A new species of *Versteria* (Cestoda: Taeniidae) parasitizing *Galictis cuja* (Carnivora: Mustelidae) from Patagonia, Argentina: Morphological and molecular characterization

Estefanía Bagnato a, *, Carmen Gilardoni b, Gabriel Mario Martin a, c, María Celina Digiani d

a Laboratorio de Investigaciones en Evolución y Biodiversidad (LIEB), Facultad de Ciencias Naturales y Ciencias de la Salud, Universidad Nacional de la Patagonia San Juan Bosco (UNPSJB), Ruta Nacional N° 259, 16.8 Km, 9200, Esquel, Chubut, Argentina

b Laboratorio de Parasitología (LAPA), Instituto de Biología de Organismos Marines (IBOMAR)-Centro Científico Tecnológico, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Centro Nacional Patagónico (CENPAT), Boulevard Almirante Brown 2915, 9120, Puerto Madryn, Chubut, Argentina

c Centro de Estudios en Ciencias de la Tierra, Geociencias, Facultad de Ciencias Naturales y Ciencias de la Salud, Universidad Nacional de la Patagonia San Juan Bosco (UNPSJB), 9200, Esquel, Chubut, Argentina

d CONICET, División Zoología Invertebrados, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Paseo del Bosque s/n, 1900, La Plata, Buenos Aires, Argentina

**Abstract**

Via morphological and molecular analysis, we describe a new species of taenid from Patagonia (Argentina): *Versteria cuja* n. sp., parasitizing the Lesser grison *Galictis cuja* (Molina) (Carnivora: Mustelidae). This is the first report of a species of *Versteria* in Argentina and for a native mustelid. The new species (the third in the genus *Versteria*) is proposed using an integrative taxonomic approach, based on traditional morphology (distinctive and morphometric diagnostic characters), genetic distances and phylogeny based on molecular data, the distinct geographical distribution, and the different definitive host species. *Versteria cuja* n. sp. mainly differs from *Versteria mustelae* (Gmelin, 1790) (from Europe) in the number of testes (54–85 vs. 83–127 in *V. mustelae*), the rostellum size (39–75 vs. 85–180 μm in *V. mustelae*), the genital atrium size (170–420 vs. 68–91 μm in *V. mustelae*) and in the hooks’ shape. It also differs from the African species *Versteria brachyacantha* (Baer and Fain, 1951) by having smaller measurements regarding the main diagnostic characters, i.e. size of scolex, rostellum and suckers, number, size and shape of rostellar hooks, number of testes, and by having smooth cirrus (vs. cirrus covered with hair-like bristles in *V. brachyacantha*). Phylogenetical analysis using cox1 showed our specimens clustering with North American isolates of *Versteria* sp. in a well-supported American clade (mean genetic divergence 0.024), separated from another clade composed of different isolates of *V. mustelae* (0.093). The close relationship between the new species and the North American species, known as “zoonotic” *Versteria* sp., and responsible for fatal infections by metacestodes in free-ranging wildlife (rodents), captive primates and immunosuppressed people, lead us to think that the zoonotic potential of *Versteria cuja* n. sp. should not be disregarded.

**1. Introduction**

The family Taeniidae comprises four genera: *Echinococcus* Rudolphi, 1801, *Hydatigera* Lamarck, 1816, *Taenia* Linnaeus, 1758 and *Versteria* Nakao, Lavikainen, Iwaki, Haukisalmi, Konyaev, Oku, Okamoto and Ito (2013) (Nakao et al., 2013). The genus *Echinococcus* consists of nine species (Nakao et al., 2007), *Hydatigera* consists of four species, *Taenia* consists of nearly 44 species (Loos-Frank, 2000; Haukisalmi et al., 2011, 2016; Hoberg, 2006; Rossin et al., 2010; Wu et al., 2021), and *Versteria* consists of two nominal species: *Versteria mustelae* (Gmelin, 1790) (type species), and *Versteria brachyacantha* (Baer and Fain, 1951) (Nakao et al., 2013; Lavikainen et al., 2016). Nakao et al. (2013) proposed the genus *Versteria* to accommodate the two species formerly included in *Taenia: Taenia mustelae* Gmelin, 1790 and *Taenia brachyacantha* Baer and Fain (1951) (Nakao et al., 2013). Phylogenies inferred from both nuclear and mitochondrial protein-coding genes had demonstrated *T. mustelae*...
as sister to *Echinococcus*, and the species showing a basal position among members of *Taenia* in a phylogeny based on nuclear 18S rRNA (*Nakao et al., 2013*). A basal position of *T. multiceps* was also shown in a morphological phylogeny by *Hoberg et al.* (2000). Morphologically, *Versteria* was differentiated from *Taenia* mainly on morphometric characters: miniature rostellar hooks, small sizes of scolex, rostellum and suckers, short strobila and small number of testes (*Nakao et al., 2013*). The type host of *Versteria mustelae* is *Mustela nivalis* L. (Carnivora: Mustelidae) from Europe (type material of Rudolph (1819) (=*Taenia tenuicollis*) deposited in the Vienna Museum, according to *Freeman* (1956)). Subsequently, the species was reported parasitizing species of *Mustela L.*, *Martes P. in Neogale Gray Baryshnikov* and *Abramov* (Mustelidae) from Europe, Russia, Japan and North America, and *B. caryon gabbii* (Allen) (Procyonidae) from Brazil (*Hoberg et al., 1990; Iwaki et al., 1995; Loos-Frank, 2000; Magalhães Pinto et al., 2009; Lee et al., 2016*). *Versteria brachyacantha* was described from the musteled *Poeciloge albinucha* (Gray) in Africa (*Baer and Fain, 1951*) (type locality Butare, present Republic of Rwanda). This species was included in *Versteria* because it is morphologically very similar to *V. mustelae*, although molecular data are not available (*Nakao et al., 2013*). Up to now, species of *Versteria* are known to use mustelids and procyonids as definitive hosts and rodents as intermediate hosts (*Lavikainen, 2014; Niedringshaus et al., 2021*).

