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Phylogenetic relationships and systematics of tapeworms of the family Davaineidae (Cestoda, Cyclophyllidea), with emphasis on species in rodents

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Abstract: The present study aims at clarifying the poorly known phylogenetic relationships and systematics of cestodes of the family Davaineidae Braun, 1900 (Cyclophyllidea), primarily the genus *Raillietina* Fuhrmann, 1920 and of the subfamily Inermicapsiferinae (Anoplocephalidae) from mammals (mostly rodents, 31 new isolates) and birds (eight new isolates). Phylogenetic analyses are based on sequences of the large subunit ribosomal RNA gene (28S) and mitochondrial NADH dehydrogenase subunit 1 gene (*nad1*). The main phylogenetic pattern emerging from the present analysis is the presence of three independent lineages within the main clade of the subfamily Davaineinae, one of which is almost entirely confined to species from rodents and the other two show a mixture of species from birds and mammals. It is suggested that the major diversification of the main clade took place in birds, possibly in galliforms. The subsequent diversification included repeated host shifts from birds to mammals and to other birds, and from rodents to other mammals, showing that colonisation of new host lineages has been the main driver in the diversification of davaineine cestodes. It is also shown that all isolates of *Inermicapsifer* Janicki, 1910, mainly from rodents, form a monophyletic group positioned among *Raillietina* spp. in the "rodent lineage", indicating that the genus *Inermicapsifer* is a member of the family Davaineidae. This means that the subfamily Inermicapsiferinae and the family Inermicapsiferidae should be treated as synonyms of the Davaineidae, specifically the subfamily Davaineinae. Three additional genera generally included in the Inermicapsiferinae, i.e. *Metacapsifer* Spasskii, 1951, *Pericapsifer* Spasskii, 1951 and *Thysanotaenia* Beddard, 1911, are also assigned here to the Davaineidae (subfamily Davaineinae). *Raillietina* spp. were present in all three main lineages and appeared as multiple independent sublineages from bird and mammalian hosts, verifying the non-monophyly of the genus *Raillietina* and suggesting a presence of multiple new species and genera.

Keywords: Davaineinae, Anoplocephalidae, *Raillietina*, *Inermicapsifer*, Inermicapsiferinae, phylogeny, 28S, *nad1*

Cestodes of the family Davaineidae Braun, 1900 (Cyclophyllidea) comprise a species-rich group with a worldwide distribution in birds and mammals. The family includes at least 450 nominal species and 37 genera (Mariaux et al. 2017). Approximately 80 nominal species have been described from mammals, of which ca. 35 species

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are from rodents (Movsesyan 2003a,b, Caira et al. 2024). The Davaineidae is currently considered to include two subfamilies, i.e. Davaineinae and Idiogeninae, although Ophryocotylinae and Cotugniinae have sometimes been regarded as valid subfamilies. For the most recent overview of morphologic features and classification of davaineids, see Mariaux et al. (2017).

The monophyly of the family, to the exclusion of the genus *Ophryocotyle* Friis, 1870, was supported by the molecular phylogenetic analysis of Waeschenbach and Littlewood (2017), part of the Planetary Biodiversity Inventory project "Tapeworms from vertebrate bowels of the Earth" (Caira and Jensen 2017). The monophyly of the Davaineidae, including Ophryocotylinae, was also supported by the phylogenetic analysis of Hoberg et al. (1999) based on morphologic characters. The phylogenetic position of the Davaineidae with respect to other cyclophyllidean families remains undefined, although it is probably a relatively early diverging group (Waeschenbach and Littlewood 2017). However, the analysis of Hoberg et al. (1999) suggested a derived phylogenetic position for the Davaineidae.

The phylogenetic relationships between species and genera of davaineid cestodes are largely unknown. The existing studies have dealt with very limited assemblages, including mainly species of *Raillietina* Fuhrmann, 1920 from birds (O'Callaghan 2004, Littlewood et al. 2008, Butboonchoo et al. 2016, Siddiqui et al. 2023). Individual DNA sequences of *Raillietina* species from mammals (rodents, pangolin) have been published by Oliveira Simões et al. (2017), Tuli et al. (2022) and Panti-May et al. (2023). In the latter studies, the species from mammals were positioned among species from birds, suggesting that they originated as a consequence of a shift from the latter hosts.

The genus *Raillietina*, of the subfamily Davaineinae, is by far the most species-rich genus of davaineid cestodes, with ca. 200 nominal species, of which ca. 165 species in birds and ca. 35 species in mammals, most of the latter species (22) in rodents (Movsesyan 2003a, Caira et al. 2024). The prevailing taxonomic concept of the genus *Raillietina* is, however, very wide and evidently artificial, including all species with unilateral genital pores and multiple eggs in each parenchymatous capsule (Jones and Bray 1994, Movsesyan 2003a). Not surprisingly, the molecular phylogenetic analysis of Littlewood et al. (2008), based on six species of *Raillietina* and *Fuhrmannetta malakartis* Mahon, 1958 from birds, strongly suggested that the former genus is not monophyletic. However, large-scale phylogenetic analyses that would allow us to consider new classification schemes for the genus *Raillietina* are still lacking.

The cestodes of the Inermicapsiferinae, a subfamily traditionally assigned to the family Anoplocephalidae (see Spasskii 1951, Schmidt 1986 and Beveridge 1994), are morphologically very similar to the davaineid cestodes, particularly *Raillietina*, the absence of a rostellum and armature in the former group being the only clear-cut morphologic difference between these taxa. Lopez-Neyra (1954, 1955) transferred several davaineid genera to the Anoplocephalidae, while Baer and Fain (1955) transferred inermicapsiferines (then included in the Linstowiidae) to the subfamily Davaineinae. The latter action was supported by Mettrick and Weir (1963) and also later by Spasskii (1996). However, the monophyly and systematic position of inermicapsiferine cestodes has not been tested using molecular phylogenetic methods.

The present study aims at clarifying the poorly known phylogenetic relationships of davaineid cestodes of birds and mammals (particularly rodents) and inermicapsiferine cestodes of rodents using phylogenetic analyses based on nuclear and mitochondrial DNA sequences. The observed phylogenetic relationships are used to infer patterns of diversification and systematic relationships in the Davaineinae, particularly in the genera *Raillietina* and *Inermicapsifer* Janicki, 1901.

MATERIALS AND METHODS

Material

The present material includes 39 new isolates of davaineid and inermicapsiferine cestodes from mammals (31 isolates, mostly from rodents) and birds (eight isolates) (Table 1). The isolates were assigned to the genera *Raillietina*, including the type species *Raillietina tetragona* (Molin, 1858) from the domestic fowl, *Paroniella* Fuhrmann, 1920, *Delamuretta* Spasskii, 1977, *Calostaurus* Sandars, 1957, *Skrjabinia* Fuhrmann, 1920, *Cotugnia* Diamare, 1893 and *Inermicapsifer* according to the keys of Jones and Bray (1994) and Beveridge (1994). Species-level identification was based on the descriptions available in Movsesyan (2003a,b) and in Caira et al. (2024). However, most of the isolates of *Raillietina* and *Inermicapsifer* from rodents could not be identified to species. The present material includes three isolates from Cape Verde, which were treated as *Thysanotaenia congolensis* Dronen, Simcik, Scharninghausen et Pitts, 1999 (Inermicapsiferinae) by Świderski et al. (2015a,b) and Miquel et al. (2016). However, they are placed here in the genus *Inermicapsifer* (species "4") due to a lack of significant morphologic differences between the present specimens from Cape Verde and Africa, and the *Inermicapsifer* species described from African rodents (Caira et al. 2024). For the host species and other background information of the present isolates, see Table 1.

