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A new species of *Versteria* (Cestoda: Taeniidae) parasitizing *Neogale vison* and *Lontra canadensis* (Carnivora: Mustelidae) from Western Canada

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Research Paper

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Abstract

Via molecular and morphological analyses, we describe adult specimens of a new species of *Versteria* (Cestoda: Taeniidae) infecting mink and river otter (Carnivora: Mustelidae) in Western Canada, as well as larval forms from muskrat and mink. These sequences closely matched those reported from adult specimens from Colorado and Oregon, as well as larval infections in humans and a captive orangutan. We describe here a new species from British Columbia and Alberta (Canada), *Versteria rafei* n. sp., based upon morphological diagnostic characteristics and genetic distance and phylogeny. *Versteria rafei* n. sp. differs from the three other described species of the genus in the smaller scolex and cirrus sac. It also differs from *V. mustelae* (Eurasia) and *V. cuja* (South America) by having an armed cirrus, which is covered in hair-like bristles, and in the shape of its hooks, with a long thorn-like blade, and short or long handle (*vs.* a short sharply curved blade and no difference in handle size in previously described species). The poorly known *V. brachyacantha* (Central Africa) also has an armed cirrus and similarly shaped hooks. However, it differs from the new species in the number and size of hooks. Phylogenetic analysis of the *cox1* and *nad1* mitochondrial regions showed that our specimens clustered with isolates from undescribed adults and larval infections in North America, and separate from *V. cuja*, confirming them to be a distinct species from the American Clade.

Introduction

Characterising the diversity and taxonomic relationships of taeniid tapeworms (Platyhelminthes: Cestoda) has been historically complicated, though of significance because of their socioeconomic impact on domestic livestock and human health (Hoberg 2006; Lavikainen *et al.* 2008). Recent research using molecular tools to organise the family Taeniidae has driven the erection of the genus *Versteria* (Nakao *et al.* 2013), with the type species *Taenia mustelae* Gmelin, 1790 [syn. *Taenia tenuicollis* (Rudolphi, 1819), *Taenia brevicollis* (Rudolphi, 1819), *Fimbriotaenia mustelae* (Gmelin, 1790)] (Nakao *et al.* 2007; Lavikainen *et al.* 2008; Knapp *et al.* 2011; Nakao *et al.* 2013). This genus remains poorly characterised, with only three nominal species described (Bagnato *et al.* 2022); at the time of its erection, only two species were included, *V. mustelae* and *V. brachyacantha* (Baer and Fain 1951), the latter based solely on morphological similarities as no sequences were available (Nakao *et al.* 2013). This separation was supported by previous studies, which noted that *V. mustelae* was basal in *Taenia* based on morphometric diagnostic characteristics such as its minute rostellar hooks, small scolex, rostellum, and suckers, a limited number of testes, and relatively short strobila (Hoberg *et al.* 2000). It was these unique characteristics that had previously defined the assignment of specimens to *V. mustelae*, the species something of a catch-all for the small-hooked taeniids of mustelids (Freeman 1956; Nakao *et al.* 2013). This species was redescribed numerous times in the 20th century; large variances in the range of diagnostically important characteristics were reported between North America, Europe, and Asia (Table 1) (Thienemann 1906; Skinker 1935; Locker 1955; Freeman 1956; Abuladze 1964; Wahl 1967; Verster 1969; Iwaki *et al.* 1995). Nakao *et al.* (2013) noted that differences in the cysticerci of Palearctic and Nearctic *V. mustelae* might suggest cryptic species. Lavikainen *et al.* (2008) reported little genetic differentiation among Palearctic specimens, while no analysis was made between Nearctic examples.

Molecular characterisation of zoonotic infections and recent morphological analysis of specimens suggest additional taxa related to Versteria are present in North America. Recent reports of echinococcus-like infections have identified taeniid cysticerci in human patients from Pennsylvania (USA) and New Brunswick (Canada) (Barkati *et al.* 2019; Lehman *et al.* 2019) as well as in an orangutan *Pongo pygmaeus* at the Milwaukee County Zoo, Wisconsin, that was born in Colorado (USA) (Lee *et al.* 2016; Deplazes *et al.* 2019). Molecular analysis of these infections identified the

Table 1. Comparison of measurements for *Versteria.* Morphometric ranges for diagnostic characteristics for other species of *Versteria* synthesized and compared against those taken for *V. rafei* n. sp. from this study. Measurements given in micrometers. Reports that did not differentiate long hooks from short hooks and only provided a general measurement are presented as a single range. Abbreviations: LH, large hooks; SH, small hooks; L, length; W, width

Species	*V. rafei*	*V. cuja*	*V. mustelae* (syn. *T. tenuicollis*)							*V. brachyacantha*
			North America			Eurasia				
Location	Canada	Argentina	Domestic	Ontario	Minnesota	Switzerland	Russia	Germany	Japan	Central Africa
Source	This study	(Bagnato *et al.* 2022)	(Freeman 1956)	(Skinker 1935)	(Freeman 1956)	(Wahl 1967)	(Abuladze 1964)	(Thienemann 1906)	(Iwaki *et al.* 1995)	(Baer and Fain 1951)
Width	384–1003	1117–1941	1630	2400	1710	1900		2000	2000	
Scolex	170–220	276–345	200–350	237–303	230–310	300	449–477	260–550	351–593	480
Rostellum	39–67	39–75	70–97	61–77	70–90	91	108	133	74–120	126
Sucker	81–101	87–151	97	77–110	97–125	130–150	167–186	100–133	118–186	176
Sucker/scolex	0.46–0.48	0.32–0.49	0.28–0.41	0.32–0.36	0.40–0.42	0.43–0.5	0.37–0.39	0.24–0.38	0.31–0.34	0.37
Hooks	c.42–48	c.48	c.48–66	c.42	c.47–59	c.37–43	50	c.36–72	c.44–58	c.54
Hook length (LH/SH)	10–17	12–17	15–20	15–16	14–17	19–20	18–21/ 12–15	16–24 / 13–21	17–22	26–28
Testes	c.84–117	c.54–85		c.90–125		c.100–110	c.114	c.60–114	c.80–141	c.100–145
Testes size (L/W)	16–36	–	49–58/ 42–51	39–55	51–76/ 28–49	26–39/ 17–27	–	42–67/ 37–52	31–75/ 26–75	
Genital atrium (L/W)	105–157/ 55–115	170–420 (L only)	–	193–225/ 127–155	–	68–91 (L only)	–	–	–	–
Cirrus	armed, hairlike bristles	unarmed, smooth	–	–	–	–	–	–	unarmed	armed, hairlike bristles
Cirrus–sac L	105–176	210–311	–	193–220	–	229–274	352–369	–	187–319	240–280
Cirrus–sac W	46–95	130–185	–	130–154	–	123–146	158–176	–	64–201	120
Uterine branches	c.18–28	c.12–28	c.16–23	c.10–19	c.10–15	c.28 (avg)	c.14–16	c.12–18	c.17–25	14–17
Eggs (L/W)	18–22/ 15–18	–	–	17–20	–	22.4–24.6/ 17.9–21.3	–	20–23/ 24–28	25–27/ 20–25	–
Host	*N. vison*	*Galictis cuja*	*N. vison*	*N. vison*	*M. erminea*	*M. erminea*	–	*M. nivalis*	*M. nivalis*	*Poecilogale albinucha*