Recent studies suggested that in the Nearctic two *Versteria* species are found. One of them is a *Versteria* sp. reported from Wisconsin (host *Mustela erminea* L.) closely related with *V. mustelae*; and the other one is a “zoonoctic” lineage of *Versteria* sp. reported from *M. erminea, Neogale vison* (Schreber), a captive orangutan, and humans from Colorado, Oregon, Wisconsin (Zoo) and Pennsylvania (*Lee et al., 2016; Niedringshaus et al., 2021*). None of these North American species are morphologically described, although they are molecularly characterized through cytchrome c oxidase subunit 1 gene (cox1) (*Goldberg et al., 2014; Lee et al., 2016; Lehman et al., 2019*). Additionally, two cases of metacestodes infection in humans by a species of *Versteria* were reported in Atlantic Canada and Pennsylvania, identified through the mitochondrial 12S rRNA gene and 18 rRNA gene, without morphological description (*Barkati et al., 2019; Deplazes et al., 2019*). The taxonomic uncertainty of this group complicates the understanding of the natural history of the two *Versteria* species occurring in North America given that ancient reports of *V. mustelae* in both definitive and intermediate hosts in this region (*Skinker, 1935; Locker, 1955; Freeman, 1956; Verster, 1969*) could be any of these species (see Niedringshaus et al., 2021).

In South America, up to now, there was only one report of adult *Versteria*, in which *Magalhães Pinto et al.* (2009) reported *V. mustelae* parasitizing the Bushy-tailed olingo *B. gabbii* from Brazil. However, the morphological description was based only on one specimen without gravid proglottids, and there was no molecular characterization. In Patagonia Argentina, there are three species of native mustelids, among them the Lesser grison, *Galictis cuja* (Molina) which has a wide distribution range from southern Peru, western Bolivia, eastern and southern Brazil to Paraguay, Uruguay, central and southern Chile and southern Argentina, occupying a wide variety of habitats (*Lariviere and Jennings, 2009; Chebez et al., 2014*). For a summary of the helminths found in the Lesser grison throughout its distribution, see *Bagnato et al.* (2022).

In this paper we describe a new species of *Versteria*, based on adults from the small intestine of *Galictis cuja* from northwestern Patagonia, Chubut province, Argentina, for which we provide morphological, molecular and phylogenetic data.

2. Materials and methods

2.1. Study area and sample collection

Between December 2018 and December 2019, six specimens (four males and two females) of *Galictis cuja* (Geoffroy, 1831) were collected and transported to the laboratory. The collection sites were between Esquel and Trevelin cities [43° 2.5’S; 71° 27.06’W, (Ge1, Ge2); 42° 58.5’S; 71° 23.8’W (Ge3-Ge5); 42° 59.1’S; 71° 29.29’W (Ge6)], Chubut province, Argentina. Three specimens were necropsied fresh and three kept frozen (at –18 °C) until further examination. Cestodes were collected individually from the small intestine. Most specimens were fixed in 4% formalin/distilled water, and preserved and stored in 70% ethanol; some others were stored directly in absolute ethanol for molecular analysis. Specimens designated for morphological studies were stained with Semichon’s acetic carmine, Langeron’s carmine or Gömöri’s trichrome, dehydrated in a graduated ethanol series, cleared in eugenol and mounted in Canada balsam for examination under Leica DM500 (Leica, Wetzlar, Germany) light microscope. Drawings were made with the aid of a drawing attachment and photographs were taken with a Leica IICC50W camera with software connected to the microscope. The measurements are given in micrometers (μm) as range, followed by mean in parentheses. Type specimens of *Versteria cuja* n. sp. were deposited in the Helminthological Collection of the Museo de La Plata (MLP-He), La Plata, Argentina. Specimens of *Galictis cuja* were deposited in the Mammal Collection of the Laboratorio de Investigaciones en Evolución y Biodiversidad (LIEB-M), Esquel, Argentina.