Molecular and phylogenetic analysis

The present molecular analysis utilises sequences of the large subunit ribosomal RNA gene (28S) and mitochondrial NADH dehydrogenase subunit 1 gene (*nad1*). For 28S, DNA was amplified using two alternative pairs of primers: (i) XZ-1 (forward, 5'-ACCCGCTGAA**T**TTAAGCATAT-3') of Waeschenbach et al. (2007), which differs from the original XZ-1 of Van der Auwera et al. (1994) by having one "Y" replaced with "T" (in bold), and 1500R (reverse, 5'-GCTATCCTGAGGGAAACTTCG-3') of Littlewood et al. (2008) (ca. 1500 bp), and (ii) U178 (forward, 5'-GCACCCGCTGAAYTTAAG-3') and L1642 (reverse, 5'-CCAGCGCCATCCATTTTCA-3' (ca. 1500 bp), both from Lockyer et al. (2003). For *nad1*, DNA was amplified with primers Cyclo_Nad1F (forward, 5'-GGNTATTSTCARTNTCGTAAGGG-3') and Cyclo_trnNR (reverse, 5'-TT CYTGAAGTTAACAGCATCA-3') (ca. 800 bp) of Littlewood et al. (2008).

Standard 50 µl PCR was performed using cycling conditions of Waeschenbach et al. (2007) and Littlewood et al. (2008) for

Table 1. GenBank accession numbers, voucher numbers and background information for isolates used in the present phylogenetic analyses. The position of *Calostaurus* spp. and *Raillietina* spp. (Mexico) within the clade B is relatively poorly supported (Fig. 1). Depositories of vouchers: "MZB", Museu de Ciències Naturals de Barcelona, Spain; "FMNH", Finnish Museum of Natural History; "CNHE", Colección Nacional de Helmintos, Mexico; "SAM", South Australian Museum.

Cestode species	DNA code	Host species	Host family	Country	Locality	Vouchers	GenBank (28S)	GenBank (*nad1*)	Source
Clade A									
Raillietina sp. 1	DV7	*Bandicota indica*	Muridae	Vietnam	Hong Gnu	MZB 2024-2584	-	OR795878	Present study
Raillietina sp. 2	DN9	*Rattus exulans*	Muridae	Vietnam	Cao Lanh	MZB 2024-2583	-	OR795877	Present study
Raillietina sp. 3	BU4	*Leopoldamys sabanus*	Muridae	Thailand	Loei	MZB 2024-2577	-	OR795870	Present study
Raillietina sp. 3	CI3	*Rattus tanezumi*	Muridae	Laos	Luang Prabang	MZB 2024-2578	-	OR795875	Present study
Raillietina sp. 3	CZ0	*B. indica*	Muridae	Laos	Lak	MZB 2024-2579	-	OR795876	Present study
Raillietina sp. 4	DA4	*Leopoldamys. herberti*	Muridae	Laos	Lak	MZB 2024-2580	OR805586	KP171527	Present study
Raillietina sp. 5	W54	*Nesomys rufus*	Nesomyidae	Madagascar	Ranomafana	FMNH KN.43440	KP171523	KP171526	Present study
Raillietina sp. 6	BV3	*Nesomys audeberti*	Nesomyidae	Madagascar	Ranomafana	FMNH KN.43441	OR805581	-	Present study
Raillietina sp. 7	BT4	*Laonastes aenigmamus*	Diatomyidae	Laos	Khammouane	FMNH KN.43437	OR805579	OR795867	Present study
Raillietina sp. 8	BT5	*L. aenigmamus*	Diatomyidae	Laos	Khammouane	FMNH KN.43439	OR805580	OR795868	Present study
Raillietina sp. 9	BT2	*L. aenigmamus*	Diatomyidae	Laos	Hinboum	FMNH KN.43438	OR805583	OR795869	Present study
Raillietina sp.	CZ8	*L. herberti*	Muridae	Laos	Lak	MZB 2024-2582	OR805585	-	Present study
Delamuretta sp.	BU5	*L. sabanus*	Muridae	Thailand	Loei	-	-	OR795871	Present study
Delamuretta sp.	CI1	*R. tanezumi*	Muridae	Laos	Luang Prabang	FMNH KN.43444, MZB 2024-2581	OR805584	OR795874	Present study
Inermicapsifer sp. 1	EP3	*Lemniscomys rosalia*	Muridae	South Africa	Mooinooi	-	OR805589	OR795886	Present study
Inermicapsifer sp. 1	ES4	*L. rosalia*	Muridae	South Africa	Zeerust	FMNH, KN.43449, KN.3648	OR805590	OR795882	Present study
Inermicapsifer sp. 2	ES9	*Aethomys chrysophilus*	Muridae	South Africa	Mooinooi	-	-	OR795884	Present study
Inermicapsifer sp. 2	ET5	*A. chrysophilus*	Muridae	South Africa	Zeerust	FMNH KN.43448	-	OR795885	Present study
Inermicapsifer sp. 3	EI4	*Smutsia temminckii*	Manidae	South Africa	-	-	-	OR795881	Present study
Inermicapsifer sp. 4	CY0	*Rattus rattus*	Muridae	Cape Verde	Santiago Island	-	-	KP171528	Present study
Inermicapsifer sp. 4	CY7	*R. rattus*	Muridae	Cape Verde	Santiago Island	FMNH KN.43447	KP171522	-	Present study
Inermicapsifer sp. 4	CY2	*Mus musculus*	Muridae	Cape Verde	Santiago Island	FMNH KN.43446	KP171521	KP171529	Present study
Inermicapsifer sp. 4	ES5	*Mastomys coucha*	Muridae	South Africa	Kaalplaas	FMNH KN.3647	OR805591	OR795883	Present study
Inermicapsifer sp. 5	FB3	*Rhabdomys dilectus*	Muridae	South Africa	Alice, East Cape	FMNH KN.3649	OR805594	-	Present study
Clade B									
Raillietina coreensis Honda, 1939	CA7	*Apodemus agrarius*	Muridae	Korea	DMZ[1]	FMNH KN.43434	KP171525	KP171531	Present study
R. echinobothrida (Megnin, 1880)	EV0	*Gallus gallus*	Phasianidae	Thailand	Udon Thani	-	-	OR795887	Present study
R. tetragona (Molin, 1858)	EV1	*G. gallus*	Phasianidae	Thailand	Udon Thani	-	OR805592	OR795888	Present study
R. tetragona	EV6	*G. gallus*	Phasianidae	Thailand	Udon Thani	-	OR805593	OR795889	Present study
R. sonini Spasskaya et Spasskii, 1971	-	*Dendrocopos syriacus*	Picidae	Bulgaria	Sofia	-	EU665462	EU665490	Littlewood et al. 2008
R. tunetensis Joyeux et Houdemer, 1928	-	*Leptotila verreauxi*	Columbidae	Costa Rica	Guanacaste	-	EU665459	EU665487	Littlewood et al. 2008
Raillietina sp.	-	*Crax rubra*	Cracidae	Costa Rica	Guanacaste	-	EU665458	EU665486	Littlewood et al. 2008
Raillietina sp.	-	*Leuconotopicus villosus*	Picidae	USA	Nebraska	-	EU665460	EU665488	Littlewood et al. 2008
Raillietina sp.	-	*L. villosus*	Picidae	USA	Nebraska	-	EU665461	EU665489	Littlewood et al. 2008
Raillietina sp.	-	*Manis pentadactyla*	Manidae	China	Guangdong	-	-	OL597540	Tuli et al. 2022
Raillietina sp.	-	*Ototylomys phyllotis*	Cricetidae	Mexico	Quintana Roo	CNHE 11960-71	OR271645	-	Panti-May et al. 2023
Raillietina sp.	-	*Sigmodon toltecus*	Cricetidae	Mexico	Yucatan	CNHE 10790-74	OR271650	-	Panti-May et al. 2023
Fuhrmannetta malakartis Mahon, 1958	-	*Coturnix coturnix*	Phasianidae	Bulgaria	Khaskovo	-	EU665457	EU665485	Littlewood et al. 2008