parasite as belonging to *Versteria*, and molecular characterisation suggested that there may be two or more *Versteria* species in the Nearctic (Lee *et al.* 2016; Niedringhaus *et al.* 2022). Because of this, and due to the wide variation in reported morphological characteristics between North American and European samples, previous identifications of *V. mustelae* from North America may have been incorrect. Lee *et al.* (2016) investigated mustelids in Oregon, Colorado, and Wisconsin to identify adults of the unnamed species, producing a single specimen each from a mink *Neogale vison* in Oregon, an ermine *Mustela erminea* in Wisconsin, and an ermine in Colorado. Unfortunately, the samples were fragmented, were without scoleces, and could not be fully described. Molecular analysis confirmed the specimens from Oregon and Colorado to be the same lineage as those identified from the orangutan infection, while the specimen from Wisconsin clustered closer to haplotypes from the Palearctic confirming there are likely multiple species present in North America (Lee *et al.* 2016). Recently, a third species was added, *Versteria cuja* Bagnato, Gilardoni, Martin & Digiani, 2022, identified from the lesser grison *Galictis cuja* in Argentina and molecularly distinct from the Nearctic haplotypes available (Bagnato *et al.* 2022).

Previous studies of parasites identified as *V. mustelae*, which likely included two or more species in North America, have reported various hosts. Definitive hosts included ermine, mink, and marten *Martes americana* (Skinker 1935; Locker 1955; Freeman 1956; Miller and Harkema 1964; Jennings *et al.* 1982). Intermediate hosts included multiple species of terrestrial rodents, including members of *Microtus* and *Peromyscus*, fox squirrels *Sciurus niger* in Michigan, and muskrats *Ondatra zibethicus* in Alaska, British Columbia, and Illinois (Skinker 1935; Locker 1955; Langham *et al.* 1990). A fatal infection of *Versteria* sp. in a muskrat from Pennsylvania was sequenced. It clustered with infections from a human in Pennsylvania, the orangutan mentioned above, and adult parasite specimens from Oregon and Colorado (Niedringhaus *et al.* 2022). This zoonotic infection by *Versteria* sp. is cause for concern and identification of the adults and their hosts is essential in the mitigation of infection in humans.

In this article, we describe this unnamed species as *Versteria rafei* n. sp. from the intestines of *Neogale vison* based on morphological and molecular data. We report infections identified in *N. vison*, *Lontra canadensis*, and *Ondatra zibethicus* and larval infections in the livers of *N. vison* and *O. zibethicus* from British Columbia and Alberta, Canada.

Materials and methods

Carcasses of *L. canadensis* (n=155) and *N. vison* (n=106) were obtained from licensed fur trappers in Alberta and British Columbia (BC), Canada, during the 2020–21 and 2021–22 trapping seasons. Muskrats *O. zibethicus* (n=41) were only collected during the 2020–21 trapping season from Alberta. Carcasses were frozen after skinning and kept at -20°C, except during shipping or transport, until necropsy. Cestodes were collected during necropsy and preserved and stored in 70% ethanol or 90% ethanol for molecular analysis. For morphological analysis, specimens were stained with acetic acid carmine, dehydrated in a graduated ethanol series, cleared in clove oil, and mounted permanently in Canada balsam for examination by light microscope. Photomicrographs were taken on an Olympus BX51 compound microscope mounted with DP25 camera attachment (Olympus, Tokyo), which calculated scale and included a scale bar in the picture. Measurements of various morphological features were then taken using the software ImageJ (Schneider *et al.* 2012). Measurements are given in µm unless otherwise stated, with average and standard deviation in parentheses.

Molecular analysis

Molecular identification was carried out based on the *nad1* mitochondrial region (Forward NDJ11: 5'-AGATTCGTAAGGGGCCTAATA-3', Reverse NDJ2a: 5'-ACCACTAACTAATTCACTTTC-3') and the *cox1* mitochondrial region (Forward JB3: 5'--5TTTTTTGGGCATCCTGAGGTTTAT-3', Reverse JB4.5: 5'--TAAAGAAAGAACATAATGAAAATG-3') using primers suggested for taeniids following the suggested cycling instructions of the authors (Bowles and McManus 1993; Bowles *et al.* 1994; Trachsel *et al.* 2007). DNA was extracted from terminal proglottids using DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) using manufacturer's protocols. Amplification and sequencing were conducted with a total volume of 25µl using PCR Master Mix (Invitrogen) according to the manufacturer's instructions. PCR products were cleaned using EXOSAP^{TM} Express PCR Product Cleanup Reagent (USB Corporation, Cleveland, OH, USA) following manufacturer's instructions. Sanger sequencing was performed by the University of Alberta's Molecular Biology Service Unit (Edmonton, Canada) or by the Genetic Analysis Service, Department of Anatomy, University of Otago (Dunedin, New Zealand). The produced sequences were trimmed and compared against those available in GenBank using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Exemplar sequences have been deposited in GenBank.

Phylogenetic analysis

Sequences were aligned using ClustalW as implemented in MEGA11 software (Kumar *et al.* 2018) against sequences from related parasites in GenBank. The resulting alignments were used to create a phylogenetic tree by Maximum-likelihood method and the Tamura-Nei model (Tamura 1992; Tamura and Nei 1993) bootstrapped at 500 replicates. Estimates of evolutionary divergence were quantified by calculating pairwise distance conducted in MEGA11 (Tamura *et al.* 2021). The analyses involved 8 (*nad1*) and 12 (*cox1*) nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There was a total of 452 (*nad1*) and 347 (*cox1*) positions in the final dataset.

