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VERSITA

A new genus of the family Hymenolepididae (Cestoda) from *Sephanoides sephaniodes* (Apodiformes, Trochilidae) in Northern Patagonia (Chile)

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Abstract

A new species of hymenolepidid cestodes from *Sephanoides sephaniodes* (Trochilidae) found in Chile is described. The most characteristic features of *Colibrilepis pusilla* gen. nov., sp. nov. are the lack of rostellum, a cirrus sac with a thick-walled distal end (separated by a constriction) and protruding into genital atrium, a thick-walled saccular uterus filling entire median field of the gravid proglottis and the small number of eggs containing thick walled embryophores with polar swellings. *Staphylepis* is the most similar genus but differs in its apical structure because of the presence of a rudimentary rostellum. Moreover, molecular phylogenetic analyses show that *Staphylepis* and *Colibrilepis* are not sister taxa.

Keywords

Hymenolepididae, Hymenolepidinae, Cestoda, *Sephanoides sephaniodes*, Chile

Introduction

In the frame of a large-scale survey of tapeworms biodiversity worldwide, a fieldtrip aimed at inventorying the diversity of avian parasites was conducted at the Huinay Scientific Field Station (HSFS) in November-December 2008. During this expedition, we have discovered a new rostellum-lacking hymenolepidid cestode in a Green-backed Firecrown, *Sephanoides sephaniodes* (Lesson, 1827), a bird of the family Trochilidae (Apodiformes). We found 13 cestode specimens that could be used in this description.

Materials and Methods

Birds were caught with mist nets, kept in ornithological cotton bags, quickly euthanized with chloroform and dissected immediately after their death. The gastrointestinal tracts were removed through an opening in the abdomen and examined under a stereomicroscope. Cestodes were relaxed a few minutes in water and some proglottides were preserved in 95% ethanol for molecular analysis, the rest were fixed in hot 4% formalin and preserved in 75% ethanol for identification.

Samples for morphological identification were stained with alcoholic hydrochloric carmine solution and mounted on slides in Canada balsam following de Chambrier *et al*. (2009). Specimens were studied by light microscopy, including Nomarski's differential interference contrast.

We studied also comparative materials of other similar hymenolepidid genera. These included *Amazilolepis trinidadensis* Schmidt et Dailey, 1992, 2 paratypes, US National Parasite Collection (USNPC 818589), and *Staphylepis ambilateralis* Mariaux et Vaucher, 1991, holotype and paratypes (MHNG-PLAT-15997, 15998, 15999).

The phylogenetic relationship of our new material with 6 other hymenolepidid taxa was studied on the basis of their partial 28S (lsrDNA) and nad1 nucleotide sequences. A parasite of mammals, *Hymenolepis diminuta* (Rudolphi, 1819), was used as outgroup. The dataset comprised a specimen of *Staphylepis* Spasskii and Oshmarin, 1954, the closest genus to *Colibrilepis*, as well as 4 samples of Hymenolepidinae from Passeriformes belonging to *Passerilepis* Spasskii et Spas-

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skaya, 1954 and *Variolepis* Spasskii and Spasskaya, 1954 (Table I). All samples for molecular studies came from the Natural History Museum of Geneva (MHNG), except *H. diminuta* used as outgroup.

DNA was extracted with PeqLab peqGOLD Tissue DNA Mini Kit (v.07.07) following the manufacturer's instructions. PCR amplification were performed on part of the 28S rDNA gene (lsrDNA) using primers of Hillis and Dixon (1991) and Barker *et al*. (1993) as modified in Zehnder and Mariaux (1999) (28s1: TACCCGCTGAACTTAAGCATAT; 28s2: CTCCTTGGTCCGTGTTTCAAGAC), and on complete nad1 using original Cyclo_trnNR and modified Cyclo_nad1F primer of Littlewood *et al*. (2008) (Cyclo_trnNR: TTCTTGAAGTTAACAGCATCA; Cyclo_nad1F: GGTTATTGTCAGTATCG TAAGGG). The PCR was performed with PeqLab peqGOLD *Taq*-DNA-Polymerase « all inclusive » (v.01.07) using samples containing 37.7 µL of ddH_2O water, 5 µL of PCR buffer Y, 2 µL of $MgCl_2$, 1 µL of dNTP, 1 µL of each primer, 0.3 µL of Taq Polymerase and 5 µL of DNA.