Table 1. continued

Paroniella urogalli (Modeer, 1790)	BW7	*Lagopus lagopus*	Phasianidae	Finland	-	FMNH KN.43442	-	KP171532	Present study
Paroniella sp.	AX3	*Neotoma cinerea*	Cricetidae	USA	Montana	FMNH KN.43443	-	KP171530	Present study
Calostaurus macropus (Ortlepp, 1922)	BZ1	*Thylogale stigmatica*	Macropodidae	Australia	Queensland	SAM 23948	OR805582	-	Present study
C. thylogale Beveridge, 1975	BY9	*Thylogale billardierii*	Macropodidae	Australia	Tasmania	SAM 9592	KP171524	-	Present study
Clade C									
Raillietina australis (Krabbe, 1869)	-	*Dromaius novaehollandiae*	Casuariidae	Australia	Victoria	-	AF286914	EU665484	Olson et al. 2001, Littlewood et al. 2008
R. fuhrmanni (Southwell, 1922)	EV9	*Columba livia*	Columbidae	Thailand	Udon Thani	FMNH KN.43435	OR805596	OR795891	Present study
R. trapezoides (Janicki, 1904)	EC6	*Rhabdomys pumilio*	Muridae	South Africa	Dronfield	-	OR805588	-	Present study
R. trapezoides	ED0	*R. pumilio*	Muridae	South Africa	Dronfield	FMNH KN.43436, KN.3650	OR805587	OR795879	Present study
R. trapezoides	EF4	*Micaelamys namaquensis*	Muridae	South Africa	Bethulie	-	-	OR795880	Present study
Other species									
Cotugnia sp.	EV7	*Columba livia*	Columbidae	Thailand	Udon Thani	FMNH KN.43445	-	OR795890	Present study
Skrjabinia cesticillus (Molin, 1858)	BW8	*Tetrao urogallus*	Phasianidae	Finland	Korpilahti	-	-	OR795872	Present study
S. cesticillus	BW9	*T. urogallus*	Phasianidae	Finland	Korpilahti	-	-	OR795873	Present study

[1] DMZ, demilitarized zone between South and North Korea.

28S and *nad1*, respectively. Successfully amplified DNA was purified using E.Z.N.A.™ Cycle Pure Kit (OMEGABio-Tek). Purified PCR products were directly sequenced using dye terminators and visualised with an ABI 3730xl DNA analyser. Assembled sequences were submitted to GenBank (Table 1).

Sequences were aligned with Muscle (Edgar 2004); ambiguously aligned sites and gaps were deleted. The best substitution models, selected by the Bayesian information criterion implemented in MEGA11 (Tamura et al. 2021), were HKY+G, GTR+G and GTR+G+I for 28S, *nad1* and their concatenated data, respectively.

The Bayesian phylogeny inference (Huelsenbeck et al. 2001) and the Maximum Likelihood (ML) method were used for assessing phylogenetic relationships among the isolates. The Bayesian analysis was performed using MrBayes v. 3.1 (Ronquist and Huelsenbeck 2003) implemented in Geneious Pro v. 5.3.6. (http://www.geneious.com). MrBayes was run for 5 million generations, sampled every 1,000 generations, and 500,000 generations were discarded as burn-in. Node support was expressed as posterior probabilities, values > 0.95 being considered significant. Bootstrap support for the ML method implemented in MEGA11 was based on 1,000 replications, with values > 0.75 considered significant.

Three separate phylogenetic analyses were performed, i.e. for 28S, *nad1* and their concatenated data. The lengths of the final alignments for these data sets were 674 bp, 708 bp and 1,885 bp, respectively. Due to amplification problems, especially for the 28S primers, the composition of the sequence data sets for the two markers is not identical. In addition to the present material, 28S and *nad1* sequences for ten species of davaineids from birds and mammals were retrieved from GenBank (Table 1).

The monophyly and phylogenetic relationships of davaineids among cyclophyllidean cestodes were tested using the 28S sequences listed in Table 1, with additional sequences retrieved from GenBank, representing the families Anoplocephalidae *sensu stricto*, Hymenolepididae, Dilepididae, Catenotaeniidae and Linstowiidae (Fig. 1). *Mesocestoides* sp. and *Tetrabothrius forsteri* (Krefft, 1871) were used as outgroup species for the 28S analysis, and *Hymenolepis diminuta* (Rudolphi, 1819) and *Andrya rhopalocephala* (Riehm, 1881) for the *nad1* and concatenated (28S + *nad1*) analyses. Sequences of outgroup species were obtained from GenBank.

RESULTS

The 28S phylogeny (Fig. 1) showed that the davaineids form a strongly supported clade among cyclophyllidean cestodes, but their relationships with other families remained unresolved. The Davaineidae, including *Inermicapsifer* spp., is clearly not phylogenetically related with the Anoplocephalidae *sensu stricto*, the latter family forming a strongly supported clade with the Hymenolepididae and Dilepididae. The position of the Linstowiidae (*Mathevotaenia* sp.) supports the validity and independence of the latter family with respect to other cyclophyllidean families, including the Anoplocephalidae *sensu stricto* These patterns are largely consistent with those of Waeschenbach and Littlewood (2017).

