* from *O. niuginii* by the more numerous uterine diverticula (72–84 in the former species vs. only 28–36 in *O. niuginii*) and the postequatorial position of the gonopore in *O. papuensis* versus the equatorial gonopore in the latter species

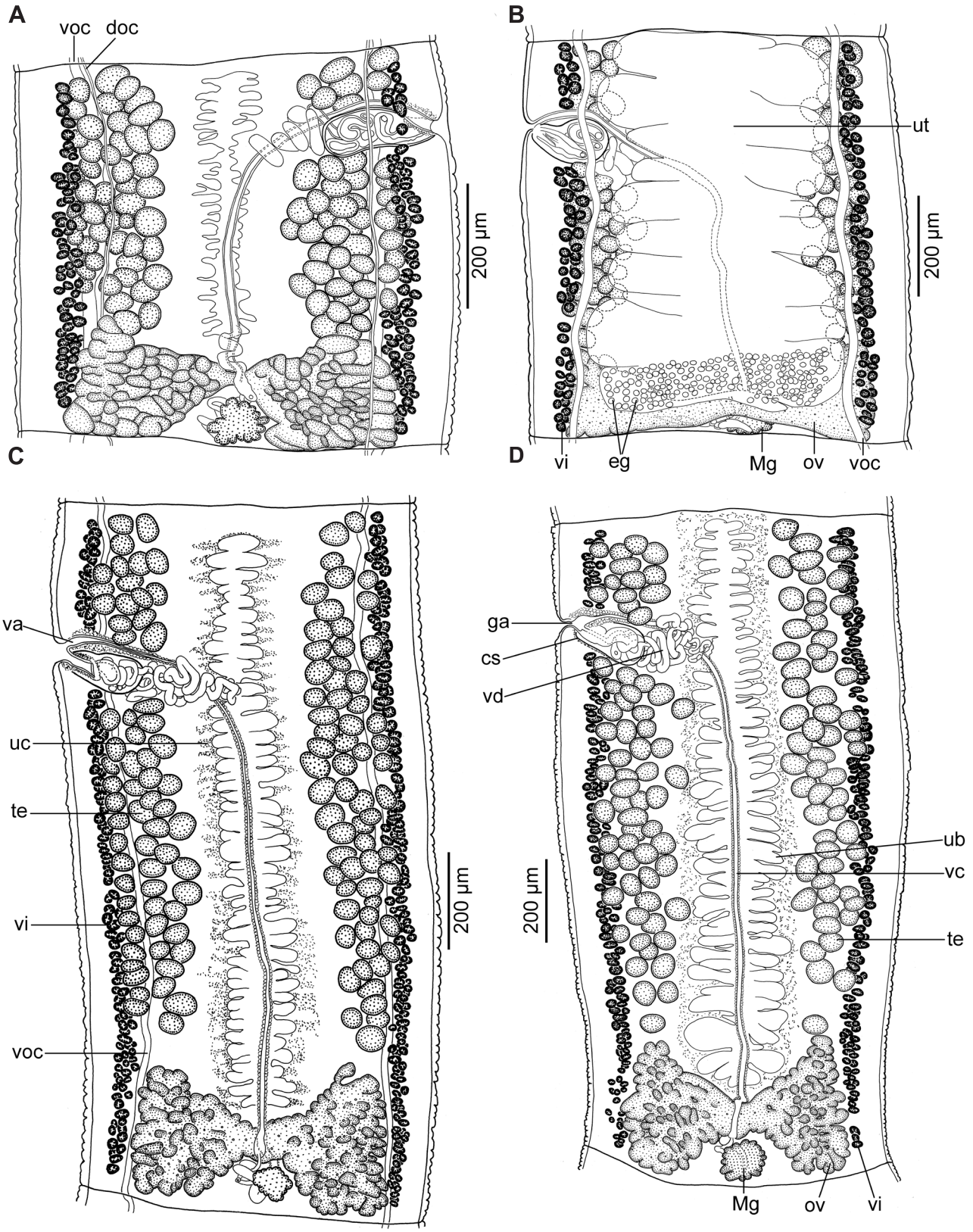

Fig. 3. Mature and gravid proglottids of *Ophiotaenia saphena* Osler, 1931 from *Rana clamitans* Latreille (US 793a, 786a), Arkansas, USA (MHNG-PLAT- 0129872, 0129871) (A, B), and mature and pregravid proglottids of *O. saphena* from *Rana catesbeiana* (Shaw) (US 800b), Arkansas, USA (IPCAS C-917) (C, D). Dorsal view (A, C, D), ventral view (B). *Abbreviations*: cs – cirrus sac; doc – dorsal osmoregulatory canal; eg – eggs; ga – genital atrium; Mg – Mehlis' glands; ov – ovary; te – testes; ub – uterine branches (diverticula); uc – uterine lateral cells; ut – uterus; va – vagina; vc – vaginal canal; vd – vas deferens; vi – vitelline follicles; voc – ventral osmoregulatory canal.