Results

Adult cestodes identified as *Versteria* sp. were found in all three species (n_{mink} = 8 [7.5%]; n_{otter}= 5 [3.2%]; $n_{muskrat}$ = 2 [4.9%]). Larval infections were identified in the livers of mink and muskrat in Alberta (n_{mink} = 4 [3.7%]; $n_{muskrat}$= 18 [43.9%]), at intensities ranging from 3 cysts to over 50. The mink infected with over 50 cysts in the liver was also notable for concurrently being infected with 21 adults.

Description of new species

Family. Taeniidae Ludwig, 1886

Genus. Versteria Nakao, Lavikainen, Iwaki, Haukisalmi, Konyaev, Oku, Okamoto, & Ito, 2013

Species. Versteria rafei n. sp.

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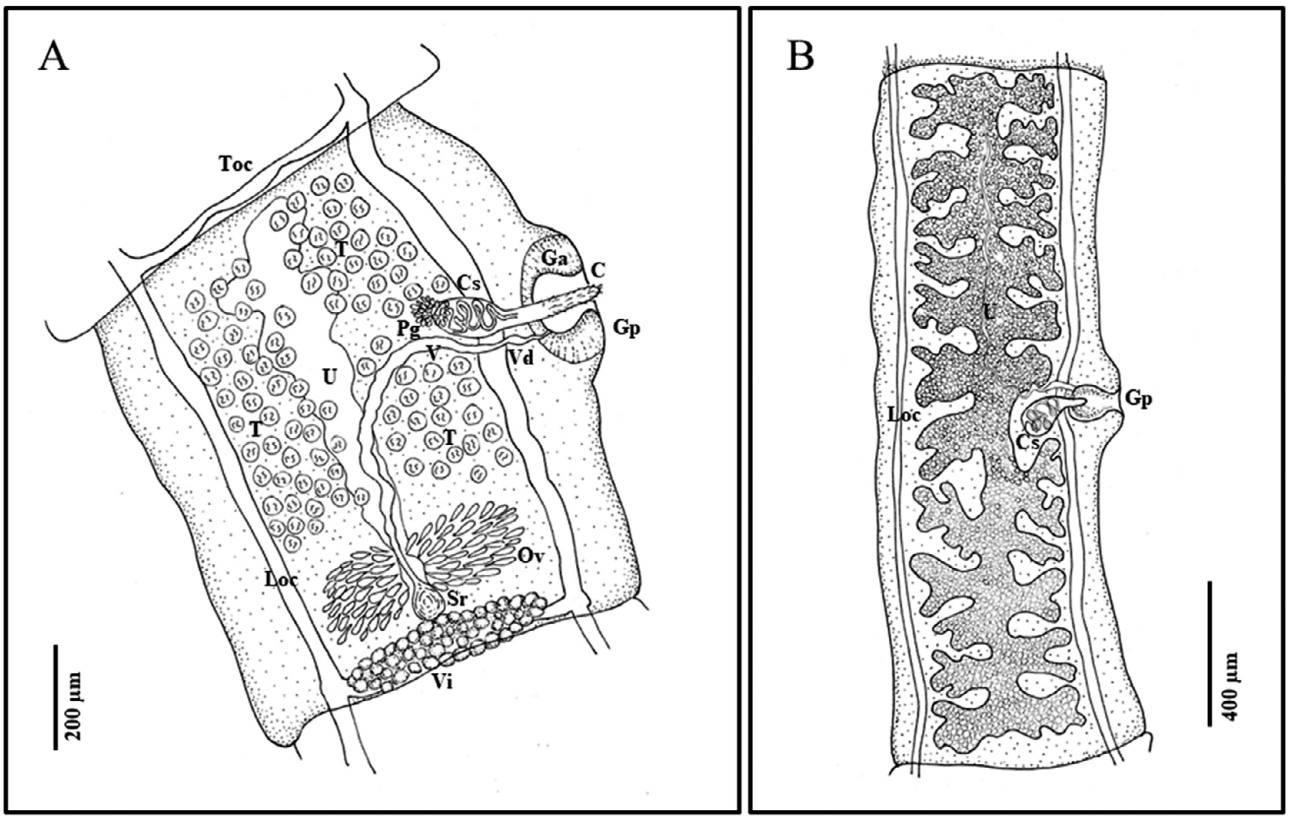

Figure 1. Line drawings of *Versteria rafei* n. sp. (Cestoda: Taeniidae**).** Examples from the North American Mink (*Neogale vison*) from British Columbia, Canada. A) mature proglottid; B) gravid proglottid. Abbreviations: C, cirrus; Ci, cirrus sac; Ga, genital atrium; Gp, genital pore; Loc, longitudinal osmoregulatory canal; Ov, ovary; Pg, prostatic gland; Sr, seminal receptable; T, testes; Toc, transverse osmoregulatory canal; U, uterus; V, vagina; Vd, vas deferens; Vi, vitellarium. Illustrated by B. Presswell.

Material studied. Ten complete adults and strobilar fragments with and without scoleces from 10–20 adults, which included immature, mature, and gravid proglottids.

Description. Strobila short, complete gravid specimens ranging from 200 to over 1000 mm (Figure 1). Scolex small, 170–220 (188 ±16 SD) wide, with rostellum 39–67 (54±9 SD) in diameter at widest point (n=14). Suckers large in relation to scolex, 81–101 (90±6 SD) wide (Figure 2). Neck tapering immediately posterior to suckers, 88–120 (100±9 SD) wide (n=14). Rostellum with double crown of very small hooks, ranging in number from 21–24 per row (42–48 total) (n=5); scoleces almost always found without hooks, or with only a few hooks remaining. Hooks with sharp curved blade, long and stout guard with bulbous epiphyseal thickening, and a short or long, straight handle with narrow epiphyseal thickening; hooks in posterior circle with short handle, thick base, and thorn-like shape, in anterior circle with long handle and thin base (Figure 2E, F). Little difference in length between the short and long handled hooks, short 10–15 (12.2±1.6 SD) (n=25) and long 13–17 in length (14.9±1.4 SD) (n=25). Proglottids craspedote: immature proglottids broader than long, mature and gravid proglottids longer than wide. Mature proglottids with length/width ratio of 1:1.07–1.91 (1:1.51±0.22 SD), gravid proglottids with length/width ratio of 1:1.03–4.37 (1:1.97±0.70 SD). Mature proglottids 629–1323 (904±152 SD) long and 384–1003 (610±120 SD) wide (n=71). Gravid proglottids 1203–3489 (1860±582 SD) long, 726–1241 (979±187 SD) wide; 18–28 uterine branches (n=30). Genital pores alternate irregularly, usually slightly anterior of middle of proglottid, protruding in older proglottids. Genital atrium well developed, with muscular sphincter, rounder than oval when relaxed, forming big genital papilla (Figure 2B); in mature proglottids 57–162 (122±24 SD) deep and 52–141 (95±586 SD) wide when relaxed (n=71); in gravid proglottids, 97–201 (146±29 SD) deep and 65–180 (130±25 SD) in width when relaxed (n=30). Longitudinal osmoregulatory canals 28 (±7 SD) wide (n=14); transverse connecting canals 18 (±4 SD) wide (n=14) in mature proglottids.