The *nad1* phylogeny (Fig. 2) showed that *Skrjabinia cesticillus* (Molin, 1858) is sister to the the "main clade"

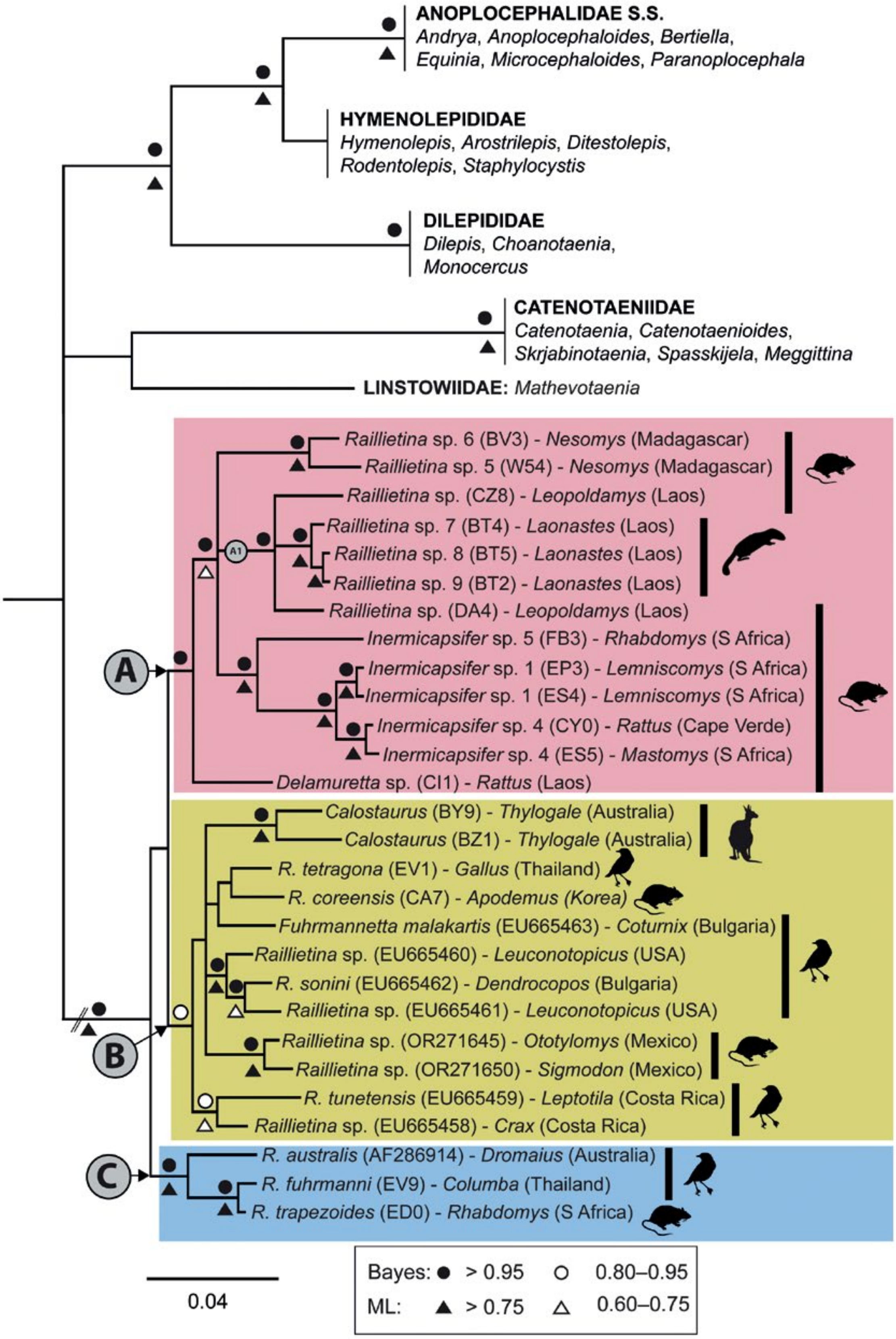

Fig. 1. Phylogenetic relationships between the cyclophyllidean families Davaineidae, Anoplocephalidae *sensu stricto*, Hymenolepididae, Dilepididae and Linstowiidae, and between isolates of the family Davaineidae (including *Inermicapsifer* spp.) based on sequences of the large subunit ribosomal RNA gene (28S). The topology is based on Bayesian phylogeny inference.

of the subfamily Davaineinae. *Cotugnia* sp. did not, however, group with other davaineids (Fig. 2). *Cotugnia* has previously been assigned either to a separate subfamily, the Cotugniinae (see Movsesyan 2003b), or to the subfamily Davaineinae (see Jones and Bray 1994); the present result supports the former view.

The phylogenies based on the three sequence data sets (Figs. 1–3) all revealed a presence of three supported lineages (A–C) within the main clade of davaineines, although the support for lineage B was relatively low (Bayesian posterior probability, 0.86) in the 28S data (Fig. 1). The relationships between lineages A–C remained poorly resolved.

Lineage A consisted almost entirely of parasites of rodents, primarily of the subfamily Murinae (family Muridae) from South-East Asia, South Africa and Cape Verde in the Atlantic Ocean, but also endemic Malagasy rodents (*Nesomys* spp.) of the family Nesomyidae and *Laonastes aenigmamus* Jenkins, Kilpatrick, Robinson et Timmins of the family Diatomyidae from Laos (Table 1). Lineage A included four sublineages, two of which consisted of *Raillietina* spp. from South-East Asia, one of *Raillietina* spp. from Madagascar and one of *Inermicapsifer* spp. from South Africa and Cape Verde (Figs. 1–3). One of the South-East Asian lineages ("A1") and the *Inermicapsifer* clade appeared as well supported sister lineages, especially in the concatenated data (Fig. 3). The *Inermicapsifer* clade also included an isolate from the pangolin *Smutsia temminckii* (Smuts) (Maniidae) from South Africa (Fig. 2), which is probably *Inermicapsifer rhodesiensis* Mettrick, 1959, described from the same host species from the present-day Zimbabwe (Mettrick 1959). The structure of the phylogenetic trees suggests that within clade A there are at least ten independent species of *Raillietina* and possibly five independent species of *Inermicapsifer* (Table 1). Lineage A also included a species of *Delamuretta* which appeared as a basal sublineage and is possibly sister to the clade formed by the rest of species within this lineage.

Lineage B was dominated by species from birds, but it also included five species from a diverse assemblage of mammals. The analysis based on the concatenated data (Fig. 3) suggested that clade B is split in two main sublineages, one of which includes two species from birds (i.e. *Raillietina tunetensis* Joyeux et Houdemer, 1928 and *Raillietina* sp.) and the other includes the rest of the species within lineage B. The bird cestodes within lineage B consisted of several species of *Raillietina* and also *Paroniella urogalli* (Modeer, 1790) and *F. malakartis*. Lineage B also included the type species *R. tetragona* from galliform birds, but its phylogenetic position remained undefined, except that it showed a slightly supported relationship with *Raillietina echinobothrida* (Megnin, 1880) in the *nad1* data (Fig. 2) and a strongly supported relationship with *F. malakartis* in the concatenated data (Fig. 3).

The cestodes from mammalian hosts in lineage B included *Raillietina coreensis* Honda, 1939 from a murine rodent (*Apodemus* Kaup) from Korea, two unidentified species of *Raillietina* from cricetid rodents (*Ototylomys* Merriam, *Sigmodon* Say et Ord) from Mexico (Fig. 1) and one from a pangolin (*Manis* Linnaeus) from China (Fig. 2), *Paroniella* sp. from a cricetid rodent (*Neotoma* Say et Ord) from North America (Fig. 2) and two species of *Calostaurus* Sandars, 1957 from Australian marsupials (Diprotodontia) (Fig. 1).