The PCR programme for lsrDNA and nad1 consisted of 45 cycles of 40 sec at 93°C, 40 sec at 58°C and 60 sec at 72°C and 40 cycles of 35 sec at 94°C, 35 sec at 41°C and 90 sec at 72°C, respectively. For both genes, a 3 minutes 94°C initial denaturation and a 7 min 72°C final extension were added. DNA was purified with Roche High Pure PCR Product Purification Kit following the manufacturer's instructions. PCR products were sequenced by Macrogen Korea. New DNA sequences have been submitted to GenBank with accession number JQ950687-93 (lsrDNA) and JQ950694-700 (nad1) (Table I).

Sequences were aligned using CLUSTALX v2.0 (Larkin *et al*. 2007) and the alignments were manually refined using EPOS (v0.9) (Griebel *et al*. 2008). A partition homogeneity test (incongruence length difference) (Farris *et al*. 1995) implemented in PAUP* (v.4.0) (Swofford 2003) was done to justify multigene-based analyses. Phylogenetic analyses were carried using three different dataset: lsrDNA, nad1 and concatenated sequences of the two genes considered as 2 different partitions. The model selected for Maximum Likelihood and Bayesian inference was HKY85 with rate heterogeneity without invariant sites for lsrDNA alignment and GTR with rate heterogeneity without invariant sites for nad1 alignment, following the Akaike information criterion implemented in METAPIGA (v2.1.3) (Helears and Milinkovitch 2010). Maximum Likelihood analysis was performed using METAPIGA with consensus pruning metaGA algorithm (Lemmon and Milinkovitch 2002) with 4 populations. Analysis stopped after 200 steps without significant improvement of 0.01%. Others parameters were by default. Bayesian inference analysis were performed using MRBAYES (v.3) (Ronquist and Huelsenbeck 2003). Settings were set to nst=6, rates=gamma, ngammacat=4. 5'000'000 generations were run by 4 chains in 2 runs. Burnin was set to 25%.

Results

***Colibrilepis* gen. nov.**

Diagnosis: Hymenolepididae, Hymenolepidinae. Scolex rounded, not clearly differentiated from neck, with inconspicuous and small suckers. No rostellum or other apical structure. Suckers unarmed, weak. Longitudinal muscular bundles in strobila multiple. Proglottides with slightly-expressed (sometimes indiscernible) velum. Ventral osmoregulatory canals with transverse anastomoses along posterior margin of each proglottis. Genital pores unilateral. Genital atrium simple. Genital ducts pass dorsally to osmoregulatory canals. Testes three, in triangle, one poral, one antero-central (sometimes antero-antiporal) and one postero-antiporal. Cirrus sac elongate, crossing poral osmoregulatory canals, with distal end protruding into genital atrium. Ovary reniform, slightly lobate. Vitellarium compact, oval, postero-antiporal to ovary. Seminal receptacle globular, adjacent to or overlapping poral osmoregulatory canals. Young uterus round or oval, saccular, when fully-developed becoming

Table I. List of species, hosts, location and GenBank accession numbers

Species	Hosts	Location	GPS Coordinates DDM (alt)	lsrDNA	nad1
Hymenolepis diminuta	*Rattus norvegicus* (Rodentia)	(artificially infected)		AY157181.1	NC_002767.1
Passerilepis sp. (1)	*Bleda syndactylus* (Pycnonotidae –Passeriformes)	Djoumou, Gabon	S 1°41.37′E 13° 39.93′ (10m)	JQ950690	JQ950697
Passerilepis sp. (2)	*Stachyris nigriceps* (Timaliidae – Passeriformes)	Gombak, Malaysia	N 3°19.075′E 101° 45.895′ (350m)	JQ950693	JQ950700
Passerilepis passeris (1)	*Pycnonotus leucotis* (Pycnonotidae – Passeriformes)	Minab, Iran	N 27°8.04′E 57° 4.32′ (30m)	JQ950692	JQ950699
Passerilepis passeris (2)	*Neolestes torquatus* (Pycnonotidae, Passeriformes)	Djoumou, Gabon	S 1°41.37′E 13° 39.93′ (10m)	JQ950691	JQ950698
Staphylepis ambilateralis	*Cinnyris minullus* (Nectariniidae –Passeriformes)	Nouamou, Ivory Coast	N 5°11.12′W 2° 53.99′ (20m)	JQ950688	JQ950695
Variolepis farciminosa	*Turdus pelios* (Turdidae – Passeriformes)	CIRMF, Gabon	S 01°37.00′E 13° 34.93′ (400m)	JQ950689	JQ950696
Colibrilepis pusilla	*Sephanoides sephaniodes* (Trochilidae – Apodiformes)	Los Lagos, Chili	S 42°22.79′ – W 72° 24.91′(10m)	JQ950687	JQ950694