2.2. DNA extraction, amplification and sequencing

Sequences were generated from a mitochondrial DNA region, cytochrome c oxidase subunit 1 gene (cox1) extracted from three samples of three proglottids each using the Wizard® Genomic DNA Purification Kit (A1120), according to the manufacturer’s instructions. The regions of mtDNA were amplified by the polymerase chain reaction (PCR) using previously published oligo-nucleotide primers (*Bowles et al., 1992; Bowles and McManus, 1993a; 1993b*). Their sequences are Forward: 5’T-AAAAAGAAAGAATAAGAGGTTTTAT-3′, Reverse: 5′-TAAGAAAGAGACACAATAGAAAAAT-3′. The PCRs were performed in a final volume of 50 μl containing 4 μl template, 1X Master Mix-PCR Pegasus (EA0401, Biological Products, Argentina), 10 μM of each primer and free-nuclease water. Negative controls for the PCR were always run to control for contamination. The PCR cycle program consisted of an initial denaturation step at 94 °C for 5 min followed by 40 cycles of 45 s at 94 °C, 40 °C, and 72 °C, and a final extension step at 72 °C for 5 min. The sizes of the amplification products were assessed by electrophoresis in 1% (w/v) Tris–borate/EDTA (TBE) agarose gels and ethidium bromide staining. Relevant bands were sent to purify and sequencing (Macrogen, Korea). The sequences have been deposited in GenBank. Sequences from the same genus and/or family that the species recorded in the present study were taken from GenBank and then compared using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.3. Sequence alignments and phylogenetic analysis

Sequences here obtained were compared with 17 cox1 sequences (including larval and strobilated stages) of *Versteria* occurring in carnivores, herbivores and/or rodents, representing 13 taxa from different geographical regions (*Table 1*). The concatenated alignments were performed using Multiple Alignment Fast Fourier Transform software (MAFFT software, available at http://www.ebi.ac.uk/Tools/msa/mafft/).

Phylogenetic molecular analyses were conducted on the aligned cox1 sequences and were inferred by both Maximum-Likelihood (ML) method using MEGA11 (*Tamura et al., 2021*) and by Bayesian Inference (BI) using Mr. Bayes program (v3.2.6, available at http://www.phylogeny.fr/one_task.cgi?task_type=mrbayes, Huelsenbeck and Ronquist, 2001; Duret et al., 2008, 2010*). The species *Echinococcus multilocularis* Leuckart, 1863 and *Echinococcus ortleppi* López-Neyra and Soler Planas, 1943 were used as outgroups. Regarding ML, to determine the nucleotide substitution model that gave the best fit to our data set, the MEGA11 software which held the JModel test analysis was employed, with model
selection based on the Akaike information criterion (AIC). Results indicated that the Hasegawa-Kishino-Yano model (HKY) was the most appropriate. The percentage of trees in which the associated taxa clustered together is shown above the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete gamma distribution was used to model evolutionary rate differences among sites (including rate variation across sites was fixed to 0.5). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site (below the branches). This analysis involved 18 nucleotide sequences, and the number of base changes was calculated using the p-distance method with a gamma distribution setting of 0.5. All ambiguous positions were removed for each sequence pair (pairwise deletion option). A total of 380 positions were used in the final dataset. All ambiguous positions were removed for each sequence pair (pairwise deletion option). A total of 380 positions were used in the final dataset.

### 3. Results

#### 3.1. Description of the new species

**Taeniid taxa included in the phylogenetic analysis with information on:** mammalian host, stage, locality, GenBank accession number (partial cytochrome oxidase subunit I, cox1, gene sequences), references and abbreviations used on phylogenetic tree. Data on *Versteria cuja* n. sp. in bold.