Male reproductive system. Testes 85–117 in number (n=71), generally sub-spherical in shape, 16–36 (23±4 SD) in diameter (n=131). Testicular fields confluent anteriorly, situated between longitudinal osmoregulatory canals, from anterior margin of proglottid to anterior margin of ovary. Antero-poral field with fewest testes; antero-poral and postero-poral fields interrupted by vagina and cirrus sac (Figure 1). Cirrus sac ovoid in shape and relatively small, 105–195 (150±21 SD) long and 46–113 (82±29 SD) wide in mature proglottids (n=71), and 145–242 (183±22 SD) long and 87–139 (109±15 SD) wide in gravid proglottids (n=30). Vas deferens forming loops inside and outside cirrus sac, surrounded by prostatic cells. Cirrus armed with hair-like bristles in a spiral pattern.

Female reproductive system. Ovary two-winged, wings roughly equal in size, lobed. Shape and size vary depending on stage of development; in mature proglottids at posterior, 248–390 (293±62 SD) wide (n=10); in mature to gravid proglottids, round and strongly staining, often just posterior of center (Figure 2). Vitellarium just posterior of ovary and seminal receptacle, at posterior edge of proglottid, averaging 337 (±34 SD) wide; proglottid width to ovary width ratio 1:0.29–0.49 (1:0.41±0.08 SD) (n=10). Vagina wide, enters genital pore posteriorly opening of cirrus sac and

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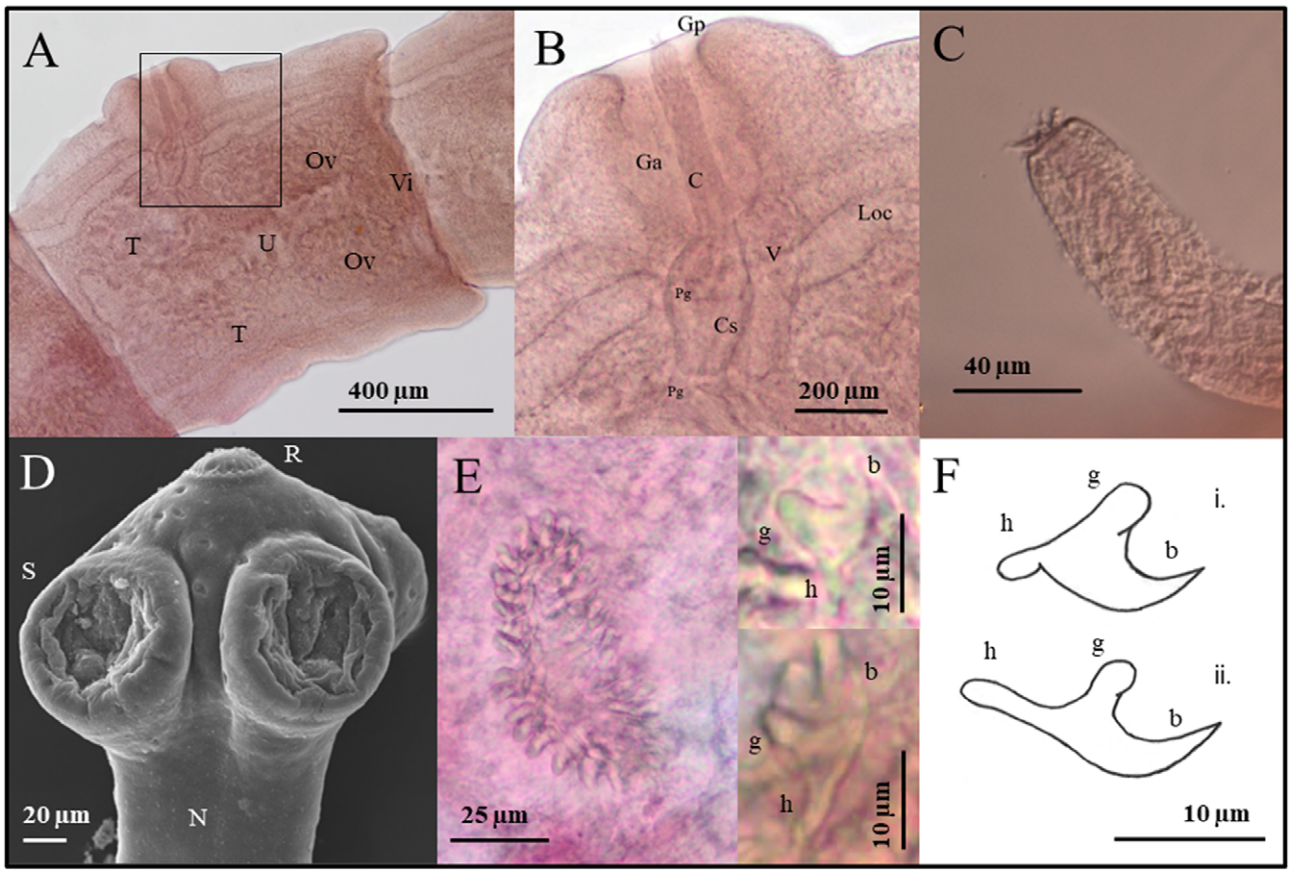

Figure 2. Specimens of *Versteria rafei* n sp. Examples from the North American mink (*Neogale vison*) from British Columbia, Canada. A) mature proglottid of an adult worm, stained with acetic acid carmine, cleared in clove oil, and mounted permanently in Canada balsam; B) close-up of cirrus sac and genital pore; box from panel A; C) partially everted cirrus of adult showing bristles; D) scanning electron microscope image of scolex (missing hooks), showing the rostellum and suckers, magnification at 995x; E) double crown of rostellar hooks, prepared by staining in acetic acid carmine and then squashing the scolex on a glass slide with a cover slip and rotating, with close-ups of hooks with short and long guards; F) line drawing of hooks. Abbreviations: b, blade; C, cirrus; Ci, cirrus sac; g, guard; Ga, genital atrium; Gp, genital pore; h, handle; Loc, longitudinal osmoregulatory canal; N, neck; Ov, ovary; Pg, prostatic gland; R, rostellum; S, sucker; T, testes; U, uterus; V, vagina; Vi, vitellarium.

directed centrally and posteriorly before connecting with seminal receptacle. Uterus fills medium portion of proglottid, with outpocketings at irregular intervals along its length, largest at anterior end of uterus (Figure 1). Gravid proglottids with 18–28 total lateral branches (n=30), often with secondary and sometimes tertiary bifurcations. Eggs (embryophores) sub-ovoid, 18–22 in length and 14–18 in diameter (n=9).