almost spherical, thick-walled, filling entire median field; with small number of eggs. Eggs with thick embryophore with polar swellings. Parasite of Apodiformes (Trochilidae). Type-species: *Colibrilepis pusilla* sp. nov.

Etymology: The genus name is derived from the French vernacular name of the host (Colibri). It is of feminine grammatical gender.

***Colibrilepis pusilla* sp. nov.**

Type-host: *Sephanoides sephaniodes* (Picaflor chico, Green-backed Firecrown), (Apodiformes, Trochilidae).

Site: small intestine.

Prevalence: 1/8 (12.5%).

Intensity: 13 specimens.

Type-locality: Chile, Los Lagos Province, Comau Fjord, Huinay Research Field Station, near powerplant. S 42°22.79 W 72°24.91. Altitude 10 m, 6 December 2008.

Type-specimen: Holotype and paratypes: MHNG-PLAT-79504, Paratypes: MHNG-PLAT-79505; deposited at the Museum of Natural History of Geneva, Department of Invertebrates.

Etymology: The species name is from the Latin "*pusillus*" = "very weak, insignificant", which refers to the structures of the scolex.

Description (Figs 1–6)

Hymenolepididae, Hymenolepidinae. Worm of medium size. Proglottides weakly craspedote (Figs 3, 5, 6), always broader