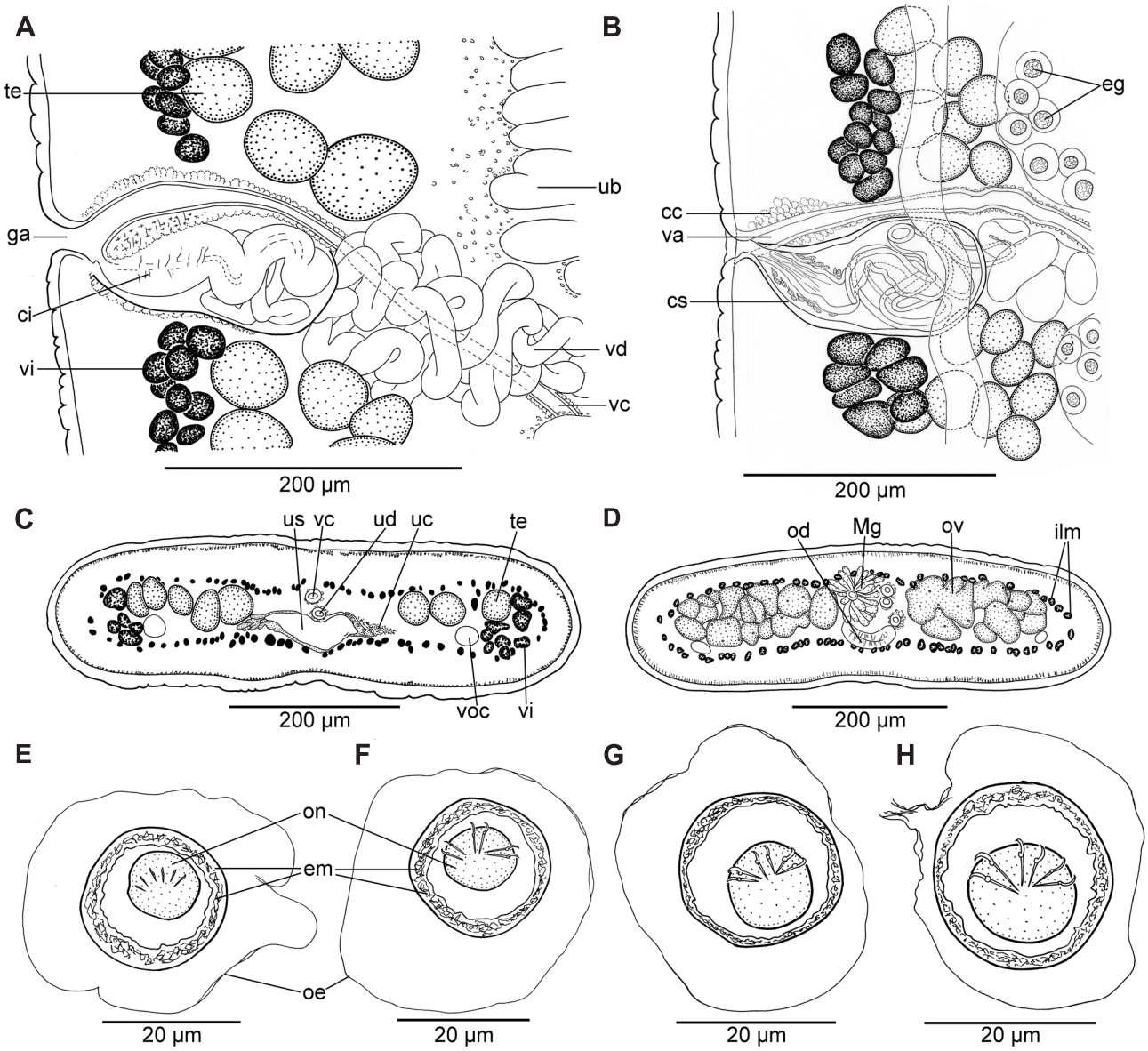

Fig. 4. *Ophiotaenia saphena* Osler, 1931 from *Rana catesbeiana* (Shaw) (US 800b), Arkansas, USA (IPCAS C-917) (A), and from *Rana clamitans* Latreille (US 786a, 793a), Arkansas, USA (MHNG-PLAT-0129871, 0129872) (B–H). **A**, **B** – terminal genitalia, dorsally and ventrally, respectively; **C**, **D** – cross-sections at level of testes and uterus, and ovary, respectively; **E** – eggs without fully formed embryonic hooks; **F**–**H** –fully formed eggs with fully developed embryonic hooks in oncosphere. *Abbreviations*: cc – chromophilic cells around vagina; ci – cirrus; cs – cirrus sac; eg – eggs; em – bilayered embryophore; ga – genital atrium; ilm – internal longitudinal musculature; Mg – Mehlis' glands; od – oviduct; oe – outer envelope; on – oncosphere; ov – ovary; te – testes; ub – uterine branches (diverticula); uc – uterine lateral cells; ud – uteroduct; us – uterine stem; va – vagina; vc – vaginal canal; vd – vas deferens; vi – vitelline follicles; voc – ventral osmoregulatory canal.

(Bursey et al. 2008). In addition, *O. papuensis* is smaller (total length of 25–49 mm, averaging 40 mm) than *O. niuginii* (strobila 170 mm long – Schmidt 1975).

8. *Ophiotaenia ranae* Yamaguti, 1938

Syns *Ophiotaenia ranarum* Iwata et Matsuda, 1938; *Batrachotaenia ranae* (Yamaguti, 1938) Freze, 1965

Type and only host: Dark-spotted frog, *Rana nigromaculata* Hallowell (currently recognised as *Pelophylax nigromaculatus* (Hallowell) – Frost 2022).

Type locality: Lake Kobata near Kyoto, Japan.

Distribution: Japan (Honshu).

Type material: Likely does not exist.

Material studied: None.

Morphological description: Iwata and Matsuda (1938), Yamaguti (1938).

Representative DNA sequences and phylogenetic relationships: No molecular data are available.

References: Iwata and Matsuda (1938), Yamaguti (1938, 1943), Goldberg and Bursey (2002).

Comments. The species was described by Yamaguti (1938) on the basis of a large number of tapeworms found in the small intestine of the dark-spotted frog, *R. nigromaculata*, from Lake Kobata near Kyoto. The description was

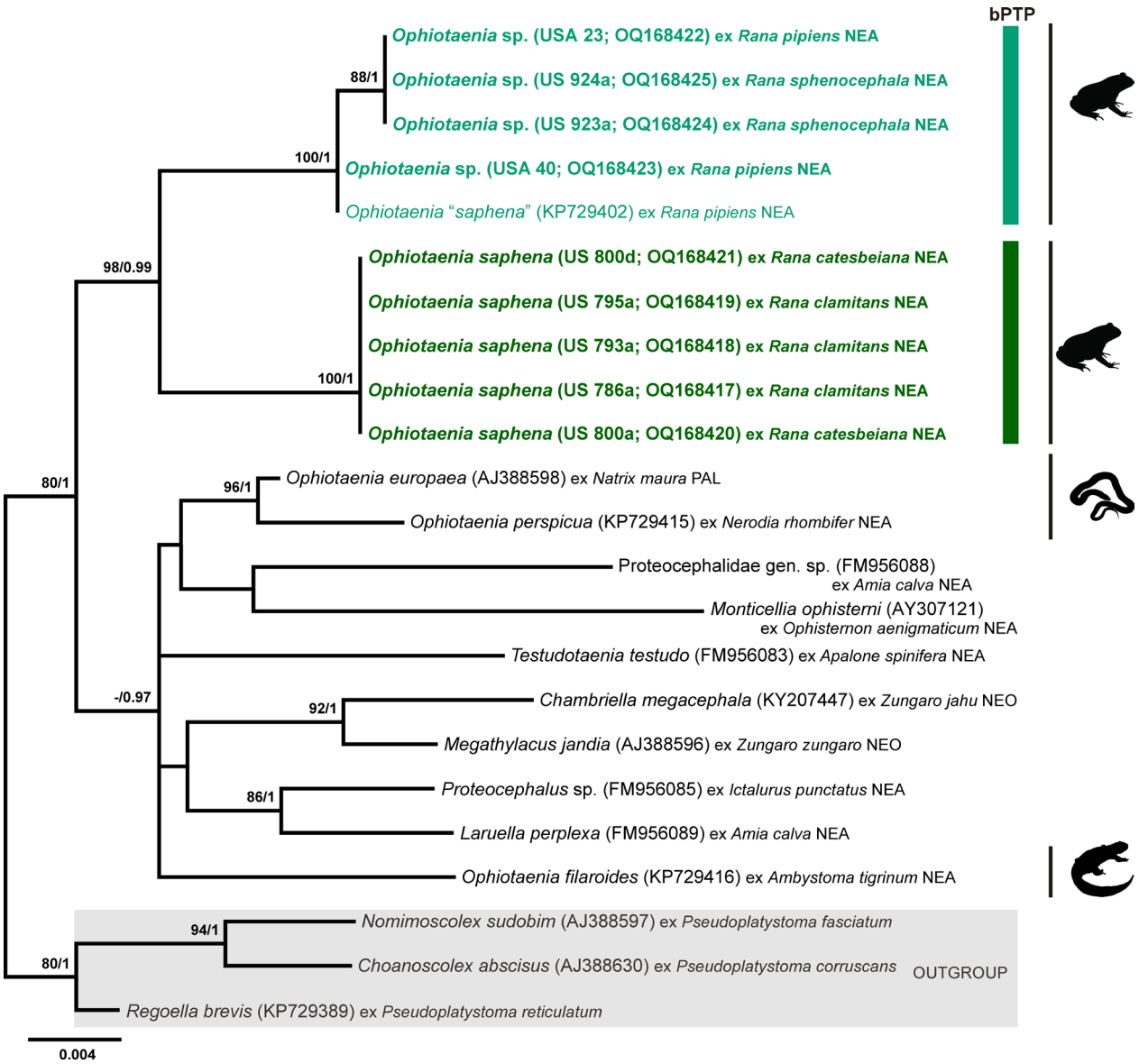

Fig. 5. Maximum likelihood phylogram of *lsr*DNA for selected members of the Proteocephalidae with hosts for *Ophiotaenia* spp. indicated by silhouettes. Bootstrap support from maximum likelihood (ML) and Bayesian inference (BI) nodal support are indicated as ML/BI; values < 0.90 (BI) and < 70 (ML) are not shown. The scale bar indicates the expected number of substitutions per site. The newly generated sequences are highlighted in bold and colour. Species boundaries of novel isolated of *Ophiotaenia* spp. inferred with bPTP approach, conducted on the *lsr*DNA Bayesian tree, are plotted on the right-hand side. *Abbreviations*: NEA – Nearctic region; NEO – Neotropical region; PAL – Palaearctic region. Symbols of other hosts (teleost fish and a spiny softshelled turtle) are not provided.

detailed, but relatively few measurements were given, including somewhat confusing information about the highly variable number of testes ("Their number varies greatly according to proglottides; the outer group consists of 10–45 testes and the inner of 25–70, the total number not exceeding 115 on one side").