| Taeniid species | Mammalian host | Stage | Locality | GenBank Accession Number | References | Abbreviation on tree |
|-----------------|----------------|-------|----------|--------------------------|------------|----------------------|
| *Versteria*     |                |       |          |                          |            |                      |
| *Versteria cuja* n. sp. | *Gallicits cuja* (Molina) | adult | Argentina | OL345572 | This study | Versteria cuja n. sp., Arg, Ge3 |
| *Versteria sp.* | *Pongo pygmaeus* L. (Hominiidae) | metacestode | USA (ZooWISUS) | KF303340.1 | Goldberg et al. (2014) | Versteria sp., ZooWISUS, Pp |
| *Versteria sp.* | *Mustela erminea* L. (Mustelidae) | adult | USA (WIUS) | KT223035.1 | Lee et al. (2016) | Versteria sp., WIUS, Me |
| *Versteria sp.* | *Mustela erminea* | adult | USA (CoUS) | KT223033.1 | Lee et al. (2016) | Versteria sp., CoUS, Me |
| *Versteria sp.* | *Neogale vison* (Schreber) (Mustelidae) | adult | USA (OrUS) | KT223034.1 | Lee et al. (2016) | Versteria sp., OrUS, Nv |
| *Versteria sp.* | *Human* (Hominiidae) | metacestode | USA (PeUS) | MK681866.1 | Lehman et al. (2019) | Versteria sp., PeUS, Hu |
| *V. mustelae* (Gmelin, 1790) | *Mustela lutreola* (L.) (Mustelidae) | adult | Spain (Sp) | MH431789.1 | Fournier-Chambrillon et al. (2018) | Versteria mustelae, Sp, Mi |
| *V. mustelae* | *Myodes glareolus* Schreber (Cricetidae) (Mg) | metacestode | Finland (Fi) | EU544559.1 | Lavikainen et al. (2008) | Versteria mustelae, Fi, Mg |
| *V. mustelae* | *Myodes rufocanus* (Sundevall) (Cricetidae) (Mruf) | metacestode | Finland (Fi) | EU544566.1 | Lavikainen et al. (2008) | Versteria mustelae, Fi, Mruf |
| *V. mustelae* | *Myodes rutulus* (Pallas) (Cricetidae) (Mrut) | metacestode | Finland (Fi) | EU544567.1 | Lavikainen et al. (2008) | Versteria mustelae, Fi, Mrut |
| *V. mustelae* | *Myodes rufocanus* | metacestode | Russia (Ru) | EU544568.1 | Lavikainen et al. (2008) | Versteria mustelae, Ru, Mrut |
| *V. mustelae* | *Myops schisticolor* Lilljeborg (Cricetidae) (Ms) | metacestode | Russia (Ru) | EU544571.1 | Lavikainen et al. (2008) | Versteria mustelae, Ru, Ms |
| *V. mustelae* | *Mustela nivalis* L. (Mustelidae) (Mn) | adult | Japan (Ja) | AB732960.1 | Nakao et al. (2013) | Versteria mustelae, Ja, Mn |
| *V. mustelae* | *Eohippus baxteri* (Thomson) (Spalacidae) (Eb) | metacestode | China (Ch) | KC089934.1 | Zhao et al. (2014) | Versteria mustelae, Ch, Eb |
| *V. mustelae* | *Neogale vison* | adult | Poland (Po) | MW476516.1 | Klockiewicz et al. (2021) | Versteria mustelae, Po, Nb |
| *Echinococcus (outgroups)* | *Echinococcus* multilocularis Leuckart, 1863 | adult | USA (US) | AB461419 | Nakao et al. (2009) | Echinococcus multilocularis, US, Vu |
| *E. ortleppi* López-Neyra and Soler Planas, 1943 | *Cattle* (Ca) | hydatid cyst | Argentina | NC011122 | Nakao et al. (2007) | Echinococcus ortleppi, Arg, Ca |

Chain Monte Carlo (MCMC) chains were run for 10,000 generations, sampling every 10 generations, with the first 250 sampled trees discarded as “burn-in”. Finally, a 50% majority rule consensus tree was constructed.
Table 2
Comparison of the main morphometrical diagnostic characters of the two nominal species of *Versteria* against *Versteria cuja* n. sp. Measurements in micrometers. **Abbreviations:** DH, definitive host; IH, intermediate host; L, length; LH, large hooks; SH, small hooks; W, width.