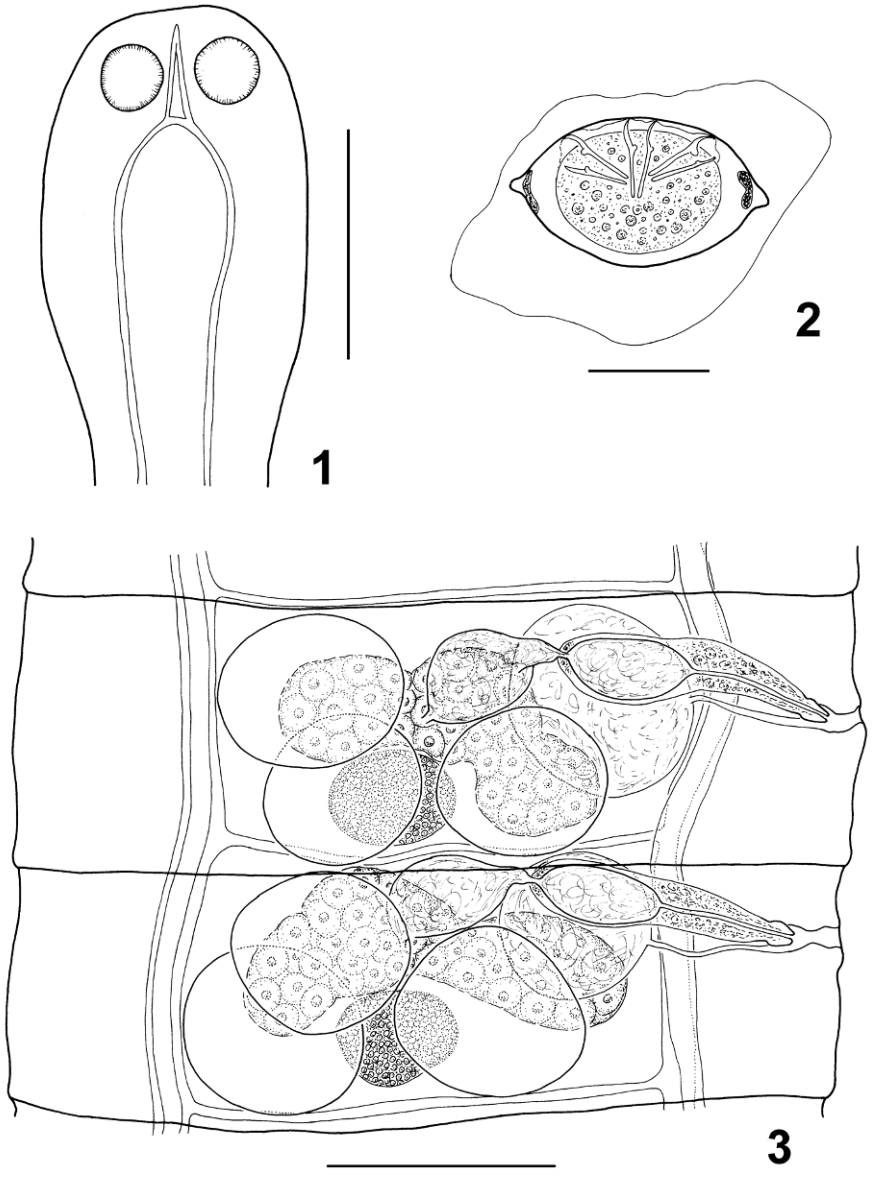

Fig. 1–3. *Colibrilepis pusilla* n. gen., n. sp. 1. Scolex. **2.** Egg. **3.** Hermaphroditic mature proglottides; note variation in position of testes. Scale bars: 1, 200 µm; 2, 20 µm; 3, 100 µm

than long (from about 4 times in premature to less than twice in gravid). Longitudinal muscular bundles of strobila multiple. Scolex anteriorly rounded, not clearly separated from neck (Fig. 1). Suckers anterior, inconspicuous, small, circular and unarmed, with weakly developed musculature. No rostellum or other apical structure, including glandular ones. Neck long. Ventral osmoregulatory canals with transverse anastomosis along posterior margin of each proglottis. Dorsal osmoregulatory canals without anastomoses. Strobilar development protandrous. Genital pores unilateral, dextral, opening in anterior half of proglottis margin. Genital atrium simple, tubular (Fig. 4); no atrial sphincter but slightly denser cells around atrium. Genital ducts pass dorsally to osmoregulatory canals.

Testes three, rounded, dorsal to female gonads; in triangle, one poral, one antero-central (sometimes antero-antiporal) and one postero-antiporal (Fig. 3). External seminal vesicle rounded, close to anterior proglottis margin, anterior and dorsal to female gonads (Figs 3, 4). Internal seminal vesicle fills up to half of cirrus sac. Cirrus sac elongate-oval, thin-walled, crossing poral osmoregulatory canals, almost half of it extending into median field; with larger, intensely stained cells in its central part distal to the internal seminal vesicle; distal part of cirrus sac thick-walled, separated by constriction, protruded into genital atrium. Cirrus unarmed.

Ovary reniform, slightly lobate, ventral to testes. Vitellarium compact, oval or with slightly-irregular shape, slightly antiporal, posterior to ovary (Fig. 3). Seminal receptacle round, reach up to the size of a testis, clearly distinguished from vagina, ventral to cirrus sac, adjacent or overlapping poral osmoregulatory canals. Vagina tubular, posterior to cir-