This species was distinguished primarily by the paramuscular position of some vitelline follicles ("The finely acinous vitellaria .... intruding into the cortex through the space between the inner longitudinal muscle bundles"); see Figs 9 and 10 in Yamaguti (1938) and de Chambrier (1990) for the definition of the paramuscular position of the vitelline follicles in proteocephalids. It should be noted that another distinguishing feature of *O. ranae* mentioned by Yamaguti (1938), namely the presence of some testes lateral to the osmoregulatory ducts, also occurs in other frog proteocephalids (see distribution of testes in *O. saphena* – Fig. 3).

In the same year as Yamaguti (1938), Iwata and Matsuda (1938) described the apparently conspecific tapeworm *O. ranarum* from the dark-spotted frog from Kōtōen near Osaka. Type specimens were deposited at the parasitological collection of the Institute for Research in Microbial Diseases, Osaka Imperial University (Coll. No. 301), but were not available to the present authors.

Yamaguti (1943) successfully infected the copepod *Mesocyclops dybowskii* (Landé) (= *Thermocyclops dybowskii*) with eggs of *O. ranae*. The same author also studied the life cycle of this parasite under laboratory conditions and assumed that only one intermediate host, a copepod, is required to complete its developmental cycle. Goldberg and Bursey (2002)

found an individual of *O. ranae* in one of 18 formalin-fixed *P. nigromaculatus* (prevalence 6%) from Honshu, but did not characterise it morphologically. The voucher specimen is deposited in the USNM (1386509), but could not be examined by the present authors due to COVID-19 limitations.

9. *Ophiotaenia saphena* Osler, 1931 Figs 1–4; Table 1

Syns *Crepidobothrium saphena* (Osler, 1931) Ingles, 1936; *Batrachotaenia saphena* (Osler, 1931) Freze, 1965

Type host: Green frog, *Rana clamitans* Latreille.

Additional hosts: American bullfrog, *Rana catesbeiana* (confirmed molecularly); Northern leopard frog, *Rana pipiens* Schreber (to be confirmed).

Type locality: Roadside ditches running into Burt Lake and Fontinalis Run on Burt Lake, Cheboygan County, Michigan, USA.

Distribution: USA (Arkansas – new geographical record, Iowa – but see Comments, Michigan, Wisconsin), Canada (New Brunswick).

Type material: USNM 1367067 (holotype).

Material studied: Tapeworms from *R. clamitans*: one large, but not mature specimen from host of field code USA 14, Winnebago, Wisconsin, USA, collected by V.V. Tkach on 3 May 1999 (MHNG-PLAT-0035371); six immature specimens from host USA 4, Rivermoor Drive, Sheboygan, Wisconsin, USA, V.V. Tkach, 3 May 1999 (MHNG-PLAT-0032850, 0035547); one immature specimen from host USA 3gén (hologenophore), Winnebago County, Wisconsin, V.V. Tkach, 3 May 1999 (MHNG-PLAT-0032849); three mature specimens from hosts US 786a, 793a and 795a, and one scolex for SEM (US 928), Blue Haze Pond, north of Mena, Polk Co., Arkansas, USA, V.V. Tkach, C.T. McAllister and T. Scholz, 13 June 2019 (IPCAS C-918; MHNG-PLAT-0129871, 0129872).

Tapeworms from *R. catesbeiana*: Three complete specimens from host of field code US 800a–c, Blue Haze Pond, north of Mena, Polk County, Arkansas, collected by V.V. Tkach, C.T. McAllister and T. Scholz on 13 June 2019 (IPCAS C-917; MHNG-PLAT-0150418).

Tapeworms from *R. pipiens* (Fig. 1E, F): One long, but not mature specimen from USA 5gén (hologenophore – sequenced as PBI-502 by de Chambrier et al. 2015), Oshkosh, Wisconsin, USA, V.V. Tkach, 14 May 1999 (MHNG-PLAT-0032851) and several fragments including three scoleces and cross sections of specimens from the same host (USA 5) (MHNG-PLAT-0034675, 0035299); fragments of three immature specimens (two with scoleces) from host USA 23, Ocean Springs, Mississippi, USA, V.V. Tkach, 3 May 2009 (MHNG-PLAT-0063342). Based on molecular data, these tapeworms belong to another, putative new species conspecific with *O.* "*saphena*" (KP729402).

Morphological description: Osler (1931); present paper (Figs 1–4; measurements in Table 1).

Representative DNA sequences and phylogenetic relationships: partial *lsr*DNA, five sequences (OQ168417–OQ168421); COI mtDNA, five sequences (OQ200060–OQ200064).

References: Osler (1931), Dyer (1991), Andrews et al. (1992), McAlpine (1997), McAlpine and Burt (1999), Muzzall et al. (2001).

Redescription (based on two entire specimens and part of another specimen from *R. clamitans* US 786a, 793a and 795a, and three entire specimens from *R. catesbeiana* US 800a–c – measurements in parentheses, all from Arkansas; other measurements in Table 1).

Proteocephalidae. Large worms, up to 12 cm (12 cm) long and 1.5 mm (0.9 mm) wide, flattened dorsoventrally, with proglottids elongated, up to 1.5 mm (1.6 mm) long. Strobila acraspedote, anapolytic, with about 120 (355) immature proglottids, 14 (25) mature proglottids, 12 (9) pregravid proglottids and 14 (not present) gravid proglottids; in total about 160 (389) proglottids. Immature proglottids wider than long to quadrate, with length: width ratio 0.29–1.00 (0.55–1.00); mature proglottids wider than long, with length: width ratio 0.75–1.00 (0.84–1.22) (Fig. 3A); pregravid and gravid proglottids wider than long to longer than wide, with length: width ratio 0.86–1.59 (1.61–2.46) (Fig. 3B–D). Tegument thick, 15–20 (10–20) in thickness.

Scolex spherical, aspinose (Figs 1, 2), wider than neck, with four uniloculate, spherical suckers directed laterally (Figs 1, 2); degenerate apical organ present (organ atrophies during parasite maturation in definitive host – Thomas 1931), with gland and other cells forming 75–80 long and 55–65 wide region without definite limitation from surrounding tissue (Fig. 1A,B). Two groups of cells with granular content posteromedian to suckers (Fig. 1A,B). Neck up to 280 wide (275); unsegmented zone posterior to scolex to first recognisable proglottids long (up to 15 mm).

Inner longitudinal musculature well-developed, consisting of bundles of muscular fibers (Fig. 4C,D). Osmoregulatory canals slightly sinuous, median to vitelline follicles, crossing lateral margin of ovarian wings, lateral (external) to all but few lateral-most testes (Fig. 3A,B). Ventral canals thin-walled, wide, 20–40 (15–25) in diameter; dorsal canals thick-walled, about 5 (4) in diameter, situated at same level as ventral canals or slightly more median (Fig. 3A). Genital ducts run between osmoregulatory canals (Fig. 3A).