| Versteria Species | Versteria cuja n. sp. | Versteria mustela (Gmelin, 1790) | Versteria brachyacantha (Baer and Fain, 1951) |
|-------------------|----------------------|----------------------------------|-----------------------------------------------|
| **Locality**      | Argentina            | USA                              | Republic of Rwanda                            |
| Scolex            | 276-345              | 237-303                          | 480                                           |
| Rostellum         | 39-75                | 61-77                            | 126                                           |
| Suckers           | 87-151               | 77-110                           | 176                                           |
| N° hooks          | 48                   | 48                               | 54                                            |
| Hooks size (LH, SH) | 12-17               | 12-17                            | 26-28                                         |
| N° testes         | 54-85                | 90-125                           | 100-145                                       |
| Cirrus-sac L      | 210-311              | 193-220                          | 240-280                                       |
| Cirrus-sac W      | 130-185              | 130-154                          | 120                                           |
| Cirrus unarmed, smooth | -                  | -                               | armed, hairlike bristles                      |
| Genital atrium size | 170-420             | -                                | -                                             |
| Uterus branched (12-28) | 10-19               | 10-19                            | 14-16                                         |
| IH                | Ctenomys sp. (Ctenomyidae), unpublished data | Rodents (various species of Cricetidae, Muridae, Spalacidae) Eulipotyphla (Talpidae) | Pocigalopale albinucha (Gray) (Mustelidae) |
| DH                | Galictis cuja (Molina) (Mustelidae) | Mustelids [Martes, Neogale, Mustela] Procyonids (Bassaricyon gabbii (Allen))] | -                                             |
Table 3
Intra- and interspecific pairwise divergence within genus Versteria in the complete sequences of mitochondrial cox1 gene. Abbreviations: AH, accidental host; IH, intermediate host; DH, definitive host.

| Versteria species | Geographical distribution | Mean intraspecific pairwise distance | Mean interspecific pairwise distance |
|-------------------|---------------------------|-------------------------------------|-------------------------------------|
| **Intraspecific**  |                           |                                     |                                     |
| *Versteria cuja* n. sp. (DH: mustelid) | South America (Argentina) | 0.000                               |                                     |
| *Versteria sp.* (AH: primates, human, DH: mustelid) | North America (Wisconsin Zoo, Colorado, Oregon, Pennsylvania) | 0.011                               |                                     |
| *Versteria mustelae* (Gmelin, 1790) (IH: rodents, DH: mustelids) | Between | 0.006                               |                                     |
| European isolates |                           |                                     |                                     |
| European vs. Japanese isolates | (Spain, Poland, Finland-Japan) | 0.035                               |                                     |
| European vs. Chinese isolates | (Spain, Poland, Finland-China) | 0.026                               |                                     |
| European vs. Siberian isolates | (Spain, Poland, Finland-Siberia) | 0.033                               |                                     |
| European vs. US isolates | (Spain, Poland, Finland-China) | 0.029                               |                                     |
| European vs. Chinese isolates | (Spain, Poland, Finland-China) | 0.026                               |                                     |
| Siberian vs. Japanese isolates | Siberia-Japan | 0.008                               |                                     |
| Siberian vs. US isolates | Siberia-Wisconsin | 0.040                               |                                     |
| Japanese vs. US isolates | Japan-Wisconsin | 0.032                               |                                     |
| Siberian vs. Chinese isolates | Siberia-China | 0.032                               |                                     |
| Japanese vs. Chinese isolates | Japan-China | 0.024                               |                                     |
| US vs. Chinese isolates | Wisconsin-China | 0.022                               |                                     |
| **Interspecific**  |                           |                                     |                                     |
| V. cuja n. sp. vs. V. cuja n. sp. |                                     | 0.024                               |                                     |
| V. cuja n. sp. vs. V. mustelae |                                     | 0.093                               |                                     |
| V. mustelae vs. V. mustelae |                                     | 0.096                               |                                     |