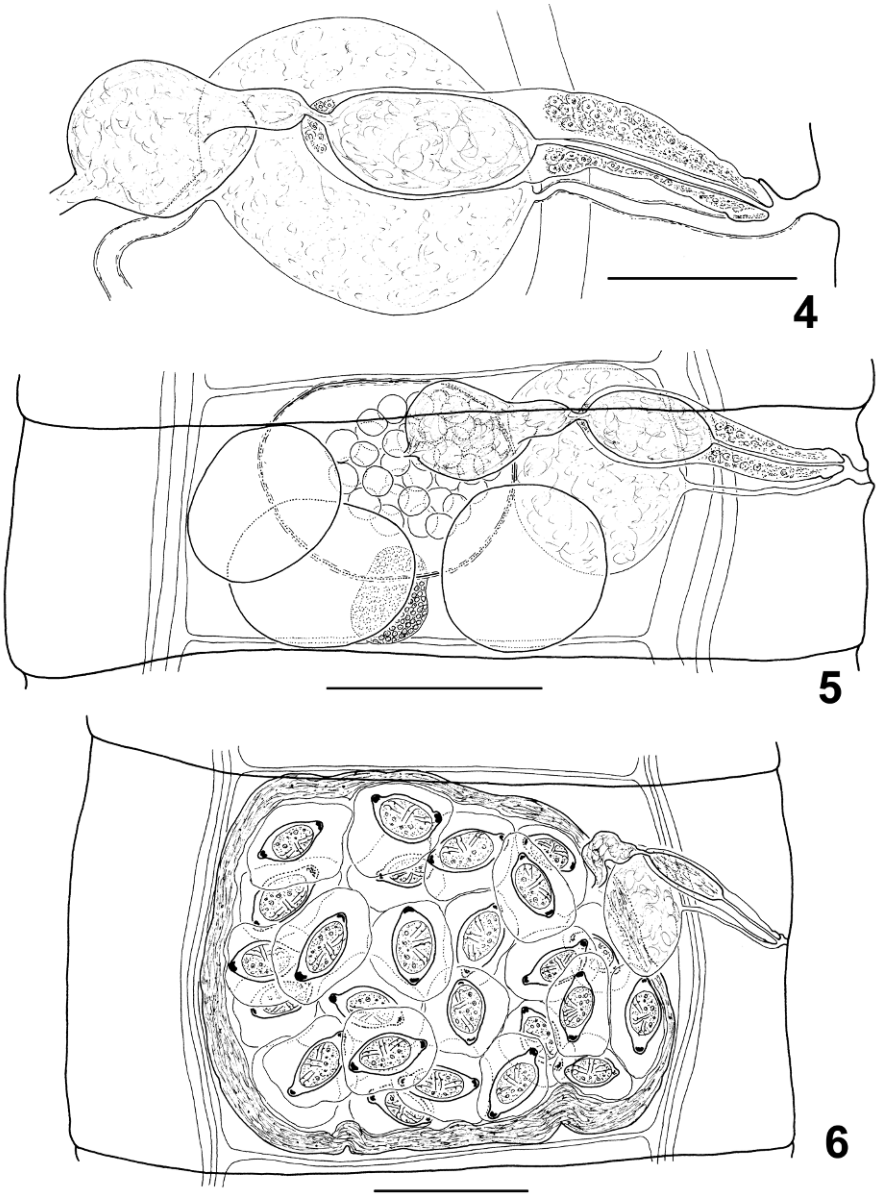

Fig. 4–6. *Colibrilepis pusilla* n. gen., n. sp. **4.** Genital ducts. **5.** Postmature proglottis. **6.** Gravid proglottis. Scale bars: 4, 50 µm; 5, 6, 100 µm