Testes spherical to oval, in one or two layers and in two fields between vitelline follicles; fields partly separated by osmoregulatory canals (Fig. 3A,C). Testes reach anterior margin of proglottids anteriorly and ovary posteriorly (Fig. 3A–D). Vas deferens strongly coiled, never reaching mid-line of proglottid, occupying small elongated area (Fig. 3A,C). Cirrus-sac elongated, thick-walled, 100–210 (155–200) long by 50–90 (60–80) wide (Fig. 4A,B); length: width ratio 1.66–2.67 (2.05–2.91). Cirrus occupies 50–80% (55–60%) of length of cirrus-sac (Fig. 4A,B). Genital atrium narrow and shallow; genital pores irregularly alternating, conspicuously pre-equatorial (Fig. 3A–D).

Ovary large, bilobed and broader laterally, 345–795 (545–670) wide (Figs 3A–D). Ovary length represents 20–32% of proglottid length. Vagina almost always anterior (anterior in 121 proglottids of 126 (96%), posterior in 5 proglottids) to cirrus-sac, without vaginal sphincter, lined with thin layer of cells in its terminal (distal) part (Fig. 4B). Mehlis' gland 70–100 (70–100) in diameter (Fig. 3A,D).

Vitelline follicles oval to elongate, arranged in two lateral, longitudinal bands on lateral sides of proglottid (Fig. 3A–D) not interrupted dorsally on poral side at level of ter-

minal genitalia (cirrus-sac and vagina), partly overlapped by lateral-most testes (Fig. 3A,C,D). Follicles closely approaching, but not reaching, anterior or posterior margin of proglottids (Fig. 3A–D).

Primordium of uterine stem ventral (Fig. 3A). Formation of uterus follows type 2 of de Chambrier et al. (2004). In terminal proglottids, uterine diverticula represent up to 73% of proglottid width. Eggs spherical, outer (hyaline) envelope 32–40 in diameter; embryonic hooks 5–7 long (Fig. 4E–H).

Comments. The species was described by Osler (1931) from tapeworms found in *R. clamitans* from Cheboygan County in Michigan, USA. The original description was relatively detailed, but the illustrations, although numerous, did not provide much information about the anatomy of the worms. The illustrations of mature and gravid proglottids and those of cross sections were rather schematic, the course of the osmoregulatory ducts, shown as perfectly straight, was apparently simplified, a more detailed illustration of the terminal genitalia was lacking, and the vas deferens was incorrectly referred to as the ejaculatory duct in the legend to Fig. 2 of Osler (1931) *Ophiotaenia saphena* was placed in *Crepidobothrium* Monticelli, 1900 by Ingles (1936), and in *Batrachotaenia* Rudin, 1917 by Freze (1965), but most authors followed the original generic assignment to *Ophiotaenia*, including Yamaguti (1959), Schmidt (1986), and de Chambrier et al. (2017).

Comparison of the new material of *O. saphena* from the type host revealed some minor differences from the data of Osler (1931). In the present study, a concentration of cells was observed in the scolex apex (Fig. 1C,D), but without obvious demarcation from the surrounding tissue. This deviates slightly from the drawing of what Osler (1931 – her Fig. 1) called an apical organ. A difference was also noted in the position of the genital pores: Osler (1931) wrote that the genital pore is located at the end of the anterior third of the proglottids (about 33%), but in our material it was more anterior (15–23%). Another minor difference between our material and the original description is the slightly larger relative size of the ovary (8.9–15.4% in the new material vs. 8.4–8.9% in the drawings of the mature and gravid proglottids, i.e., Figs 9 and 10 in the original description (Osler 1931).

The tapeworms of *R. catesbeiana* differ little from those of *O. saphena* found in the type host from the same pond near Mena, Arkansas, except for two morphological and biometrical characteristics (Figs 1–4 and Table 1). Tapeworms of *R. clamitans* have shorter, nearly square proglottids (Fig. 3A,B), whereas specimens of *R. catesbeiana* have longer proglottids (Fig. 3C,D). The most striking difference between these sympatric tapeworms is in the number of uterine diverticula, which are much more numerous in specimens of *R. catesbeiana* compared to those of the type host (Table 1). This difference may be related into some exent to the more elongate (longer) proglottids of the tapeworms of *R. catesbeiana*. Since the molecular data confirmed the species identity of all tapeworms from both frog hosts, the difference in the number of uterine diverticula is thought to represent host-related, intraspecific variability.

The records of *O. saphena* in *R. pipiens* by Dyer (1991), McAlpine (1997), and McAlpine and Burt (1999) should be confirmed. Thomas (1931, 1934a, b) studied the life cycle of *O. saphena* and found that eggs deposited in tap water did not contain fully developed oncospheres (without embryonic hooks); the oncospheres developed in the water within three days. In contrast, eggs from frog faeces contained fully developed oncospheres (Thomas 1931). Copepods of all three species used in the experiments, i.e., *Macrocyclops albidus* (Jurine), *Megacyclops viridis* (Jurine), and *Mesocyclops leuckarti* (Claus), ingested eggs, but only in *M. viridis* were fully developed metacestodes found (after 12–14 days). The functional apical (fifth) sucker, which was well developed in larvae from copepods, hypetrophies in juvenile tapeworms in frogs, but then atrophies and eventually becomes vestigial in the adult worm (Thomas 1931).

Thomas (1934b) successfully infected frog tadpoles with copepods (*M. viridis*) infected with larvae of *O. saphena* and found plerocercoids in nine of 12 tadpoles 16 days after experimental infection. The author also found mature tapeworms in newly metamorphosed frogs, which indicates that adult frogs have eaten infected copepods or infected tadpoles. The experimental data provided by Thomas (1931, 1934a, b) indicate that only a single intermediate host, a cyclopid copepod, is required to complete the developmental cycle of *O. saphena*.

10. *Ophiotaenia* sp.

Syn. *Proteocephalus saphena* of de Chambrier et al. (2015)

Hosts: American bullfrog, *Rana catesbeiana* Shaw; Southern leopard frog (*Rana sphenocephala* Cope).

Distribution: USA (Minnesota, Mississippi, North Carolina, Wisconsin).

Material studied: One long, but not mature specimen from *R. pipiens* (USA 5gén; hologenophore – sequenced as PBI-502 by de Chambrier et al. 2015), Oshkosh, Wisconsin, USA, V.V. Tkach, 14 May 1999 (MHNG-PLAT-0032851) and several fragments including three scoleces and cross sections of specimens from the same host (USA 5) (MHNG-PLAT-0034675, 0035299); fragments of three immature specimens (two with scoleces) from host USA 23, Ocean Springs, Mississippi, USA, V.V. Tkach, 3 May 2009 (MHNG-PLAT-0063342); one specimen from host USA 40, Riverwood Road, Wisconsin, USA, V.V. Tkach, 9 May 2009, Winnebago County, (MHNG-PLAT-0036377; one specimen from *R. sphenocephala* (US 923), origin unknown, probably Florida; one specimen from *R. sphenocephala* (US 924), Mankato, Minnesota, USA, V.V. Tkach, 21 May 2019.

Representative DNA sequences and phylogenetic relationships: Sequence of the *lsr*DNA of sample from *R. pipiens* (PBI-502; KP729402) is available (de Chambrier et al. 2015). Novel partial *lsr*DNA, four sequences (OQ168422–OQ168425); COI mtDNA, four sequences (OQ200065–OQ200068).

Reference: de Chambrier et al. (2015).