The small, strongly supported lineage C consisted of two species of *Raillietina* from birds, i.e. *Raillietina australis* (Krabbe, 1869) from *Dromaius* Vieillot (emu) from Australia and *Raillietina fuhrmanni* (Southwell, 1922) from *Columba* Linnaeus from Thailand, and *Raillietina trapezoides* (Janicki, 1904) from South African murine rodents, the latter two species showing a strongly supported relationship (Figs. 1–3).

DISCUSSION

Phylogenetic patterns

The main phylogenetic pattern emerging from the present analysis is the presence of three independent lineages within the main clade of the subfamily Davaineinae, one of which (A) is almost entirely confined to species from rodents and the other two (B, C) show a mixture of species from birds and mammals. Based on the facts that lineages B and C evidently originated in birds and that the basal genera *Cotugnia* and *Skrjabinia* are parasites of the same host group, the most parsimonius assumption is that the original hosts of the main clade of the Davaineinae are found among birds. The species in the genus *Skrjabinia*, the sister group of the main davaineine clade, are predominantly parasites of galliform birds (Movsesyan 2003a). Further, the presence of five species of cestodes from galliforms in lineage B (Fig. 2) leads us to suggest that this group of birds harboured the tapeworm lineage that gave rise to the main davaineine clade. However, the basal species in lineage C (*Raillietina australis*) is a parasite of ratite birds (*Dromaius*), which appeared before galliform birds. Therefore, we cannot rule out the possibility that the origin of the Davaineinae is with the ratites. The Australian emu, *Dromaius novaehollandiae* (Latham), has at least five host-specific species of *Raillietina* (see O'Callaghan et al. 2000).

The three main lineages, particularly those dominated by parasites of birds (B, C), showed very extensive geographic distributions. For example, the range of clade B covers Europe, China, Korea, South-East Asia, North America and Central America (Fig. 2), and possibly also Australia and Mexico (Fig. 1). It is probable that the high mobility of birds, including their migratory behaviour, is the main factor explaining the observed pattern.

The major diversification of the precursor of the main clade of davaineines into three lineages (A–C) remains partly obscure, but it is clear that there was an early colonisation of rodents (lineage A) from birds, probably galliforms. The species in lineage A are almost exclusively parasites of murine rodents (Figs. 1–3). In the *nad1* and concatenated trees, the basal relationships within lineages A were unresolved, but the structure of the 28S tree (Fig. 1) suggests that there is a basal South-East Asian species (isolate CI1), which is sister to the clade formed by the rest of the species in the lineage A. The presence of sev-

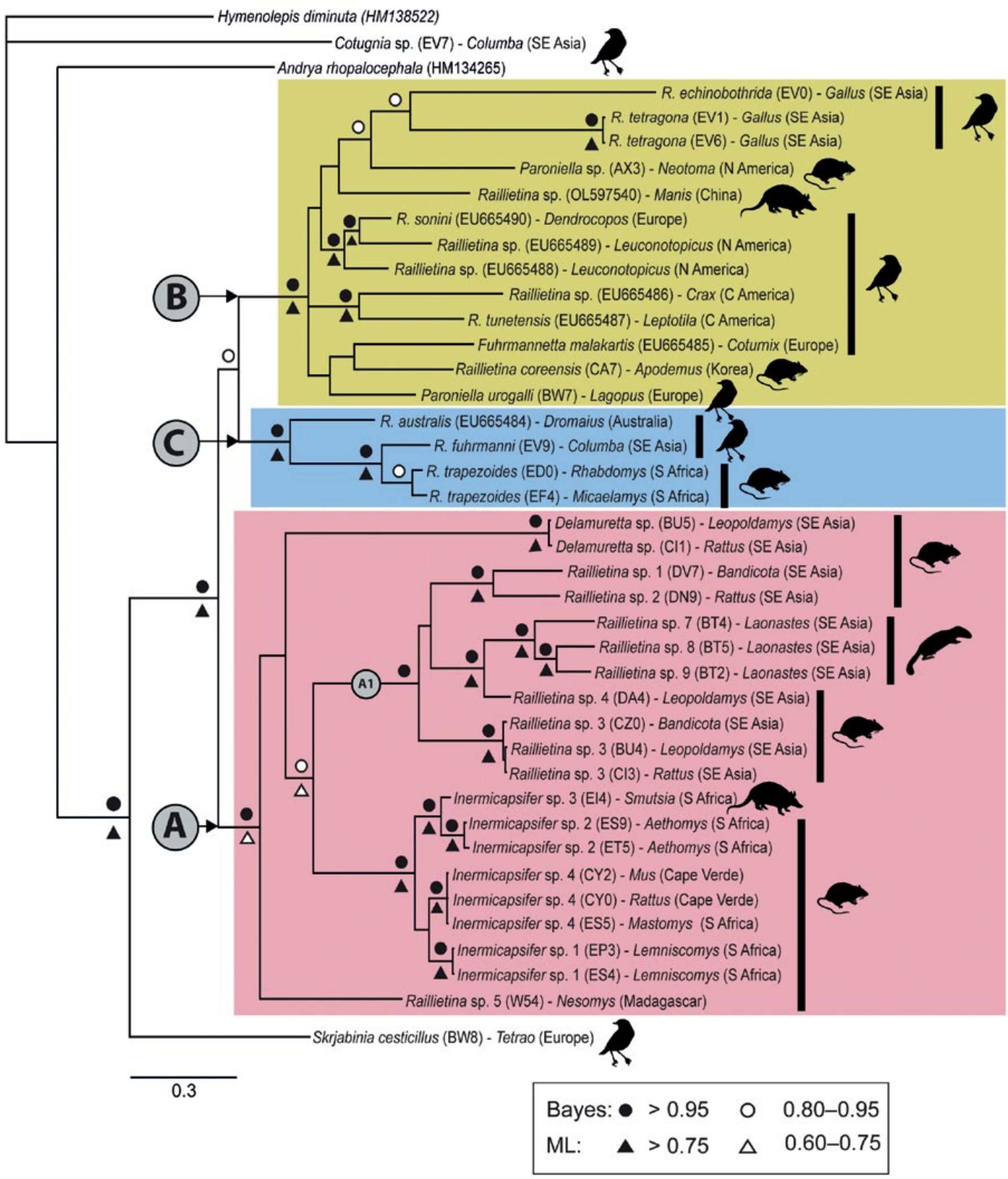

Fig. 2. Phylogenetic relationships between isolates of the family Davaineidae (including *Inermicapsifer* spp.) based on sequences of the mitochondrial NADH dehydrogenase subunit 1 gene (*nad1*). The topology is based on Bayesian phylogeny inference.

eral additional isolates from South-East Asia in the main sublineage suggests that lineage A originated in South-East Asian murines. Sublineage A1 probably also originated in South-East Asian murines, with a subsequent colonisation of African murines, leading to divergence and diversification of the *Inermicapsifer* clade. These patterns are consistent with the phylogenetic history for the subfamily Murinae, which probably appeared in South-East Asia and subsequently colonised Africa and other regions (Schenk et al. 2013).