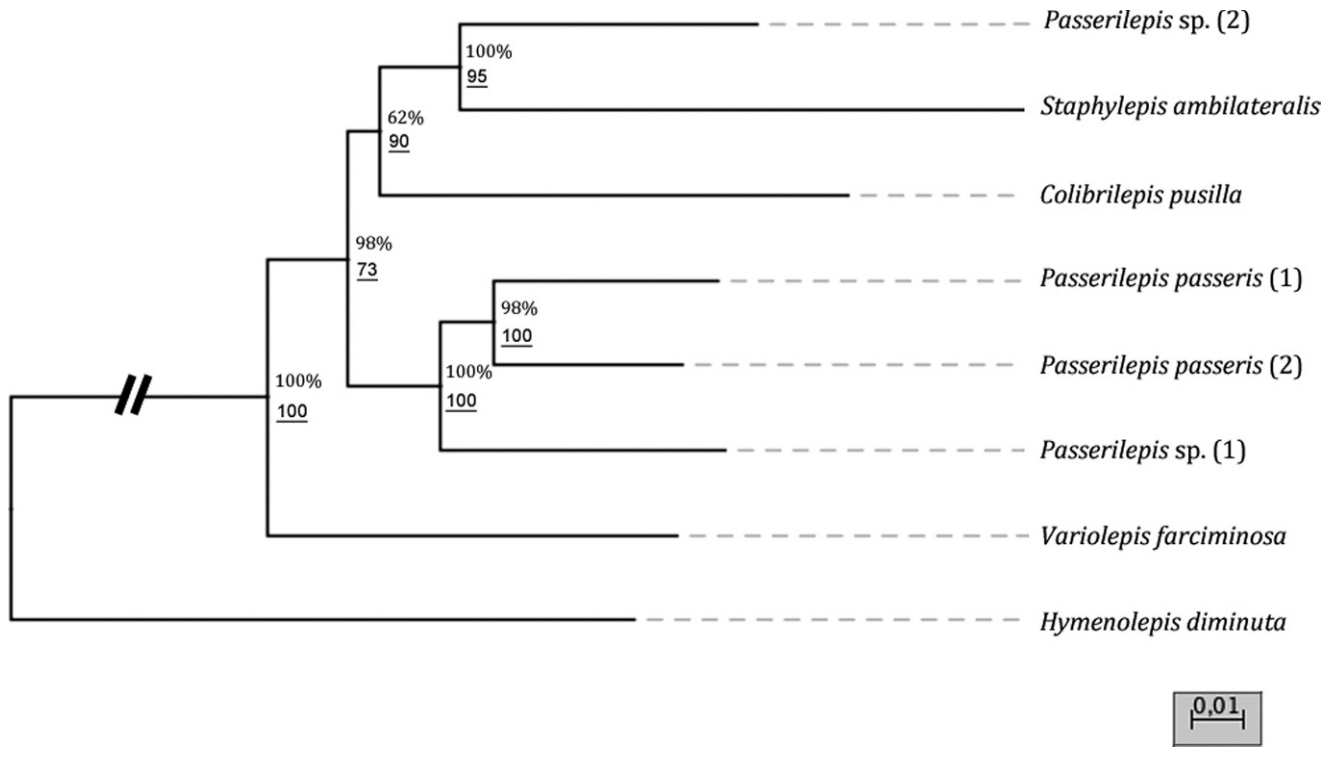

Fig. 7. Phylogenetic analyses for combined gene partitions (lsrDNA + nad1). ML analysis. BI posterior probabilities underlined and bootstrap values of maximum likelihood test are given as percentage

rus sac, unarmed (Fig. 4). Young uterus saccular, round to oval (Fig. 5), fills half of proglottis; gravid uterus, thick-walled, fills entire median field (Fig. 6), with small number of eggs (~50). Eggs with thin irregular outer shell (observed in whole-mounts only); embryophore thick, lemon-shaped, with polar prolongations; oncosphere elliptical (Fig. 2).

Dimensions (in micrometers if not specified otherwise): Total length of gravid specimens: 46–59 mm (50.6, n = 10); max. width of strobila: 405–525 (458, n = 10); number of proglottides in gravid specimens: 405–525 (458, n = 10); scolex: 143–272 (229, n = 9); suckers, diameter: 51–71 (59, n = 26); dorsal canals, diameter (max.): 11 (n = 10); ventral canals, diameter (max.): 28 (n = 10); testes, diameter: 60–80 (70, n = 60); cirrus sac: 102–135 x 22–32 (118 x 26, n = 55); external seminal vesicle, diameter: 21–49 (38, n = 60); internal seminal vesicle: 38–66 x 21–32 (53 x 25, n = 60); vitellarium, diameter: 27–60 (48, n = 60); seminal receptacle, diameter: 49–67 (51, n = 60); embryophores, diameter: 26–48 (36, n = 55); oncospheres, diameter: 17–32 (26, n = 55); embryonic hooks, length: 14–17 (15.5, n = 30).

Molecular analysis

LsrDNA fragments were 1752 to 1881 base pairs (bp) long and nad1 fragments were 751 to 771 bp. LsrDNA alignment was 1893 bp and nad1 alignment was 787 bp. The congruence of these partitions was supported by the ILD test (P = 0.36).

Trees based on multigene and lsrDNA alignments have the same topology (Fig. 7). *Colibrilepis* shares an ancestor with the group formed by *Passerilepis* sp. (2) and *Staphylepis* (bootstrap support (BS) 62%, posterior probability (PP) 90, based on multigene analysis). *Passerilepis* sp. (1) and the two *P. passeris* form a group very well supported (BS 100%, PP 100). These 2 clades are sister-groups and *Variolepis* is basal to them. The topology of the nad1 tree is similar except for the position of *Colibrilepis* who is basal to the *Passerilepis-Staphylepis* clade (tree not shown).