folded (Fig. 2A–C). Suckers 87–151 (109) in length (n = 26) by 73–126 (106) wide (n = 13) (Fig. 1A). Neck 768–1,958 (1,274) in length (n = 9) by 264–450 (358) maximum width (n = 10) (Fig. 1A). Prolongtids apolyptic and cespode. Immature prolongtids 113–733 (458) in length (n = 216) by 476–1,489 (916) wide (pw, prolongtids width) (n = 216) (Fig. 3A), length/width ratio 0.2–0.8 (0.5) (n = 216). Mature prolongtids 476–2,027 (1,230) in length (n = 171) by 1,117–1,941 (1,535) maximum width (n = 171) (Figs. 1B and 3B), length/width ratio 0.4–1.1 (0.8) (n = 171). Gravid prolongtids 1,474–3,437 (3,159) in length (n = 88) by 1,117–1,945 (1,681) maximum width (n = 87) (Fig. 1C), length/ width ratio 1.0–2.9 (1.9) (n = 87). Gravid prolongtids becoming more elongate posteriorly. Genital pores irregularly alternating (Fig. 3B), protruding, opening from anterior to roughly at middle of proglottid margin. Genital atrium subspherical, well-developed, 170–420 (315) deep (n = 202) (Fig. 1B and C, 3B-D). Longitudinal osmoregulatory canals 48 wide (n = 4); transverse connecting canals narrower, 45 wide (n = 4). Distance between longitudinal canals 477–1,237 (984) (n = 125) in immature prolongtids (Figs. 3A), 926–1,273 (1,080) (n = 33) in mature prolongtids, and 685–1,290 (1,055) (n = 27) in gravid prolongtids. Terminal genital ducts pass longitudinal osmoregulatory canals ventrally (Fig. 1B). Testes subpherical, relatively few, 54–85 (68) (n = 28) in number, mostly anterior to female organs; 25–54 (46) major diameter (n = 38) by 20–57 (43) minor diameter (n = 38) (Figs. 1B and 3B). Testicular fields confluent anteriorly, and situated between longitudinal osmoregulatory canals, from anterior proglottid margin to anterior margin of ovary, few in postero-poral field 12–36 (18) (n = 23). Relative length of testicular fields (length of the antero-poral field/length of the postero-poral field) 0.9–1.4 (1.1) (n = 26). Cirrus sac elliptical to sub spherical, 210–311 (259) transverse diameter (n = 81) (cl, cirrus sac length) by 130–185 (159) longitudinal diameter (n = 81) in mature prolongtids (Figs. 1B), 167–330 (261) transverse diameter (n = 22) by 159–200 (176) longitudinal diameter (n = 22) in gravid prolongtids, usually overlapping or extending across longitudinal ventral canal (Fig. 1C). Relative length of cirrus sac (cl/pw) 0.13–0.22 (0.17) (n = 100). Cirrus smooth, 417–1,014 (659) in length (n = 12) by 33–54 (42) wide (n = 12) (Figs. 1B and 3C). Deferent duct forming loops inside and outside cirrus sac, surrounded by prostatic cells (Figs. 1B, 3B–C, E). Ovary posteriori, median, bilobed, 327–458 (396) in length (n = 44) by 444–929 (634) total width (ow, ovary width) (n = 44). Poral and aporal lobes subequal in size (Figs. 1B and 3B). Relative ovary width on prolongtids width (ow/pw) 0.35–0.44 (0.40) (n = 44). Vitellarium posterior to ovary, transversely elongated, 135–194 (166) in length (n = 49) by 375–691 (544) wide (vw, vitellarium width) (n = 49) (Figs. 1B and 3B). Relative vitellarium with on prolongtids width (vw/pw) 0.30–0.42 (0.35) (n = 49). Relative vitellarium on ovary width on (vw/ow) 0.76–1.02 (0.89) (n = 47). Maximum size of seminal receptacle 135–152 (144) in length (n = 10) by 84–117 (100) wide (n = 10) (Figs. 1B and 3F). Ootype and Mehlis’ gland sub spherical, 78–191 (129) in length (n = 30) by 41–120 (88) wide (n = 29) (Fig. 1B). Vagina running posterior to cirrus sac and deferent duct, slightly widened and undulating. Vaginal sphincter absent. Vaginal pore posterior to male pore (Figs. 1B, 3B-D). Uterus appearing as median longitudinal stem; gravid uterus with several lateral branches, 12–28 (20) (n = 43) in number, arising regularly, each one with a secondary bifurcation (Fig. 1C). Eggs spherical or sub spherical, 25–31 (27) major diameter (n = 67) by 22–29 (25) minor diameter (n = 67), with embryophore or outer shell 1–4 (3) thick (n = 68) (Figs. 1C, 3G–H).

3.2. Taxonomic summary

**Type definitive host:** Galictis cuja (Molina) (Carnivora: Mustelidae).

**Type locality:** Between Esquel and Trevelin cities (43°2.504′S, 71°27.056′W), National Route N 259, Chubut province, Argentina.

**Site of infection:** Small intestine.

**Prevalence and intensity of infection:** Three out of six Lesser grisons infected with 1–13 specimens by host.

**Type specimens:** Syntypes, MLP-He 7771 (13 fragments, 3 with scolex, neck and immature prolongtids, 1 with immature prolongtids, 4 mature prolongtids and 5 gravid prolongtids); vouchers (7 fragments, 2 with mature prolongtids, 1 with mature and gravid prolongtids and 4 with gravid prolongtids), MLP-He 7772.

**Host specimens deposited:** LIEB-M-1626, LIEB-M-1787 and LIEB-M-1789, skull or skull’s parts and tissues (muscle and liver).

**Etymology:** The specific epithet refers to the specific epithet in the name of the type definitive host, Galictis cuja. *cuja* latinization of the host’s common name (“cuya”) in Chile and Peru, used in the original description of Mustela cuja Molina (see Mouchard, 2019).

GenBank accession numbers: OL345569, OL345573 and OL345572 (cox1, adults).