The present study includes the first report of davaineid cestodes in endemic Malagasy nesomyid rodents (*Nesomys* spp.) of the subfamily Nesomyinae. The phylogenetic position of these cestodes indicates an early divergence within lineage A. The Malagasy nesomyid rodents probably originate from a single colonisation event by an African nesomyid 24–20 Mya (Poux et al. 2005), suggesting that their extant, host-specific parasites have an equally long history on Madagascar. However, a species of *Raillietina* (*R. murium* Joyeux et Baer, 1936) has been described from the black rat *Rattus rattus* from Madagascar (Joyeux and Baer 1936), which leads to an alternative hypothesis that the *Raillietina* species of the endemic nesomyids originate through a recent capture from commensal rats. However, the latter hypothesis is invalid owing to the basal phylogenetic position and high genetic divergence of the *Raillietina* species of nesomyids within lineage A. A recent survey of helminths of Malagasy rodents showed an absence of davaineid cestodes in black rats (n = 90) and *Eliurus* spp. (Nesomyidae) (n = 17) (JTL and VH – unpubl. data). There are evidently no reports of *Raillietina* from African nesomyids (subfamilies Cricetomyinae, Delanymyinae and Dendromurinae).

Laonastes aenigmamus or the Laotian rock rat ("kha-nyou") is a recently found "enigmatic" rodent that was shown to belong to the Diatomyidae, a fossil family thought to be extinct for 11 My (Dawson et al. 2006). It has a limited distribution in the karst region of Central Laos (Nicolas et al. 2012) and also in a small, adjacent region of Vietnam (Nguyen et al. 2014). Hugot et al. (2013) described a new species and genus of an oxyurid nematode from *L. aenigmamus*, but there are no published reports of its tapeworms. The structure of the present phylogenetic trees suggests strongly that the *Raillietina* species of *L. aenigmamus* originate through a single host shift from South-East Asian murine rodents (Figs. 1–3), and are therefore much more recent than the host itself.

Lineage B probably diversified initially in galliforms, with subsequent shifts to woodpeckers (Piciformes), doves (Columbiformes) and mammals. The three *Raillietina* species from woodpeckers appeared as a monophyletic group (Fig. 2), as shown earlier by Littlewood et al. (2008). Among the mammalian hosts, there were at least two tapeworm species from rodents and one from pangolins (Fig. 2), which evidently differentiated as a consequence of a shift from birds.

The 28S tree suggests that clade B also includes two additional species from cricetid rodents (*Raillietina* spp.) from Mexico and two from marsupials (*Calostaurus* spp.) (all without *nad1* data), although the support for clade B was relatively low (Fig. 1). Additional phylogenetic analyses including unpublished 28S and *nad1* sequences of *Raillietina* sp. from a "field biologist" (KT001065, KT001066; submitted to GenBank by B. A. Kendall, V. V. Tkach et al.) showed that the species in question is a parasite of birds within clade B (results not shown). This finding attests to the ability of bird-associated davaineids to infect mammalian hosts.

The original bird hosts for clade C remain unknown, but it is obvious that murine rodents were colonised by a tapeworm lineage of birds, probably columbiforms, giving rise to (the precursor of) *R. trapezoides* (Figs. 1–3).

Based on the present results, there has been in total 5–7 independent colonisations of mammals by davaineine species of birds, and two colonisations by parasites of murine rodents to other mammals (a diatomyid rodent, a pangolin). No shifts from mammals to birds were inferred. Although no explicit cophylogenetic analysis was performed, it is clear that the colonisation of mammals by parasites of birds and other mammals has played a major role in the diversification of the main davaineine clade. No unambiguous evidence for cophylogenetic events of the host and parasite lineages was found.

Systematics

The present phylogenetic analyses showed consistently that all isolates of *Inermicapsifer* form a strongly supported monophyletic group, which is positioned among *Raillietina* spp. in the "rodent lineage" (A). Therefore, the genus *Inermicapsifer* is unambiguously a member of the family Davaineidae, specifically the subfamily Davaineinae, and should be excluded from the family Anoplocephalidae. The implication is that the inermicapsiferines are davaineids which have lost the rostellum and armature in connection with their divergence from other davaineids of rodents (*Raillietina* spp.) and that the loss has occurred only once. This also means that the subfamily Inermicapsiferinae and the family Inermicapsiferidae should be treated as synonyms of the Davaineidae, specifically the subfamily Davaineinae. The independence of inermicapsiferines with respect to the Anoplocephalidae *sensu stricto* is supported by ultrastructural characteristics of spermatozoa (Miquel et al. 2016) and eggs (Świderski et al. 2015a).

The species of the genus *Inermicapsifer* are primarily parasites of hyraxes (Hyracoidea), with the type species *Inermicapsifer hyracis* (Rudolphi, 1808), and rodents, mainly in sub-Saharan Africa (see Caira et al. 2024). Morphologic evidence concerning the structure of the osmoregulatory system and position of the female glands suggests that the *Inermicapsifer* species of rodents represent a separate, new genus. However, the present analysis lacks the inermicapasiferines of hyracoids, and without knowledge of their phylogenetic position, an erection of a new genus would be premature.

In addition to *Inermicapsfer*, the genera traditionally assigned to the Inermicapsiferinae include *Metacapsifer* Spasskii, 1951, *Pericapsifer* Spasskii, 1951 and *Thysanotaenia* Beddard, 1911; *Arhynchotaenia* Pagenstecher, 1877 and *Hyracotaenia* Beddard, 1912 are junior synonyms of *Inermicapsfer* (see Spasskii 1951 and Caira et al. 2024). *Metacapsifer*, *Pericapsifer* and *Thysanotaenia*, which share the main morphological features with *Inermicapsfer*, including egg-bearing parenchymtous capsules, are here assigned to the Davaineidae (subfamily Davaineinae).

The fact that *Raillietina* spp. are present in all three main lineages (A–C) and appear as multiple independent sublineages from bird and mammalian hosts unambiguously verifies the non-monophyly of the genus *Raillietina*. Most of the *Raillietina* lineages identified here evidently represent new genera. The question which species should be included in *Raillietina* sensu stricto cannot be reliably answered based on the present results, because the phylogenetic relationships among the species in lineage B, including the type species *R. tetragona*, remained largely unresolved. However, the phylogenetic association between *R. tetragona* and *R. echinobothrida*, both from galliform birds, was strongly supported by Siddiqui et al. (2023), and slightly supported by Butboonchoo et al. (2016) and by the present