Discussion

Unarmed hymenolepidids have been described in a number of bird families, including Trochilidae (Rietschel 1934, Schmidt and Dailey 1992) and have been attributed to various genera (Czaplinski and Vaucher 1994, Schmidt 1986). In their revision of Hymenolepididae, Czaplinski and Vaucher (1994) chose to treat the parasites of birds and mammals separately, and, in the key dealing with bird parasites, Czaplinski lumped all unarmed genera within the genus *Hymenolepis* Weinland, 1858. As a consequence, the definition of the genus *Hymenolepis* became different for the two groups of hosts, and very peculiar taxa (e.g. *Woodlandia* Yamaguti, 1959 or *Arhynchotaeniella* Schmidt, 1986) were put in synonymy with *Hymenolepis* despite obvious morphological, geographical or host differences. Here, we follow

Schmidt (1986) and consider *Hymenolepis* to be restricted to mammal hosts.

Among the avian hymenolepidids possessing three testes per proglottis, there are seven genera characterized by the absence of rostellum or by the presence of unarmed rudimentary rostellum (Schmidt 1986, Schmidt and Dailey 1992); these are *Amphipetrovia* Spasskii et Spasskaja, 1954, in West Africa, *Arhynchotaeniella* Schmidt, 1986 in Ukraine and *Cloacotaenia* Wolffhügel, 1938, all the three from Anatidae; *Woodlandia* Yamaguti, 1959, found in cormorants in Asia; *Schmelzia* Yamaguti, 1959 from sandgrouses in the Old-World tropics; *Amazilolepis* Schmidt et Dailey, 1992, found in Trochilidae in Trinidad; and *Staphylepis* Spasskii et Oschmarin, 1954, found in Galliformes, Columbiformes, Anseriformes, Apodiformes and Passeriformes all over the world, including in Trochilidae and in their African Nectariniidae vicariants (see Mariaux and Vaucher 1991).

Most of these taxa can be easily distinguished from the present material. Besides their different hosts, *Amphipetrovia* has testes in a transverse row entirely antiporal to ovary; *Arhynchotaeniella* has a sclerotized vaginal clamp, a large scolex, no neck and an armed cirrus; *Cloacotaenia* has a massive globular scolex with a distinct apical glandular organ; *Woodlandia* has testes external to osmoregulatory canals; *Schmelzia* is characterized by the presence of a rudimentary rostellum surrounded by glandular cells.

Members of the genera *Amazilolepis* and *Staphylepis* are more similar to the present material and are known from Trochilidae. However, *Amazilolepis* has proglottides with strongly-developed velum, testes in a transverse row, a vagina that opens in the genital atrium anteriorly to cirrus sac, an ovary antiporal to the vitellarium and cirrus-sac extending much more into the median field than in the present material, almost to middle of proglottis (Schmidt and Dailey 1992, confirmed also by present study based on paratype specimens), and an uterus overlapping osmoregulatory canals.

González-Acuña *et al*. (2012) reported *Amazilolepis trinidadensis* from the host species studied by us. Unfortunately, their paper does not contain morphological information. In view of the external similarity between the genera *Amazilolepis* and *Colibrilepis*, we consider that their record needs morphological confirmation.

Staphylepis is the most similar to our material but differs in the apical structure because it has a rudimentary rostellum. Rietschel (1934) described *Staphylepis inhamata* from *Eupetomena macroura* (Gmelin, 1788), a South-American trochilid bird. This species is close to our material but some characteristics differ, especially the gravid uterus that is separated by three incomplete walls and the irregular alternation of the genital pores. Mariaux and Vaucher (1991) described *Staphylepis ambilateralis* from nectariniid birds in West Africa. *S. ambilateralis* is also close to our material but has a very different young uterus (horseshoe-shaped, with numerous marginal lobes forming an irregular border), a genital pore that may be dextral or sinistral, and a round and small internal seminal vesicle.

Furthermore, *Colibrilepis* has a young uterus that is clearly rounded and a gravid uterus with a small number of eggs (Fig. 6), which differentiates it from all these genera.

Our phylogenetic analyses (Fig. 7) include only a small sample of hymenolepidids but they show that *Colibrilepis pusilla* sp. nov. is never in a monophyletic group with *Staphylepis ambilateralis*, thus supporting the validity of the new genus. The exact relationship between the two genera remains however uncertain because of contradictory information from lsrDNA and nad1. These results also tend to support the multiple origins of hymenolepidids with unarmed scoleces and do not support their placement in a single genus as proposed by Czaplinski (in Czaplinski and Vaucher 1994). They also suggest a possible paraphyly of *Passerilepis* although this should be verified on a much larger dataset.

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