Taxonomic summary

Type host: Neogale vison (Carnivora: Mustelidae).

Other hosts: definitive, *Lontra canadensis* (Carnivora: Mustelidae), *Ondatra zibethicus* (Rodentia: Cricetidae); intermediate *N. vison*, *O. zibethicus.*

Type locality: Southeast Vancouver Island, British Columbia, Canada.

Known distribution: Western Canada: Alberta and British Columbia.

Site of infection: Small intestine (definitive host), liver (intermediate host).

Prevalence and intensity of infection: N. vison: 7.5% (n=106), intensity 5–54 per host; *L. canadensis*: 3.2% (n=155), intensity 3–23 per host; *O. zibethicus*: 4.9% (n=41), intensity 2–4 per host. Larval infections in the liver, *N. vison*: 3.8% (n=106); *O. zibethicus*: 43.9% (n=41).

Type specimens: Syntypes, CMNPA 2023-0008.1, CMNPA 2023-0008.2, CMNPA 2023-0008.3, Canadian Museum of Nature, Ottawa.

Etymology: The specific epithet (a noun in the genitive case) honours Dr. Rafael 'Rafe' R. Payne, a parasitologist who dedicated almost 50 years of his life to the education of undergraduate students.

GenBank accession numbers: OR448764 (*cox1*, adult, *N. vison*), OR852790 (*nad1*, adult, *N. vison*), OR852791 (*nad1*, metacestode, *N. vison*), OR863684 (*nad1*, adult, *L. canadensis*), OR852792 (*nad1*, adult, *O. zibethicus*), OR852793 (*nad1*, metacestode, *O. zibethicus*).

ZooBank access number: DBD28DD4-DBB4-4931-92A5-F5D0BD897510

Remarks: According to molecular evidence and the diagnosis given by Nakao *et al.* (2013), *Versteria rafei* n. sp. belongs to the genus *Versteria* due to its short strobila, elongate gravid proglottids, small scolex, rostellum, suckers, and double crown of very small hooks. Other characteristics of the genus include genital pores that alternate irregularly roughly at the middle of the proglottid and terminal genital ducts that pass longitudinally over the osmoregulatory canals, median, posterior female glands with bilobed ovaries, transversely elongated vitellarium posterior to the ovary, median uterus a longitudinal stem, laterally branched when gravid, and relatively small number of testes almost entirely anterior and lateral to the female organs. It differs from the diagnosis only in that its

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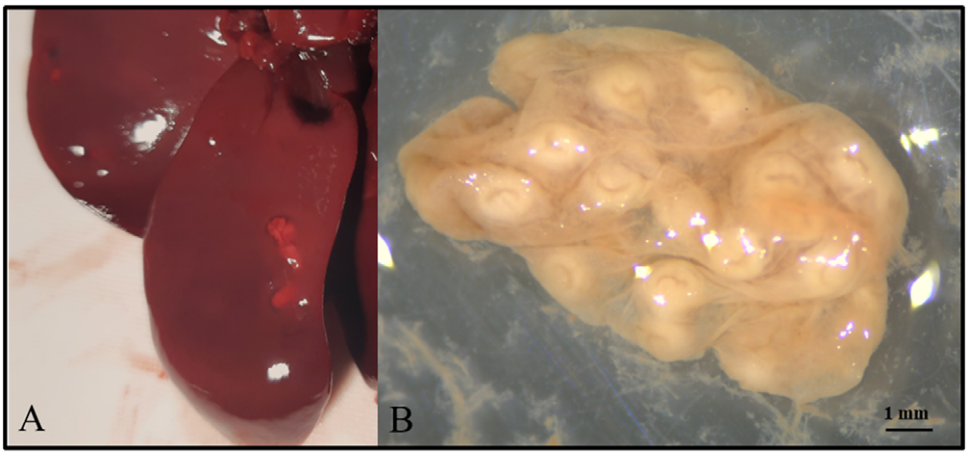

Figure 3. Cysts of *Versteria rafei* n sp. Examples from muskrat (*Ondatra zibethicus*) from Alberta, Canada. A) two cysts in a lobe of the liver; B) excised cyst from the liver showing multiple metacestodes adhered to the cyst wall.

mature proglottids are not wider than they are long. Mature proglottids are generally close to equal in length and width, though more often longer than they are wide. Descriptions of *V. mustelae*, *V. brachyacantha*, and *V. cuja* all reported mature proglottids wider than long (Table 1). However, the recent description of *V. cuja* also reported ranges for length and width that overlap in mature proglottids (476–2027 long by 1117–1941 wide) and might have included proglottids that were longer than wide. In fact, their published line drawing showed a mature proglottid longer than wide (Bagnato *et al.* 2022). Skinker (1935) noted that mature proglottids were *generally* wider than long, but that the ratio also varied. Descriptions of *V. mustelae* by Wahl (1967) and Joyeux and Baer (1936) did not provide the length-width ratio for mature proglottids. In the description by Freeman (1956) of natural and experimental infections in weasel and mink from North America, the author reported a length–width ratio of 1:0.54–1.71 in weasel *Mustela nivalis* and 1:1.63–1.22 in mink, showing a range of ratios in weasels, while all examples reported from mink were longer than wide. It is likely that this ratio is dependent on the age of the mature proglottid, wider when young as it transitions from an immature stage but then gradually longer than wide as it matures. This ratio may also be host-dependent as Freeman (1956) has reported ratios in mink that were similar to those we report from mink here. We suggest the diagnosis for *Versteria* be amended to state that immature proglottids are wider than long, gravid proglottids longer than wide, and that mature proglottids vary depending on their level of maturity and possibly host.