Comments. de Chambrier et al. (2015) submitted the *lsr*DNA sequence (isolate PBI-502; KP729402) of a tapeworm of *R. pipiens* in Wisconsin, but it differs from that

of tapeworms identified as *O. saphena* found in the type host, *R. clamitans* (Figs 5). In contrast, it is identical to the new sequence of the tapeworm of *R. pipiens* (USA 40; GBOQ168423) from Wisconsin and differs only by 0.2% (2 nt) from the sequences of the tapeworms of *R. pipiens* (USA 23; OQ168422) from Mississippi and *R. sphenocephala* (US923a; OQ168424 and US 924a; OQ168425) from USA 924a (Minnesota) and US 923a (origin unknown, probably Florida) North Carolina. These newly sequenced tapeworms were also nearly identical in COI sequences (intraspecific nucleotide divergence of 0–0.68%, i.e., 0–11 nt difference).

The molecular data suggest that these tapeworms (isolates USA 23, USA 40, US 923a and US 924a) indeed belong to a different species, which is morphologically similar to *O. saphena* but differs genetically (interspecific nucleotide divergence between *Ophiotaenia* sp. and *O. saphena*: 1.52–1.72%, i.e. 15–17 nt difference in *lsr*DNA and of 13.54–13.97%, i.e. 220–227 nt difference in mtDNA; see Supplementary File 1 for detail). The *lsr*DNA sequences of *O. saphena* were identical whereas the COI sequences differed by 0–1.66%, i.e., 0–27 nt. The results from bPTP analysis confirmed that the isolates of *Ophiotaenia* sp., i.e., USA 23, US 923a, US 924a, USA 40, and PBI-502 (KP729402), belong to the same species. However, this putative species is not formally described here due to insufficient material (only immature tapeworms).

In addition, these tapeworms (MHNG-PLAT-0032851, 0034675 and 0035299) exhibit anomalies such as the presence of two or three cirrus-sacs in a proglottid and/or a duplicated ovary in 85% of the observed proglottids (n = 39). Interestingly, some proglottids of *O. saphena* from *R. clamitans* in Wisconsin (MHNG-PLAT-0035371) also exhibit abnormalities, usually with an extra ovary near the anterior margin of the proglottid. However, the number of abnormal proglottids is much lower, only 22% of 41 proglottids, compared to 85% of proglottids in of tapeworms from *R. pipiens*.

Key to the identification of species of *Ophiotaenia* from ranid frogs in the world

1 (2) Testes in a single field 3
2 (1) Testes in twomedially separated fields 7
3 (4) Eggs in pairs; more than 100 testes (135–165); vagina anterior to cirrus-sac; apical organ present in adults. Parasites of ranids in Papua New Guinea 5
3 (4) Eggs not paired (eggs single in uterus); much fewer than 100 testes (59–78); vagina posterior to cirrus-sac; apical organ absent. Parasite of *Rana* frogs in Central America (Guatemala) ..... ***O. hernandezi***
5 (6) Gonopore postequatorial; 72–84 uterine diverticula in total; body length 25–49 mm. In *Papurana supragrisea* ..... ***O. papuensis***
5 (6) Gonopore equatorial; 28–36 uterine diverticula in total; body length 170 mm. In *Papurana arfaki* .. ***O. niuginii***
7 (8) More than 200 testes; vitelline follicles paramuscular, i.e., follicles penetrating from medulla into cortex between bundles of muscle fibres. Parasite of *Pelophylax nigromaculatus* in Japan ..... ***O. ranae***
8 (7) Fewer than 200 testes; vitelline follicles medullary, i.e., not penetrating cortex 9
9 (10) Length of cirrus-sac equals to 11–14 % of proglottid width. Parasite of *Rana aurora* in North America .... ***O. olor***
10 (9) Length of cirrus-sac equals ≥ 17% of proglottid width 11
11 (12) Diameter of suckers accounts for ≥ 50% of scolex width. Parasite of *Rana vaillanti*, Central America (Costa Rica) ..... ***O. bonneti***
12 (11) Diameter of suckers accounts for < 50% of scolex width. Parasites of ranid frogs in North America ... 13
13 (14) Vagina crosses cirrus-sac and opens posteriorly; scolex > 400 μm (455 μm) in width; total body length up to 600 mm; osmoregulatory ducts median to vitelline follicles. Parasite of *Rana catesbeiana* ***O. magna***
14 (13) Vagina anterior to cirrus-sac; scolex ≤ 350 μm wide; total body length < 300 mm; osmoregulatory ducts between vitelline follicles 15
15 (16) Atrophied apical organ present in scolex of adults; fewer than 140 testes (88–140). Parasite of *Rana clamitans*, *R. catesbeiana* (and presumably other species of *Rana*) ..... ***O. saphena***
16 (15) No evidence of apical organ in scolex; more than 135 testes (135–155). Parasite of *Rana catesbeiana* (and presumably other species of *Rana*) .. ***O. gracilis***

A tabulated overview of all species of proteocephalids reported from amphibians

Based on literature data and new measurements taken from the original descriptions (e.g., ovary surface), a tabulated overview of all 32 species of proteocephalids from three genera reported from amphibians (frogs and salamanders) is presented, with information on their hosts, distribution, and taxonomically important characteristics, including key measurements (Table 2). The validity of some species is questionable, but new material is needed for their taxonomic evaluation.

DISCUSSION

Based on new material collected from four ranid frog species in North America, including molecular data, the presence of two species, each found in two species of *Rana*, is confirmed: one species, considered conspecific with *Ophiotaenia saphena*, is found in *R. clamitans* (type host) and *R. catesbeiana*, while the other, genetically distinct species, was found in *R. pipiens* and *R. sphenocephala*. The latter taxon could be a new species of *Ophiotaenia*, but our material was insufficient for a descriptioin (only immature worms). On the other hand, thanks to the new material, it was possible to redescribe *O. saphena* and document its intraspecific variability, with the number of uterine diverticula and the shape of proglottids proving to be the most variable, probably host-related characters.

In addition, the present study summarises data on proteocephalid tapeworms of the genus *Ophiotaenia* reported from ranid frogs worldwide. However, the complete lack of molecular data on tapeworms of ranids from other zoogeographical regions does not allow us to assess the rela-

doi: 10.14411/fp.2023.009

Table 2. Tapeworms (Cestoda: Proteocephalidae) from amphibians in the world.