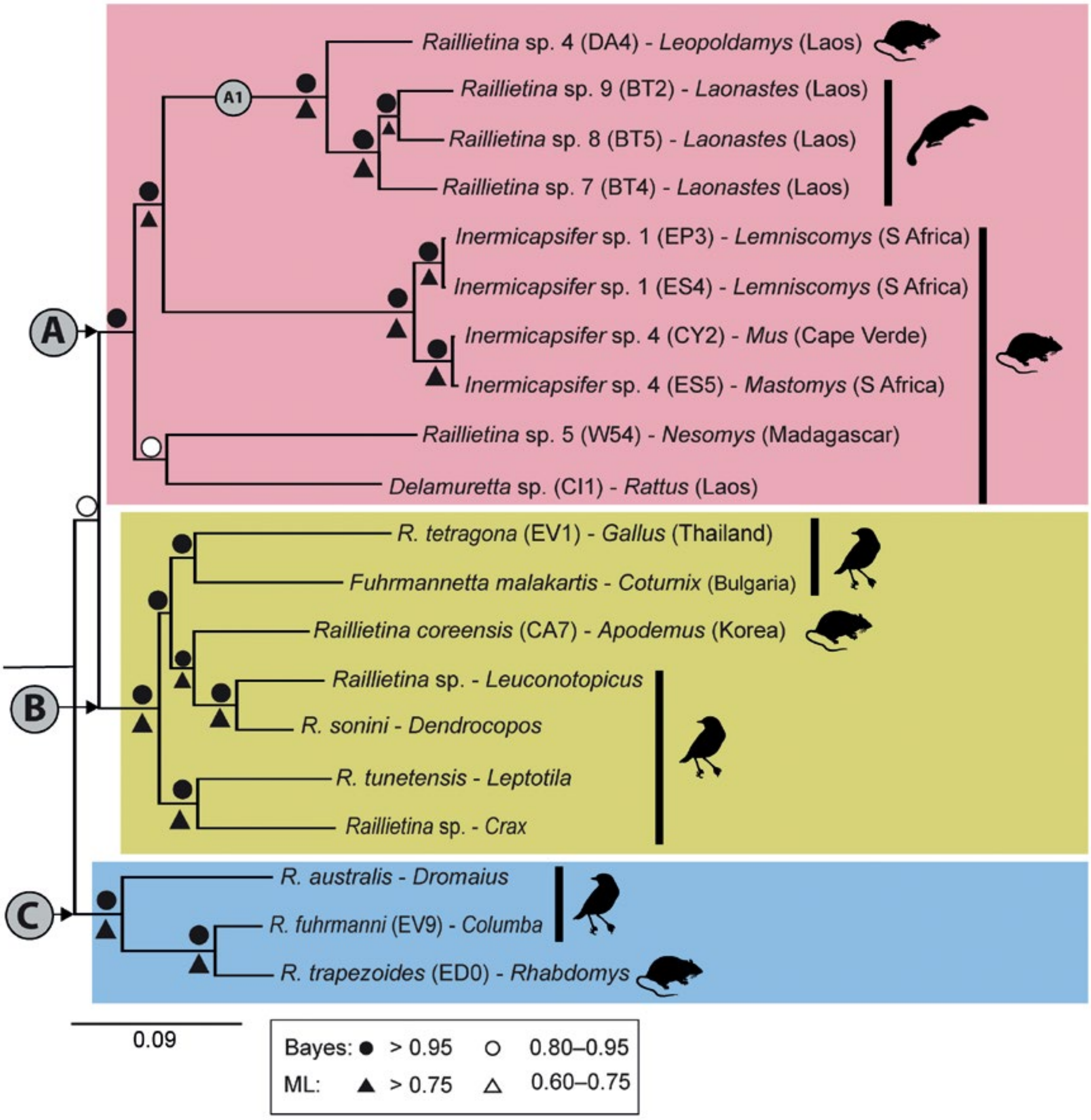

Fig. 3. Phylogenetic relationships between isolates of the family Davaineidae (including *Inermicapsifer* spp.) based on concatenated sequences of the mitochondrial NADH dehydrogenase subunit 1 gene (*nad1*) and the large subunit ribosomal RNA gene (28S). The topology is based on Bayesian phylogeny inference.

nad1 analysis (Fig. 2). The genus *Raillietina* is obviously in need of an extensive reorganisation and description of new taxa based on phylogenetic and morphologic criteria. The present results also provide evidence for non-monophyly of the genus *Paroniella*.

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Author contribution statement. The first author is responsible for the design of the study, identification of the parasites, molecular and phylogenetic analyses, and writing of the manuscript, The other authors designed and performed the collection of the host and parasite specimens, and contributed to the writing of the manuscript.

REFERENCES

Baer J.G., Fain A. 1955: Les Cestodes des Pangolins. Bull. Soc. Neuchâtel. Sci. Nat. 78: 37–52.

Beveridge I. 1994: Family Anoplocephalidae Cholodkovsky, 1902. In: L.F. Khalil, A. Jones and R.A. Bray (Eds.), Keys to the Cestode Parasites of Vertebrates. CABI, Wallingford, pp. 315–366.

Butboonchoo P., Wongsawad C., Rojanapaibul A., Chai J.-Y. 2016: Morphology and molecular phylogeny of *Raillietina* spp. (Cestoda: Cyclophyllidea: Davaineidae) from domestic chickens in Thailand. Kor. J. Parasitol. 54: 777–786.

Caira J.N., Jensen K. 2017: Planetary Biodiversity Inventory (2008–2017): Tapeworms from the Vertebrate Bowels of the Earth. University of Kansas, Natural History Museum, Special Publication No. 25, Lawrence, 464 pp.

Caira J.N., Jensen K., Barbeau E. (Eds.) 2024: Global Cestode Database. World Wide Web electronic publication, www.tapewormdb.uconn.edu.

Dawson M.R., Marivaux L., Li C.-K., Beard K.C., Métais G. 2006: A recently discovered living rodent is a survivor of a family thought to have been extinct for 11 million years. Science 311: 1456–1458.

Edgar R.C. 2004: MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucl. Acids Res. 32: 1792–1797.

Hoberg E.P., Jones A., Bray R.A. 1999: Phylogenetic analysis among the families of the Cyclophyllidea (Eucestoda) based on comparative morphology, with new hypotheses for co-evolution in vertebrates. Syst. Parasitol. 42: 51–73.

Huelsenbeck J.P., Ronquist F., Nielsen R., Bollback J.P. 2001: Bayesian inference of phylogeny and its impact on evolutionary biology. Science 294: 2310–2314.

Hugot J.P., Feliu C., Douangboupha B., Ribas A. 2013: *Laoxyuris laonasti* n. gen., n. sp. (Nematoda: Syphaciinae) parasite of *Laonastes aenigmamus* (Rodentia: Diatomyidae): morphology, biology, taxonomy, phylogeny. Infect. Genet. Evol. 16: 113–121.

Jones A., Bray R.A. 1994: Family Davaineidae Braun, 1900. In: L.F. Khalil, A. Jones, and R.A. Bray (Eds.), Keys to the Cestode Parasites of Vertebrates, CABI, Wallingford, pp. 407–441.

Joyeux C., Baer J. 1936: Helminthes des rats de Madagascar: contribution a l'etude de *Davainea madagascarensis* (Dav., 1869). Bull. Soc. Pathol. Exot. 29: 611–619.

Littlewood D.T.J., Waeschenbach A., Nikolov P.N. 2008: In search of mitochondrial markers for resolving the phylogeny of cyclophyllidean tapeworms (Platyhelminthes, Cestoda) – a test study with Davaineidae. Acta Parasitol. 53: 133–144.

Lockyer A.E., Olson P.D., Littlewood D.T.J. 2003: Utility of complete large and small subunit rRNA genes in resolving the phylogeny of the Neodermata (Platyhelminthes): implications and a review of the cercomer theory. Biol. J. Linn. Soc. 78: 155–171.

Lopez-Neyra C.R. 1954: Anoplocephalidae. Rev. Ibér. Parasitol. 14: 13–130, 22–290, 303–396.

Lopez-Neyra C.R. 1955: Anoplocephalidae. Rev. Ibér. Parasitol. 15: 33–84.