Since historically *V. mustelae* was a catch-all species for the small-hooked taeniids of mustelids, and morphological descriptions vary widely over the years and between North America and Eurasia (Table 1), it is difficult to accurately compare the species described herein to the rest of the genus. Modern molecular analysis has identified one or more distinct lineages of *Versteria* in North America, and others may exist in Eurasia (Lee *et al.* 2016; Niedringhaus *et al.* 2022), which may explain the large variation in morphological characteristics previously reported for *V. mustelae.* However, there are several distinct features that characterise *V. rafei* n. sp. from the three other species of *Versteria.*

For the main diagnostic characteristics of the scolex, rostellum, suckers, hook size and number, cirrus sac, genital atrium, and uterine branches, *V. rafei* n. sp. is distinct in the size of its scolex and sucker to scolex ratio, the size of its cirrus sac, and the depth of its genital atrium. It has the smallest scolex of all *Versteria* species (170–220 µm), with large suckers compared to the scolex (0.46–0.48 of the scolex width). In comparison, *V. cuja* in South America (276–345 µm, 1:0.32–0.49) (Bagnato *et al.* 2022), *V. brachyacantha* in Central Africa (480 µm, 1:0.37) (Baer and Fain 1951), and species reported as *V. mustelae* from Europe (260–550 µm, 1:0.24–0.50) (Thienemann 1906; Abuladze 1964; Wahl 1967) have much larger scoleces. It also has the smallest cirrus sac (105–176 µm long, 46–95 µm wide) and ovoid in shape compared to the more spherical *V. cuja* (210–311 µm long, 130–185 µm wide) (Bagnato *et al.* 2022), *V. brachyacantha* (240–280 µm long, 120 µm wide) (Baer and Fain 1951), and *V. mustelae* in Europe (229–369 µm long, 123–176 µm wide) (Abuladze 1964; Wahl 1967). The genital atrium (105–157 µm) is deeper than that reported in *V. mustelae* in Europe (68–91 µm) (Wahl 1967), but smaller than *V. cuja* (170–420 µm) (Bagnato *et al.* 2022). Note that there is no report of the atrium depth for *V. brachyacantha.* However, the published line drawing shows the genital atrium as equal to or slightly larger than the length of the cirrus sac (240–280 µm) (Baer and Fain 1951).

Besides these diagnostic characteristics, what distinguishes *V. rafei* n. sp. from *V. mustelae* and *V. cuja* is its armed cirrus (Iwaki *et al.* 1995; Bagnato *et al.* 2022), which has hair-like bristles in a spiral pattern, similar to *V. brachyacantha* (Baer and Fain 1951). The rostellar hooks of *V. rafei* n. sp. (10–17 µm) are similar in size to those of *V. cuja* (12–17 µm), but they are different in shape. *Versteria cuja* only has hooks with long handles and a short blade (Bagnato *et al.* 2022), similar to *V. mustelae*, which also has a short blade and no difference in handle size (Verster 1969). They are much smaller than the hooks of *V. brachyacantha* (26–28 µm, c.54) with fewer hooks, though there is some similarity in shape with an enlarged flattened guard, long thorn-like blade, and smallish handle even in the long-handled specimens (Baer and Fain 1951).

Metacestodes

Cysts containing cysterceri were identified in the liver of muskrats and mink ($n_{muskrat}$= 18 [43.9%]; n_{mink} = 4 [3.7%]), ranging in intensity from 1 to over 50 cysts. They were found in the parenchyma of the liver, most often easily seen in the periphery, though at times embedded within and obscured by tissue. Cyst walls were thin and transparent, the number of larvae ranging from 6 to 18, all closely applied to the inner wall of the host cyst (Figure 3). Larvae

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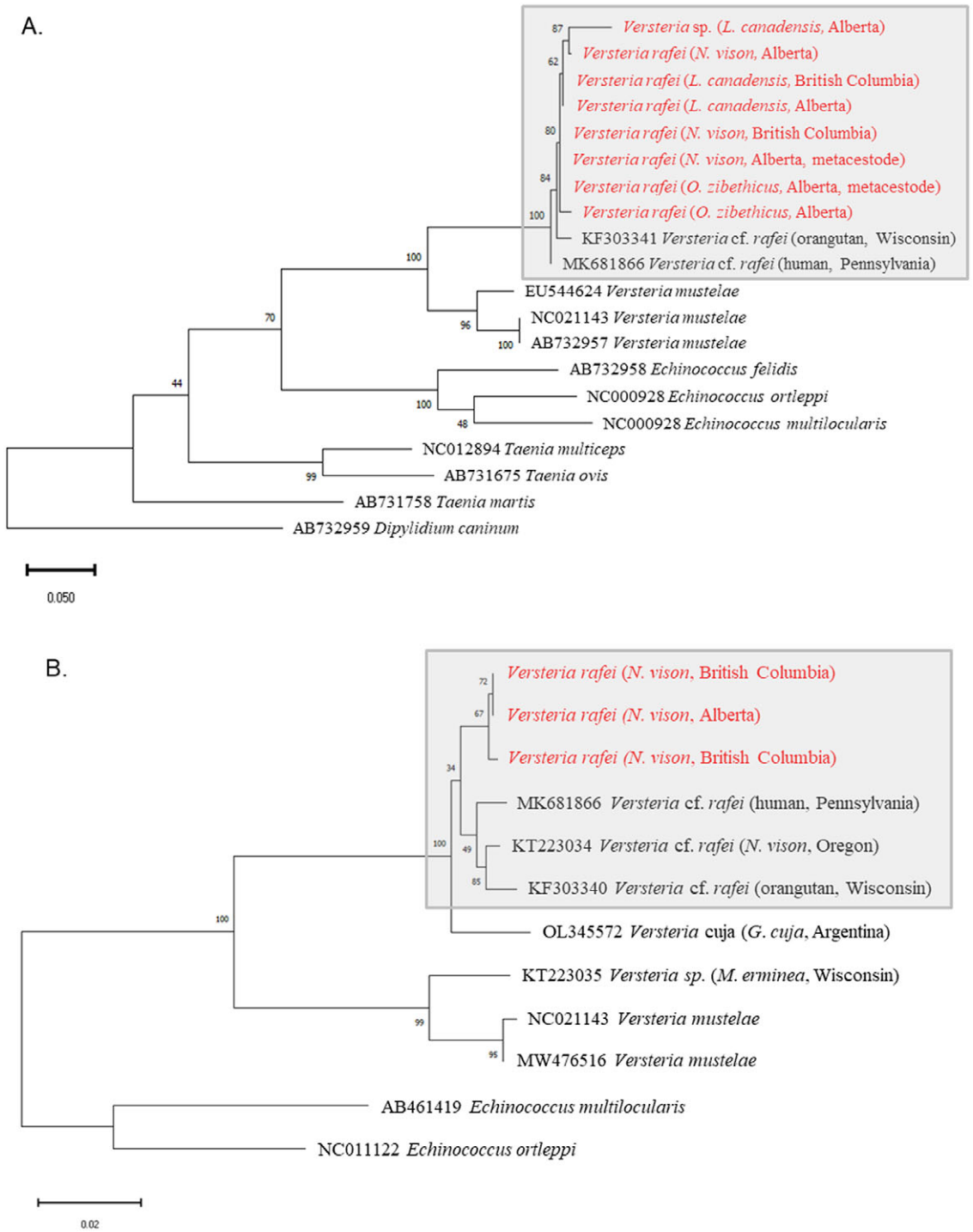