Species	Authority and year	Type host (literary)	Type host (current name)	Host family	Country	Total length (mm)	Scolex width (μm)	Apical organ	Number of testes	Number of testis fields	Relative length of cirrus-sac[1]	Position of gonopore[2]	Relative width of ovary[3]	Surface of ovary[4]	Relative width of Mehlis glands[5]	Position of vagina to cirrus-sac	Number of uterine diverticula on each side	Type of uterine development[6]	Diameter of embryophore (oncosphere)
Australotaenia grobeli	de Chambrier et de Chambrier, 2010	*Hyla moorei*	*Ranoidea moorei*	Pelodryadidae	Australia	57–98	245–420	present	46–76	2	27–33%	46–57%	55–63%	[5.9%]	[5.8%]	anterior +posterior	14–21	Type 2	18–23
A. hylae	Johnston, 1912	*Hyla aurea*	*Ranoidea aurea*	Pelodryadidae	Australia	121	340–390	present	74–106	2	17–19%	44–55%	68–71%	[9.8%]	[9.2%]	anterior+posterior	10–17	Type 2	13–14
Nomimoscolex touzeti	de Chambrier et Vaucher, 1992	*Ceratophrys cornuta*	*Ceratophrys cornuta*	Ceratophryidae	Ecuador	64–73	480–530	present	97–137	2	20–28%	33–47%	51–57%	[2.8%]	[5.9%]	anterior+posterior	24–35	Type 1	19–24
Ophiotaenia alessandrae	Marsella et de Chambrier, 2008	*Hyla boans*	*Boana boans*	Hylidae	Ecuador	138	475	absent	86–128	2	11–17%	35–53%	66–77%	[5.6%]	[7–8%]	anterior+posterior	18–25	Type 1	22–24
O. alternans	Riser, 1942	*Amphiuma tridactylum*	*Amphiuma tridactylum*	Amphiumidae	USA	290–345	425–560	present	100–120	2	33%	17–33%	[68%]	[4.8%]	[13.6%]	anterior+posterior	[33–37]	N/A	N/A
O. amphiumae	(Zeliff, 1932) Riser, 1942	*Amphiuma tridactylum*	*Amphiuma tridactylum*	Amphiumidae	USA	250	400–480	absent	100–140	2	[24–27%]	11–18%	[70–74%]	[6.8%]	N/A	anterior	50–65	N/A	N/A
O. bonariensis	Szidat et Soria, 1954	*Leptodactylus ocellatus*	*Leptodactylus latrans*	Leptodactylidae	Argentina	400–500	[300]	present	120–140	2	[23–26%]	[32–34%]	[80%]	[6.9%]	N/A	anterior (?)	23–27	Type 1	(20–27)
O. bonneti*	de Chambrier, Coquille et Brooks, 2006	*Rana vaillanti*	*Phyllomedusa vaillanti*	Ranidae	Costa Rica	380	280–385	absent	100–177	2	15–24%	15–29%	70–83%	[6.9%]	[11.9%]	anterior	18–32	Type 2	(25–30)
O. bufonis	(Vigueras, 1942) Yamaguti, 1959	*Bufo peltacephalus*	*Peltophryne peltocephala*	Bufonidae	Cuba	12–180	740	absent	180–220	2	[20%]	[44–48%]	[64–67%]	[7.1%]	N/A	anterior+posterior	[12–14]	N/A	18–20
O. calamensis	Puga et Formas, 2005	*Telmatobius dankoi*	*Telmatobius dankoi*	Telmatobiidae	Chile	45–70	225–296	absent	34–60	2	20–38%	25–50%	[63–65%]	[4.5%]	N/A	anterior+posterior	9–19	N/A	30–33
O. carpathica	(Sharpilo, Kornyushin et Lisitsyna, 1979) Schmidt, 1986	*Triturus cristatus*	*Triturus cristatus*	Salamandridae	Ukraine	40 (?)	385	present	101–128	1	25%	50–60%	[63%]	[8.8%]	[18%]	anterior+posterior	60–70	Type 1	about 20
O. ceratophryos	(Parodi et Widakowich, 1916) Cordero, 1946	*Ceratophrys ornata*	*Ceratophrys ornata*	Ceratophryidae	Argentina	380	700	absent	?	1	16–20%	[38%]	[80%]	[7.9%]	N/A	N/A	16–20	N/A	(23)
O. chandrae	de Chambrier et Gil de Pertierra, 2012	*Duttaphrynus melanostictus*	*Duttaphrynus melanostictus*	Bufonidae	India	30–50	120	absent	100–110	1	[19–23%]	[61–66%]	[73–78%]	[8.6%]	N/A	N/A	10–13	N/A	20–30
O. cryptobranchi	La Rue, 1914	*Cryptobranchus alleganiensis*	*Cryptobranchus alleganiensis*	Cryptobranchidae	USA	300	340	absent	105–160	2	17–25%	11–25%	[62–76%]	[5.6%]	[7.2%]	usually posterior	15–20	Type 2	(20)
O. ecuadorensis	Dyer, 1986	*Hypsiboas geographicus*	*Boana geographicus*	Hylidae	Ecuador	29	450	present	92–121	2	[23–32%]	[42–45%]	[60–68%]	[7.1%]	N/A	posterior	22–30	Type 1	24–34
O. filaroides	(La Rue, 1909) La Rue, 1911	*Amblystoma tigrinum*	*Ambystoma tigrinum*	Ambystomatidae	USA	80–110	365–400	absent	70–114	2	[27–29%]	[13%]	[72%]	[10.8%]	N/A	anterior	25–35	Type 2	30–31
O. gracilis**.[1]	Jones, Cheng et Gillespie, 1958	*Rana castebiana*	*Rana catesbeiana*	Ranidae	USA	100–200 (72–119)	350 (340–425)	absent	135–155 (115–140)	2	20–25% (19–23%)	17–20% (16–22%)	[72%] (69–80%)	[7.8%] (8.7–9.0%)	N/A (8.2–12.8%)	usually anterior	20–23 (40–45)	Type 1	15
O. hanumanthai	Ramadevi, 1974	*Euphlyctis cyanophlyctis*	*Euphlyctis cyanophlyctis*	Dicroglossidae	India	200	255	absent	104–114	1	17%	[47–52%]	[66–70%]	N/A	N/A	usually posterior	9–14	N/A	20
O. hernandezi*	(Flores-Barroeta, 1955) de Chambrier, Coquille et Brooks, 2006	*Rana* sp.	*Rana* sp.	Ranidae	Guatemala	N/A	880	absent	59–78	1	[24–26%]	[17–18%]	[61–73%]	[7.8–9.9%]	N/A	posterior	21–32	N/A	N/A

doi: 10.14411/fp.2023.009

***O. junglensis* sp. inq.**	(Srivastav et Capoor, 1980) Schmidt, 1986	*Hoplobatrachus tigerinus*	*Hoplobatrachus tigerinus*	Dicroglossidae	India	N/A	N/A	present	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
O. loennbergi	(Fuhrmann, 1895) La Rue, 1911	*Necturus maculosus*	*Necturus maculosus*	Proteidae	USA	190	600	absent	140	2	[23%]	[24%]	[64%]	[5.5%]	[3–4%]	anterior+posterior	N/A	N/A	N/A
O. magna*	Hannum, 1925	*Rana catesbiana* [sic!]	*Rana catesbeiana*	Ranidae	USA	600	455	absent	100–125	2	[20%]	[29%]	[68–72%]	N/A	N/A	posterior	20–25	Type 2	12
O. niuginii*	(Schmidt, 1975) de Chambrier, Scholz, Mariaux et Kuchta, 2017	*Rana arfarki*	*Papurana arfaki*	Ranidae	Papua New Guinea	170	380–385	present	135–165	1	[27%]	[48%]	[63%]	[8.6%]	[7.5%]	anterior (usually)	14–18	Type 1	(15–17); eggs in pairs
O. noei[2]	Wolffhügel, 1948	*Caudiverbera caudiverbera*	*Calyptocephalella gayi*	Calyptocephalellidae	Chile	420	410–580	absent	200–250	2	22–27%	[39%]	[50–52%]	[6.6%]	[13.7–15%]	anterior+posterior	70	Type 1	22–25
O. olor*	(Ingles, 1936) Yamaguti, 1938	*Rana aurora*	*Rana aurora*	Ranidae	USA	184 (length of 2 specimens)	312–475	absent	110–132	2	11%	20%	[61–71%]	[7.4%]	N/A	anterior (usually)	10 to 18	N/A	44 – outer envelop?
O. olseni[3]	Dyer et Altig, 1977	*Hyla geographica*	*Boana geographica*	Hylidae	Ecuador	43	430–490	absent	126–160	2	19%	[59%]	[69%]	[6.5%]	[9.2–10.6%]	posterior	17–27	N/A	28–32
O. papuensis*	(Bursey, Goldberg et Kraus, 2008) comb. n.	*Rana supragrisea*	*Papurana supragrisea*	Ranidae	Papua New Guinea	25-49	205-280	present	89-138	1	[18-20%]	[58-62%]	[53%]	[4.4%]	[8%]	[posterior]	30-42	N/A	21-27; eggs in pairs
O. oumanskyi	Gil de Pertierra et de Chambrier, 2012	*Lepidobatrachus laevis*	*Lepidobatrachus laevis*	Ceratophryidae	Paraguay	50–96	350–410	present	85–119	2	20–27%	35–61%	61–70%	6.7–8.3%	9–13%	posterior + anterior	18–25	Type 1	23–26
O. ranae*	Yamaguti, 1938	*Rana nigromaculata*	*Pelophylax nigromaculatus*	Ranidae	Japan	160	320–380	absent (in adult)	< 230	2	[20–27%]	[17–20%]	[68–71%]	[8.9-10.2%]	[7.6–8.2%]	anterior	12–13	Type 2	21
O. saphena*,[1]	Osler, 1931	*Rana clamitans*	*Rana clamitans*	Ranidae	USA	280 (70–120)	270–320 (340–425)	[present] unclear	88–120 (94–136)	2	about 17% (17–26%)	[23–26%] (15–23%)	[70–76%] (71–82%)	[8.4–8.9%] (8.9–15.4%)	12.2–18.4%	[usually anterior] anterior	14–18 (9–15)	Type 2	17–20
O. schultzei	(Hungerbühler, 1910) Dickley, 1921	*Pyxicephalus adspersus*	*Pyxicephalus adspersus*	Pyxicephalidae	South Africa	90	1000	absent	80–100	2	[16%]	[47%]	[71%]	[22.4%, but original drawing poor]	[6.3%]	posterior	N/A	N/A	(13)
O. tigrina	(Woodland, 1925) de Chambrier, Coquille et Brooks, 2006	*Rana tigrina*	*Hoplobatrachus tigerinus*	Dicroglossidae	India	30–40	235	present	70–110	1	20%	50%	[63–68%]	8,80%	[4.2–4.8%]	usually posterior	15–20	Type 1	(11)