Mariaux J., Tkach V.V., Vasileva G.P., Waeschenbach A., Beveridge I., Dimitrova Y.D., Haukisalmi V., Greiman S.E., Littlewood D.T.J., Makarikov A.A., Phillips A.J., Razafiarisolo T., Widmer V., Georgiev B.B. 2017: Cyclophyllidea van Beneden in Braun, 1900. In: J.N. Caira and K. Jensen (Eds.), Planetary Biodiversity Inventory (2008–2017): Tapeworms from Vertebrate Bowels of the Earth. University of Kansas, Natural History Museum, Special Publication No. 25, Lawrence, pp. 77–148.

Mettrick D.F. 1959: A new tapeworm, *Inermicapsifer rhodesiensis* sp. nov. from a scaly ant-eater, *Manis temminckii*, in southern Rhodesia. J. Helminthol. 33: 273–276.

Mettrick D.F., Weir J.S. 1963: Studies on the genus *Inermicapsifer* Janicki, 1910 with notes on some genera in the subfamilies Inermicapsiferinae, Linstowiinae, and Davaineinae. Proc. Helminthol. Soc. Wash. 30: 199–205.

Miquel J., Świderski Z., Feliu C. 2016: Spermatozoon ultrastructure of *Thysanotaenia congolensis* (Cyclophyllidea, Anoplocephalidae, Inermicapsiferinae): phylogenetic implications. Parasitol. Res. 115: 3083–3091.

Movsesyan S.O. 2003a: [Davaineata – Tapeworms of Animals and Man. I]. Nauka, Moskva, 395 pp. (In Russian.)

Movsesyan S.O. 2003b: [Davaineata – Tapeworms of Animals and Man. II]. Nauka, Moskva, 262 pp. (In Russian.)

Nguyen D.X., Nguyen N.X., Nguyen D.D., Dinh T.H., Le D.T., Dinh D.H. 2014: Distribution and habitat of the Laotian rock rat *Laonastes aenigmamus* Jenkins, Kilpatrick, Robinson and Timmins, 2005 (Rodentia: Diatomyidae) in Vietnam. Biodivers. Data J. 2: e4188.

Nicolas V., Herbreteau V., Couloux A., Keovichit K., Douangboupha B., Hugot J.-P. 2012: A remarkable case of micro-endemism in *Laonastes aenigmamus* (Diatomyidae, Rodentia) revealed by nuclear and mitochondrial DNA sequence data. PLoS One 7: e48145.

O'Callaghan M. 2004: Studies on the systematics of the cestodes infecting the emu, *Dromaius novaehollandiae* (Latham, 1790). Ph. D. Thesis, The University of Adelaide, Adelaide, 236 pp.

O'Callaghan M.G., Davies M., Andrews R.H. 2000: Species of *Raillietina* Fuhrmann, 1920 (Cestoda: Davaineidae) from the emu, *Dromaius novaehollandiae*. Trans. R. Soc. S. Aust. 124: 105–116.

Oliveira Simões R., Simões S.B.E., Luque J.L., Iñiguez A.M., Maldonado Júnior A. 2017: First record of *Raillietina celebensis* (Cestoda: Cyclophyllidea) in South America: redescription and phylogeny. J. Parasitol. 103: 359–365.

Olson P.D., Littlewood D.T., Bray R.A., Mariaux J. 2001: Interrelationships and evolution of the tapeworms (Platyhelminthes: Cestoda). Mol. Phylogenet. Evol. 19: 443–467.

Panti-May J.A., Moguel-Chin W.I., Hernández-Mena D.I., Cárdenas-Vargas M.H., Torres-Castro M., García-Prieto L., Digiani M.C., Hernández-Betancourt S.F., Vidal-Martínez V.M. 2023: Helminths of small rodents (Heteromyidae and Cricetidae) in the Yucatan Peninsula, Mexico: an integrative taxonomic approach to their inventory. Zootaxa 5357: 205–240.

Poux C., Madsen O., Marquard E., Vieites D.R., De Jong W.W., Vences M. 2005: Asynchronous colonisation of Madagascar by the four endemic clades of primates, tenrecs, carnivores, and rodents as inferred from nuclear genes. Syst. Biol. 54: 719–730.

Ronquist F., Huelsenbeck J.P. 2003: MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574.

Schenk J.J., Rowe K.C., Steppan S.J. 2013: Ecological opportunity and incumbency in the diversification of repeated continental colonizations by muroid rodents. Syst. Biol. 62: 837–864.

Schmidt G.D. 1986: CRC Handbook of Tapeworm Identification. CRC Press Inc., Boca Raton, 675 pp.

Siddiqui T.R., Hoque M.R., Roy B.C., Anisuzzaman, Alam M.Z., Khatun M.S., Dey A.R. 2023: Morphological and phylogenetic analysis of *Raillietina* spp. in indigenous chickens (*Gallus gallus domesticus*) in Bangladesh. Saudi J. Biol. Sci. 30: 103784.

Spasskii A.A. 1951: Anoplocephalate Tapeworms of Domestic and Wild Animals (English translation). The Academy of Sciences of the USSR, Moscow, 783 pp.

Spasskii A.A. 1996: [On the systematics of davaineids (Cestoda: Cyclophyllidea)]. In: Parazitologiya v Ukraine. Materiali konf. URNOP, posvyashh. 90-letiyu A. P. Markevicha, Kiev, pp. 88–91. (In Russian.)

Świderski Z., Miquel J., Conn D.B. 2015a: Functional ultrastructure of eggs and cellular organization of hexacanths of the cyclophyllidean cestode *Thysanotaenia congolensis*: a phylogenetic implication of obtained results. Parasitology 143: 320–333.

Świderski Z., Miquel J., Feliu C. 2015b: Functional ultrastructure of the parenchymatic capsules of the cestode *Thysanotaenia congolensis* (Cyclophyllidea, Anoplocephalidae, Inermicapsiferinae). Parasitol. Res. 114: 297–303.

Tamura K., Stecher G., Kumar S. 2021: MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Mol. Biol. Evol. 38: 3022–3027.

Tuli M.D., Li H., Pan X., Li S., Zhai J., Wu Y., Chen W., Huang W., Feng Y., Xiao L., Yuan D. 2022: Heteroplasmic mitochondrial genomes of a *Raillietina* tapeworm in wild pangolin. Parasit. Vectors 15: 204.

Van der Auwera G., Chapelle S., De Wachter R. 1994: Structure of the large ribosomal subunit RNA of *Phytophthora megasperma*, and phylogeny of oomycetes. FEBS Letters 338: 133–136.

Waeschenbach A., Littlewood D.T.J. 2017: A molecular framework for the Cestoda. In: J.N. Caira and K. Jensen (Eds.), Planetary Biodiversity Inventory (2008–2017): Tapeworms from Vertebrate Bowels of the Earth. University of Kansas, Natural History Museum, Special Publication No. 25, Lawrence, pp. 431–451.

Waeschenbach A., Webster B.L., Bray R.A., Littlewood D.T.J. 2007: Added resolution among ordinal level relationships of tapeworms (Platyhelminthes: Cestoda) with complete small and large subunit nuclear ribosomal RNA genes. Mol. Phylogenet. Evol. 45: 311–325.

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