Figure 4. Phylogenetic trees of parasites described as *Versteria rafei n. sp.* Mitochondrial sequences from this study identified in red, including host species, and location of capture in brackets for the A) *nad1* and B) *cox1* mitochondrial regions. All sequences were from adult specimens unless noted as produced from metacestodes. Sequences are compared against sequences from GenBank, identified by ascension number before their name. Sequences identified in GenBank as *Versteria* sp. or *Versteria mustelae* that are likely examples of *V. rafei* are identified as *V. cf. rafei.* One sequence collected in this study was labelled as *Versteria* sp. due to increased evolutionary distance, which suggests it may be a unique species. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree.

were covered by a thin capsule, 0.75–4 mm in diameter, with round calcareous corpuscles and invaginated small scoleces, mostly unischolex, some with up to 4 scoleces. Scoleces contained two rows of short rostellar hooks of similar size and shape as described in adult specimens.

Molecular and phylogenetic analysis

Extraction, PCR, and sequencing produced 33 *nad1* sequences from adult specimens in mink (n_{AB}=20, n_{BC}=6), otters (n_{AB}=3, n_{BC}=2), and muskrat (n_{AB}=2), and 6 *nad1* sequences from

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Table 2. Intra- and interspecific pairwise distance among *Versteria* in the *nad1* gene. Sequences collected are identified in the left column, including their location (AB=Alberta, BC=British Columbia) and host species. All are adult specimens unless indicated as metacestodes, which were collected from the host liver. One sequence is identified as *Versteria* sp. due to high pairwise distance, which may imply it is a unique species. Sequences compared against all other sequences of *V. rafei* n. sp. collected in this study to provide an average intraspecific pairwise distance, then against sequences from GenBank. All GenBank ascension numbers are included. Analyses were conducted using the Tamura-Nei model (Tamura and Nei 1993). This analysis involved 13 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 451 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura et al. 2021)

	Intraspecific pairwise distance			Interspecific pairwise distance		
	Average	*V.* cf *rafei* KF303341	*V.* cf *rafei* MK681866	*V. mustelae* EU544626	*V. mustelae* NC021143	*V. mustelae* AB732957
V. rafei n. sp. (BC, *L. canadensis*)	0.009	0.016	0.009	0.164	0.164	0.164
V. rafei n. sp. (BC, *N. vison*)	0.009	0.014	0.007	0.161	0.161	0.161
V. rafei n. sp. (AB, *O. zibethicus*)	0.009	0.021	0.014	0.170	0.170	0.170
V. rafei n. sp. (AB, *L. canadensis*)	0.009	0.016	0.009	0.164	0.164	0.164
V. rafei n. sp. (AB, *N. vison*)	0.011	0.023	0.016	0.174	0.173	0.173
V. rafei n. sp., metacestode (AB, *O. zibethicus*)	0.013	0.014	0.007	0.161	0.161	0.161
V. rafei n. sp., metacestode (AB, *N. vison*)	0.013	0.014	0.007	0.161	0.161	0.161
Versteria sp. (AB, *L. canadensis*)	0.039	0.054	0.047	0.216	0.210	0.210

metacestodes in the livers of mink (n_{AB}=2) and muskrat (n_{AB}=4). A recent study described a new species, *V. cuja*, in South America using the *cox1* mitochondrial region, so we conducted a second analysis using the *cox1* region in order to compare against this new species. Three *cox1* sequences were also produced from adult specimens in mink (n_{AB}=1, n_{BC}=2). Phylogenetic analysis of available *nad1* and *cox1* sequences in GenBank confirmed our study specimens to be a sister lineage to larval infections previously identified and likely represent a separate species from *V. cuja*; 100% of bootstrapped trees clustering the rest of the North American examples separate from *V. cuja* while still making a distinct North American clade (Figure 4). One worm from a river otter in Alberta produced a *nad1* sequence unique from the other 28, with a pairwise distance of 0.046 from *V. rafei* n. sp., which is more than the distance reported between the newly described *Versteria cuja* and *Versteria* sp. in North America, and may represent another species (Bagnato *et al.* 2022). Unfortunately, only one fragmented specimen was found, so description was not possible. However, this further supports assumptions that *Versteria* is likely a species complex in North America (Lee *et al.* 2016; Bagnato *et al.* 2022).

Intra- and interspecific variation

Average intraspecific pairwise distance for *V. rafei* n. sp. was 0.007 for the *nad1* gene (n=33) and 0.002 for the *cox1* gene (n=3) amongst our samples. For the eight samples used in the phylogenetic analysis, examination of intraspecific pairwise distance against sequences from undescribed specimens in GenBank showed close similarities, except for the specimen identified as *Versteria* sp., which had 2–3 times the number of substitutions (Table 2). Interspecific pairwise distances between our samples and sequences from *V. mustelae* in Eurasia were 10 times that of the intraspecific distances (Table 2). Sequences from *cox1* gene were also very similar to sequences previously identified as *Versteria* sp. from North America, with an average pairwise distance of 0.016 (*cox1*) (KF303340, KT223034, MK681866). Our samples also had an average interspecific pairwise distance of 0.025 compared against *V. cuja* (OL345572) and 0.104 compared against examples of *V. mustelae* from Eurasia (MW476516, NC021143) and a specimen of *Versteria* sp. from an ermine in Wisconsin that is related to the European clade (KT223035) (Lee *et al.* 2016). This is similar to previous analysis by Bagnato *et al.* (2022), which calculated an intraspecific average distance of 0.011 among undescribed specimens in North America, compared to an interspecific difference of 0.024 with *V. cuja* and 0.093 with *V. mustelae.*

The authors of the description of *V. cuja* noted that due to lower genetic distances between their specimen and other North American samples identified as *Versteria* sp., the possibility of conspecificity should not be ignored (Bagnato *et al.* 2022). However, due to strong clustering and consistent mean pairwise distances between multiple samples from North America and the sequences from *V. cuja* in South America, and distinct morphological differences, mainly the presence of an armed cirrus in *V. rafei* n. sp., we are confident in asserting that they represent two distinct species in the American Clade.