Note: *O. bonneti*, *O. calamensis* and *O. papuensis* were unintentionally omitted in the list of species of the Proteocephalidae provided by de Chambrier et al. (2017); measurements taken from drawings in original descriptions are in brackets. * Species of *Ophiotaenia* from 'true' frogs (Ranidae); [1] measurements from the present redescription in parentheses; [2] measurements taken from *O. noei* (MHNG-PLAT-0022366); [3] measurements taken from *O. olseni* (MHNG-PLAT-0018711); [4] ratio of cirrus-sac length to proglottid width; [5] position in relation to proglottid length; [6] ratio of ovary width to proglottid width; [7] ratio of ovary surface to proglottid surface; [8] ratio of Mehlis' gland width to proglottid width; [9] position of terminal vaginal canal to cirrus-sac; N/A – not available

tionships of these parasites worldwide and to reconstruct the historical colonisation of amphibian hosts by proteocephalids. Despite this limitation, we briefly discuss some issues related to species diversity, distribution, host specificity, and classification of proteocephalid tapeworms that parasitise ranid frogs.

Species diversity. We recognise only nine nominal species of *Ophiotaenia* as valid. This small number of tapeworms found in ranid frogs contrasts sharply with the high diversity of these anurans (429 species according to Frost 2022, with 30 species occurring in the USA and Canada). There may be several explanations for this disparity, such as (i) few species of the Ranidae actually harbour *Ophiotaenia* tapeworms due to the overall depauperate tapeworm fauna of amphibians; (ii) low number of the Ranidae examined for parasites, and therefore patchy knowledge of actual species diversity; (iii) low prevalence of frog infections with tapeworms; (iv) existence of species complexes that have not yet been recognised.

de Chambrier et al. (2006) recognised nine putative new species of *Ophiotaenia* in amphibians, particularly frogs, from Costa Rica, Ecuador, and Paraguay, but did not formally describe them, in part because of insufficient available material. They studied 4,718 hosts of 202 amphibian species. Extrapolating the proportion of amphibians infected with proteocephalids to the approximately 6,000 amphibian species recognised worldwide, a rough estimate of the total number of proteocephalids infecting amphibian hosts could reach as many as 270 species, nine times more than the 32 currently recognised species (see Table 2).

Geographical distribution. The ranges of *Ophiotaenia* spp. in ranid frogs are very disjunct: four species occur in the Nearctic region, two in the northern Neotropics (Costa Rica and Guatemala), one in the easternmost Palaearctic (Japan), and two in Australasia (Papua New Guinea). Regarding the number of ranid frog species examined for parasites, it is difficult to obtain reliable numbers because negative hosts are often not reported in faunal surveys. It can be assumed that only a few dozen ranid frog species have been studied by parasitologists and only a small proportion of them harboured proteocephalid tapeworms (de Chambrier et al. 2006). For example, Goldberg and Bursey (2002) found *O. ranae* in a single *Pelophylax nigromaculatus* out of 18 examined, whereas no tapeworms were found in the other five species of *Rana* Linnaeus from Japan that they dissected.

Infection rate. The prevalence of infection of amphibians with proteocephalid tapeworms is generally very low, especially in the Neotropical region (de Chambrier et al. 2006, Ammann and de Chambrier 2008, de Chambrier and Gil de Pertierra 2012). In Ecuador, only 9 (i.e., 0.41%) of 2,200 amphibians from 91 species surveyed between 1983 and 1990 were infected with proteocephalids. In Paraguay, only 7 (i.e., 0.46%) of 1,510 amphibians from 64 species were infected with *Ophiotaenia* spp. A higher prevalence was found in Costa Rica, where 33 (3.3%) of 1,008 amphibians from 47 species were infected with *Ophiotaenia* tapeworms (de Chambrier et al. 2006).

Data from North America are not as comprehensive, but published reports also indicate a relatively low prevalence of infection of ranid frogs with *Ophiotaenia* tapeworms: Bursey and DeWolf (1998) found *O. gracilis* in 5 of 62 *R. clamitans* (prevalence 8%) in Ohio. Goldberg et al. (2000) found *O. magna* in 3 of 149 *Rana blairi* frogs examined (prevalence 2%) from Colorado, Iowa, Kansas, Nebraska and Texas, but in Carson County, Texas, 3 of 21 frogs (prevalence 14%) were infected with tapeworms.

These low prevalence values mean that a large number of hosts needs to be screened for proteocephalids. This is a serious obstacle because many frogs are endangered and protected by federal and state laws in the USA, so it is rarely possible to examine more than a few host individuals. Parasitological studies of hosts from museum collections can reveal the presence of tapeworms (e.g., Goldberg and Bursey 2002), but tapeworms from hosts fixed in formalin are not suitable for morphological (they are contracted and twisted) and molecular evaluation (the DNA is damaged and fragmented); specimens from frozen hosts should also not be used for morphological studies.

Phylogenetic relationships and host associations. Molecular data are available only for the tapeworms of a few ranid frog species, all from North America (USA); they indicate their possible monophyly. However, the lack of molecular data on proteocephalids from other zoogeographical regions does not allow more reliable conclusions about the colonisation history of ranid frogs by these parasites.

Taxonomic status of *Batrachotaenia* Rudin, 1917. The genus proposed by Rudin (1917) with *Batrachotaenia schultzei* (Hungerbühler, 1910) as the type species, was never recognised by other authors, except Freze (1965). He resurrected the genus and transferred 18 species of tapeworms from frogs and salamanders previously belonging to *Ophiotaenia* to *Batrachotaenia*. This author distinguished species of *Batrachotaenia* from those of *Ophiotaenia* by the following characters: (i) scolex and neck always without 'spines' (= large spiniform microtriches) (as opposed to sometimes or usually with 'spines' in *Ophiotaenia*); (ii) mature and gravid proglottids subquadrate or slightly longer than broad (as opposed to much longer than broad in *Ophiotaenia*); (iii) extremely weak or nearly absent inner longitudinal musculature (reportedly more developed in *Ophiotaenia*); (iv) large Mehlis' gland (smaller in species of *Ophiotaenia*); and (v) different definitive hosts, i.e., amphibians *versus* reptiles (Freze 1965).