**ZooBank access number:** LSID: urn:lsid:zoobank.org:pub:3A82F343-FDD8-4205-9DE0-171F8A591FD6

3.3. Remarks

According to the diagnosis given by Nakao et al. (2013), *Versteria*
cuja n. sp. belongs to the genus *Versteria* by having a short strobila, immature and mature proglottids wider than long, gravid elongated proglottids; scolex, rostellum and suckers small, rostellum with two rows of very small hooks; genital pores alternating irregularly, opening roughly at the middle of the proglottid margin; terminal genital ducts passing longitudinal osmoregulatory canals ventrally; female glands posterior and median; ovary bilobed; vitellarium posterior to ovary, transversely elongated; vaginal sphincter absent; testes relatively few, mostly anterior to female organs; uterus appearing as median longitudinal stem; gravid uterus with several lateral branches (Nakao et al., 2013).

Comparing the specimens described herein against the two nominal species of *Versteria*, morphological- and morphometrically the three species do not differ substantially (see Table 2), although there are some differences, as described below will be described below. *Versteria mus
tela* was reported repeatedly from Europe, Asia and North America and, since its original description in 1790, there were numerous descriptions made by various authors (Thienemann, 1906; Skinker, 1935; Joyeux and
Concerning the African species _V. brachyacantha_, descriptions are much less numerous, i.e. the original description and a further redescription by Verster (1969). _Versteria cuja_ n. sp. differs from _V. brachyacantha_ by having smaller measurements regarding the main diagnostic characters, i.e. size of scolex, rostellum and suckers, number, size and shape of rostellar hooks, number of testes (Table 2, Fig. 2A–D), and by having a smooth cirrus (vs. covered with hair-like bristles in _V. brachyacantha_) (Bae and Pain, 1951). Regarding the hooks’ shape, the angle between blade and handle is more pronounced, and that between handle and guard is less pronounced in the rostellar hooks of _V. cuja_ n. sp. than in those of _V. brachyacantha_ (see Fig. 2D). Furthermore, the cirrus-sac is wider and rather subshperical in _V. cuja_ n. sp. with respect to the other two species (see Table 2).

### 3.4. Molecular and phylogenetic analysis

The PCR amplification of cox1, mtDNA from adult _Versteria cuja_ n. sp. gave three products of 373, 377 and 380 bp, respectively, of partial cox1 sequence. _Versteria cuja_ n. sp. sequences coincided in a 97.88% (369/378 bp) (MK6818866.1) with _Versteria_ sp. from Pennsylvania (human) and in a 97.85% (364/372 bp) (KT223034.1) with _Versteria_ sp. from Oregon (mink). Coxl tree topologies resulting from the ML and BI analysis were identical with BI producing higher branch support (Fig. 4).

The genus _Versteria_ formed a monophyletic group. The isolates obtained in this study appeared on the tree closely related with a group of species from USA: _Versteria_ sp. ZooWiUS (Wisconsin) from _Pongo pygmaeus_ L.,
Versteria sp. CoUS (Colorado) from Mustela erminea L., Versteria sp. OrUS (Oregon) from Neogale vison (Schreber), and Versteria sp. PeUS (Pennsylvania) from human, all of them forming a well-supported “American” clade (Table 3, Fig. 4) which is the sister group of another clade mainly composed of V. mustelae. This latter large clade is composed in turn of a “European” clade and an “Asian” clade, plus Versteria sp. from Wisconsin (USA) and V. mustelae from China (Fig. 4). According to the genetic distances (Table 3) obtained in this study, this large clade of V. mustelae could represent a species complex, well separated from the “American” clade. The pairwise distances of V. cuja n. sp. compared against the North American species were: 0.022 with Versteria sp. OrUS, 0.024 with Versteria sp. ZooWiUS and Versteria sp. CoUS, and 0.026 with Versteria sp. PeUS (the “zoonotic” Versteria sp.). Whereas the pairwise distance with Versteria sp. WiUS from Mustela erminea was 0.097, and this latter clustered with V. mustelae from Europe and Asia (Table 3, Fig. 4).

3.5. Intra- and interspecific variation between Versteria species

Pairwise divergence values of cox1 gene were utilized to numerically evaluate intra- and interspecific variation in Versteria species. One isolate of V. cuja n. sp. from South America; four isolates of Versteria sp. from North America and 11 isolates of V. mustelae from the Holarctic Region (Lehman et al., 2019b) originating from different localities were used for this evaluation. As shown in Table 3, the mean intraspecific divergence values of the complete cox1 sequences between the Holarctic isolates of V. mustelae ranged from 0.006 (between European isolates) to 0.040 (between Siberian and US isolates). Mean value between North American isolates of Versteria sp. was 0.011. Regarding the mean interspecific divergence values, they were as follows: between V. cuja n. sp. and Versteria sp. 0.024, between V. cuja n. sp. and V. mustelae 0.093 and between Versteria sp. and V. mustelae 0.096 (Table 3).