Discussion

The species described herein belongs to the genus *Versteria* (Taeniidae), forming a clade with other specimens of *Versteria* in North America and *V. cuja* (Bagnato *et al.* 2022). This clade included adult specimens identified as *Versteria* sp. in an ermine (*M. erminea*) in Colorado and a mink (*N. vison*) in Oregon (Lee *et al.* 2016), and larval infections in muskrat (*O. zibethicus*) from

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Pennsylvania (Niedringhaus *et al.* 2022), an orangutan (*P. pygmaeus*) at the Milwaukee County Zoo (Goldberg *et al.* 2014), and humans in New Brunswick (Barkati *et al.* 2019) and Pennsylvania (Lehman *et al.* 2019). According to the results of our molecular analysis, it is likely all these infections were attributable to *Versteria rafei* n. sp. and should be considered as such.

Human alveolar and cystic echinococcosis as well as taeniid cysticercosis and coenuruses have historically been diseases of concern for both humans and wildlife. These pathologies may be caused by a variety of cestode species (Deplazes *et al.* 2019). Notoriously difficult to identify in their larval form, many cases are simply recorded as 'echinococcosis' or 'cysticercosis' without species-level identification. The genus *Versteria* was erected based on molecular characterisation of *Taenia*, which clustered with *Versteria mustelae* as a closer relative of *Echinococcus* than *Taenia* (Nakao *et al.* 2013). Using molecular approaches, recent reports have identified diseases similar to alveolar and cystic echinococcosis caused by an unknown species of *Versteria* in humans as well as an orangutan in a zoo in North America, suggesting it should be considered as a differential diagnosis for cases of 'echinococcosis' (Barkati *et al.* 2019; Deplazes *et al.* 2019). In one such case, a patient even tested positive for two different ELISA tests for *Echinococcus granulosus* antigens, though negative for a confirmatory western blot (Barkati *et al.* 2019), highlighting the potential for misidentification. However, this has only been recently discovered, and it is impossible to determine how many historical cases of 'echinococcosis' or 'cysticercosis' are due to *Versteria*, or how significant a threat it is to human communities.

Here, we document infections of adult *Versteria rafei* n. sp. in both river otter and mink from Alberta and British Columbia, as well as larval infections in the livers of mink and muskrat from Alberta. Our report is the first to document infection on a broad scale for river otter and mink, with infection intensities as high as 54 worms in a single individual host. The prevalence and intensity of infection as well as the presence of mature and gravid proglottids confirm both *L. canadensis* and *N. vison* as competent definitive hosts for the newly named species. Due to historical confusion over identification of *Versteria* species, it is hard to definitively identify the intermediate hosts of *Versteria rafei* n. sp. based on the literature, but previous reports of '*T. mustelae*' and '*T. tenuicolis*' identified multiple rodent species including beaver and muskrat in North America (Locker 1955; Freeman 1956). We found larval cysts of taeniid parasites in the livers of muskrats, which were confirmed as *Versteria rafei* n. sp. based on molecular characterisation. Sequences and morphology matched those of metacestodes previously identified in muskrat by Niedringhaus *et al.* (2022), confirming *O. zibethicus* as an intermediate host. Muskrats are prey items for both river otter and mink, though mink more often include terrestrial rodents in their diet (Larivière and Walton 1998; Larivière 1999). Infection prevalence and intensity were slightly higher in mink, which may be explained by this dietary difference. Surprisingly, we also observed adult specimens of *V. rafei* in the intestines of two muskrat, and larval infections in mink. Taeniids are known to cause both adult and larval infections in the same species, such as with *Taenia* in humans and *Echinococcus* in dogs (Ito 1997; Peregrine 2015), and *V. rafei* may exhibit similar plasticity in its hosts. Muskrat, while predominantly feeding on vegetation, are also scavengers that have been reported to participate in cannibalism in winter when food is scarce (Errington *et al.* 1963). However, adult infection in muskrats is likely a rare occurrence.

The pathway of infection by metacestodes in mink is unclear. It should be noted that all mink observed with larval infections were also concurrently infected by adults of *V. rafei*. In one case, a mink from Alberta was infected with 21 adults and over 50 cysts in its liver. While we cannot definitively state the reason for this, it may indicate that hosts can be autoinfected by cysticerci; autoinfection via reverse peristalsis of gravid proglottids into the stomach or release of eggs in the intestine has been documented in other taeniid species. *Versteria*'s closest relatives, *Echinococcus* and *Taenia*, have been shown to autoinfect their hosts, in both canids and humans (Ito 1997; Peregrine 2015). If humans are competent definitive hosts for adults of *V. rafei*, this may be a threat for first nation groups in Canada who hunt and eat muskrats and may thus be infected with adults and potentially autoinfected by cysticerci (Wein and Freeman 1995; McLachlan 2014). Infections may also be of concern for trappers handling wild mustelids, which could lead to contact with eggs and therefore infection by cysticerci.

Investigating the life history and prevalence of zoonotic pathogens in the wild is essential to prevent future infections. Infections of larval *Versteria rafei* n. sp. can be fatal in various hosts, including humans, captive animals, and wildlife (Lee *et al.* 2016; Barkati *et al.* 2019; Lehman *et al.* 2019; Niedringhaus *et al.* 2022). Considering infection presents the same as *Echinococcus* species in humans (Barkati *et al.* 2019), it is possible infections by *Versteria rafei* n. sp. are more prevalent than reported and currently diagnosed as echinococcosis. *Versteria rafei* n. sp. has also been reported to encyst in patients' brains (Lehman *et al.* 2019). Since histology is not an accurate guide to the identification of larval cestode infections, molecular characterisation is essential to understand infection pathways and factors that may predispose people to infection by *Versteria* and other taeniid species. Such an approach is also needed to accurately identify the prevalence of specific parasites that may present with the same pathology but are transmitted by completely different organisms (Niedringhaus *et al.* 2022).

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Author contribution. KMS designed the research, dissected hosts, identified the parasites, conducted the phylogenetic analysis, and wrote the manuscript. JB extracted and processed DNA samples. SG and CL funded and supervised the work. BP studied and identified the parasites, drew Figure 1, and advised as an expert taxonomist.

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