A critical review of the distinguishing characters used by Freze (1965) to justify the validity of *Batrachotaenia* as a distinct genus shows that almost all of them are questionable:

(i). 'Spines' (= large spiniform microtriches; for terminology of microtriches, see Chervy 2009) are also present in three species of ranid frogs, namely *O. bonneti*, *O. niuginii* and *O. papuensis*; only the descriptions of *O. olor* and *O. saphena* explicitly state that 'spines' are absent from the scolex; therefore, this feature is not suitable as a genus-specific feature for *Batrachotaenia*. In addition, there was confusion about the terminology of surface structures of proteocephalids (hooks, hooklets, spines, giant microtri-

ches, etc.), and older studies did not use scanning electron microscopy to reliably determine the presence and nature of these surface structures.

(ii). In most species of *Ophiotaenia* from the ranids, the proglottids are subquadrate or only slightly longer than wide; only in *O. hernandezi* have elongate proglottids been reported, up to 2.5× longer than wide (Flores-Barroeta 1955).

(iii). Five tapeworms from ranid frogs possess a well developed internal longitudinal muscles, namely *O. bonneti*, *O. hernandezi*, *O. magna*, *O. ranae*, and *O. saphena*. Therefore, this trait is not suitable to characterise species of *Batrachotaenia*.

(iv). Only six of nine species previously included in *Batrachotaenia* possess a relatively large Mehlis' gland (the ratio between the width of the gland and the width of the proglottids ranges from 6.3% to 18.4%); however, in the remaining three species, namely *Ophiotaenia loennbergii* (Fuhrmann, 1895) (3–4%), *Ophiotaenia oumanskyi* de Chambrier et Gil de Pertierra, 2012 (5.2%) and *Ophiotaenia tigrina* (4.2–4.8%), the Mehlis' gland is significantly smaller (see Table 2).

(v). The host ranges of *Batrachotaenia* (amphibians) and *Ophiotaenia* (reptiles) may be able to distinguish the two putative genera, but it is uncertain whether all proteocephalids from amphibians are monophyletic (two sequenced species from frogs and one from a salamander do not form a common group – de Chambrier et al. 2015).

It is evident that most of the characters used by Freze (1965) to distinguish *Batrachotaenia* from *Ophiotaenia* are not reliable, the shape of proglottids being the only potentially suitable character. However, a comparison of the relative size of the ovary shows that species of *Ophiotaenia* from amphibians (= *Batrachotaenia* sensu Freze 1965) have a large ratio of ovary to proglottid surface area (ratio 4.5–15.4%; mean 7.6%; n = 28). In contrast, species of *Ophiotaenia* from reptiles (with the exception of taxa from the Palaearctic) have a much smaller ovary to proglottid surface ratio, i.e., a ratio of 1.1–6.5% (up to 8.5% in one species), with a mean of 3.3% (n = 60; see table 2 in de Chambrier et al. 2021). Three European species of *Ophiotaenia* from snakes differ from the other congeneric taxa parasitising reptiles in having a conspicuously larger ovary (relative size of 9.8% in *O. dubinini* Freze et Sharpilo, 1965, 12.7% in *O. europaea* Odening, 1963, and 9.1% in *O. spasskyi* Freze et Sharpilo, 1967).

It is therefore possible to distinguish most proteocephalids from amphibians and reptiles by the combination of the relative size of the ovary (but without a clear threshold) and, in part, of the Mehlis' gland, and by the shape of the proglottids. However, these characters are not sufficient to resurrect *Batrachotaenia*. Moreover, the molecular data, although very incomplete, do not support monophyly of the tapeworms of frogs and salamanders (de Chambrier et al. 2015). Therefore, the tapeworms of ranid frogs are tentatively left in *Ophiotaenia*. A new taxonomic classification of proteocephalids that parasitise amphibians and reptiles at the genus level cannot be made until their phylogenetic relationships are better known.

Based on the new morphological and molecular data obtained in the present study, the questions listed in the Introduction can be tentatively answered as follows:

(1) Do the proteocephalid tapeworms of ranid frogs form a monophyletic clade? The answer is that the Nearctic taxa appear to form a monophyletic group (and are morphologically similar), but no information is available on the five remaining nominal taxa from other zoogeographical regions, including two Neotropical species from Central America.

(2) Are these tapeworms closely related to the type species of the genus, *Ophiotaenia perspicua*, i.e., do they belong to the 'true' *Ophiotaenia* (= *Ophiotaenia* sensu stricto), or do they represent a separate genus (or genera) that should be proposed? The available molecular data do not provide a clear answer. Based on *lsr*DNA sequences, species of *Ophiotaenia* from colubrid snakes, including *O. perspicua*, do not appear closely related to proteocephalids from ranid frogs (Fig. 1 in de Chambrier et al. 2015).

(3) Are proteocephalids of ranid frogs strictly host-specific (oioxenous)? Available data suggest somewhat slightly less strict host specificity (most likely stenoxenous, i.e., intermediate specialists found in multiple congeneric hosts) in at least some species, but too few samples from a small number of ranid hosts have been evaluated. A putative new species (*O.* '*saphena*' by de Chambrier et al. 2015) was found in *R. pipiens* and *R. sphenocephala* and thus could also be considered an intermediate specialist according to the host specificity classification proposed by Kuchta et al. (2020).

(4) Do molecular data support Freze's (1965) concept for the resurrected *Batrachotaenia* Rudin, 1917? Available data are too limited and no sequences of *Ophiotaenia schultzei*, the type species of *Batrachotaenia*, are available. However, proteocephalids of frogs and salamanders are probably not closely related (de Chambrier et al. 2015).

Acknowledgements. Two anonymous reviewers provided helpful suggestions. The authors express their gratitude to Luis García-Prieto, Curator of CNHE, Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City, Mexico for information about types of *O. hernandezi*, Anna Phillips, Smithsonian National Museum of Natural History, Washington, D.C., USA for enabling to study types of *O. olor*, and to Julián Faivovich, División Herpetología, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires, Argentina for providing information about species richness of ranid frogs. Thanks are also due to Roman Kuchta for taking SEM micrographs of *O. gracilis* and *O. saphena*, Blanka Škoríková (both České Budějovice) for preparing plates with figures, Janik Pralong, Gilles Roth and Christina Lehmann-Graber (all Geneva) for technical help, and Andrea Waeschenbach, Natural History Museum, London, UK, for generous help with a species delimitation analysis (bPTP). We acknowledge Laurence (Mac) Hardy, Ouachita Mountains Biological Station, Big Fork, Arkansas, USA, for providing housing and laboratory space for processing frogs. C.T.M. was issued a Scientific Collecting Permit from the Arkansas Game and Fish Commission. This study was supported by the Institute of Parasitology (RVO 60077344), Ministry of Education, Youth and Sports of the Czech Republic (project LTAUSA18010 to T.S. and Czech-BioImaging LM2015062 to the Laboratory of Electron Microscopy), and NSF-PBI awards (grants 0818696 and 0818823).

Author contribution. TS and AdC designed the study; VVT, CTM and TS collected specimens; TS and AdC processed tapeworm specimens and examined them; OK sequenced specimens and performed phylogenetic analyses; TS wrote the manuscript except for the molecular part, which was written by OK; all authors contributed to manuscript editing.

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Received 17 August 2022 Accepted 18 January 2023 Published online 12 April 2023

Cite this article as: Scholz T., de Chambrier A., Kudlai O., Tkach V.V., McAllister C.T. 2023: A global survey of tapeworms (Cestoda: Proteocephalidae) of 'true' frogs (Amphibia: Ranidae), including a tabulated list of all proteocephalids parasitising amphibians. Folia Parasitol. 70: 009.