4. Discussion

The specimens discussed herein belong to the genus Versteria within the family Taeniidae and differed molecularly from V. mustelae (mean pairwise distance 0.093), integrating a separate clade from the latter. The genus Versteria is reported for the first time in Argentina and in a native mustelid. Our specimens clustered (mean genetic divergence, 0.024, Fig. 4) with a group of four North American Versteria sp. in a well-supported “American” clade. Of these four species, two (OrUS and CoUS) are strobilate forms which had the mink, respectively, the mink Neogale vison from Oregon and the ermine M. erminea from Colorado as definitive hosts, respectively (Fig. 4). The other two (ZooWiUS and
PeUS) correspond to metacestodes of Versteria sp. which were responsible for a fatal, respectively, for a fatal infection in a captive juvenile Bornean orangutan (born in captivity in the USA) (Goldberg et al., 2014), and for four cases of cysticercosis in immunosuppressed human patients (see Deplazes et al., 2019a; Lehman et al., 2019b).

The relatively low genetic distances between our specimens and the above-mentioned Versteria sp. suggest that their conspecificity should not be ruled out. However, it is a challenge to delimit species only on a molecular basis and without the help of the morphology, since there is no description of any of these Versteria sp. from USA. In proposing the new species, additionally to the phylogenetic molecular analysis, we also evaluated the distinctive morphological and morphometric characteristics described above, the Lesser grison Galictis cuja as a new definitive host, and the distinct geographical distribution with respect to the nominal species V. mustelae and V. brachyacantha. Concerning the V. mustelae clade, it probably constitutes a species complex, since within this clade the intraspecific variation ranged from 0.006 to 0.040 between the different isolates and different lineages could be differentiated (Europe, Siberia/Japan, US and China) (Table 3, Fig. 4). Regarding the species reported as V. mustelae from Brazil (Magalhães Pinto et al., 2009), it probably deserves revision, including further morphological studies based on more specimens (the report was based on a single immature individual), and a molecular characterization.

Recently, Niedringhaus et al. (2021) reported a fatal infection by cysticerci of Versteria sp. in a muskrat Ondatra zibethicus (L.) in Pennsylvania, USA; remarkably, in the same locality where a case of human cysticercosis was reported (Lehman et al., 2019b). The sequence (cox1) obtained from these cysticerci coincided in a 99% with those of Versteria sp. from the mink from Oregon and the ermine from Colorado. Moreover, it was integrated into the clade which also included Versteria sp. from the orangutan and from humans (Niedringhaus et al., 2021). Unfortunately, the sequence of Versteria sp. from O. zibethicus could not be incorporated into our analysis since there is no GenBank accession number in the article by Niedringhaus et al. (2021), and we did not find it in the database. The work by Niedringhaus et al. (2021) points out O. zibethicus at least as one of the natural intermediate hosts of this species. Regarding the South American species, cysticerci obtained from the liver of Ctenomys sp. (Ctenomysidae) showed sequences of cox1 identical to that of the adult of Versteria cuja n. sp (Bagnato, unpublished data).

Interestingly, though there in the Palearctic, though there are some reports of hyperinfestation by V. mustelae in definitive hosts, in the Palearctic (Fournier-Chambillon et al., 2018), there are no reports of disseminated fatal cases of V. mustelae in intermediate hosts, either natural (rodents) or other accidental hosts such as humans or other primates (Niedringhaus et al., 2021).

The identification of the Neartic taxa reported until now as Versteria sp., as well as new data on its life-cycle become critical in view of its potential for zoonotic disease. Indeed, the few cases of metacestodes reported from humans are suggesting a potentially emergent zoonosis caused by accidental ingestion of the eggs of these tapeworms (Deplazes et al., 2019; Lehman et al., 2019b).

The close relationship between the new South American species and the lineage known as “zoonotic” Versteria sp., responsible for fatal infections by metacestodes in free-ranging wildlife (rodents), captive primates and immunosuppressed people, lead us to think that the zoonotic potential of Versteria cuja n. sp. should not be discarded.

Author contributions

EB conceived and designed research, analyzed host samples, studied and identified the parasites, wrote the manuscript. CG performed the phylogenetic analysis. GMM contributed with supplies and reagents. MCD studied and identified the parasites, supervised the work. All authors read and approved the manuscript.

Note

Nucleotide sequence data reported in this paper are available in the GenBankTM database under the accession numbers: OL345569, OL345573, OL345572 (cox1).

Declaration of competing interest

None.

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