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Hidden diversity and complex transmission networks of Taeniidae and Dipylidiidae tapeworms in the Western Balkans revealed by mitochondrial markers

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Abstract

Background

Cestodes of the families Taeniidae and Dipylidiidae constitute a neglected but epidemiologically important group of parasites, including species of veterinary and zoonotic relevance. In the Western Balkans, their molecular epidemiology, host range, and transmission dynamics remain largely unresolved, limiting accurate assessment of their ecological and public health significance.

Methods

We conducted a large-scale molecular survey of cestodes collected from a broad spectrum of definitive and intermediate hosts across Croatia and Bosnia and Herzegovina over a 10-year period (2014–2024). A total of 264 specimens were analysed using mitochondrial markers (*cox1* and *nad1*), followed by phylogenetic and haplotype analyses to resolve species identity, genetic diversity, and transmission patterns.

Results

We provide the first comprehensive molecular evidence of nine taeniid species and multiple dipylidiid lineages circulating in the region. Generalist carnivores, particularly red foxes and golden jackals, emerged as key hubs of parasite diversity, harbouring the highest number of taxa and linking multiple transmission cycles. Several species (e.g. *Taenia hydatigena*, *T. krabbei*, *T. martis*) exhibited broad host associations, while phylogenetic analyses revealed extensive gene flow across geographic boundaries, indicating a continuous regional transmission system. Notably, we detected previously unrecognised genetic divergence within *Taenia pisiformis* and *Joyeuxiella* spp., suggesting cryptic diversity. Additionally, both canine and feline genotypes of *Dipylidium caninum* were identified, including evidence of cross-host transmission. The detection of zoonotic species such as *T. crassiceps* and *T. martis* further highlights the relevance of these parasite communities at the human–animal interface.

Conclusions

Our findings redefine the epidemiological landscape of cestodes in the Western Balkans, demonstrating that parasite transmission operates within highly interconnected, cross-border networks driven by generalist carnivores and shared ecological niches. This study establishes a critical molecular baseline and provides strong evidence that southeastern Europe represents an active hotspot of cestode diversity and transmission. Integrating wildlife into surveillance frameworks is essential for accurate risk assessment within a One Health context.

Introduction

Tapeworms (Cestoda, Platyhelminthes) represent a highly diverse class of parasites comprising species of considerable medical and veterinary significance. Adult cestodes inhabit the intestinal tract of all major vertebrate groups, while larval forms parasitize various tissues, organs, or body cavities of both invertebrate and vertebrate hosts [1, 2]. Currently, the class Cestoda includes approximately 5,000 valid species, classified into 19 orders [3]. Among them, families Taeniidae and Dipylidiidae are of particular public health importance due to their global distribution in mammalian definitive hosts, zoonotic potential, and economic impact [4]. The family Taeniidae comprises four genera (*Taenia*, *Echinococcus*, *Hydatigera*, and *Versteria*), while three genera (*Dipylidium*, *Joyeuxiella* and *Diplopylidium*) have been described within the family Dipylidiidae. Due to subtle morphological differences, particularly in larval stages, accurate species identification often requires the use of molecular tools [5]. Several Taeniidae and Dipylidiidae species of public health concern have been studied extensively at the molecular level [e.g. 6, 7, 8], while taxonomy of genus *Joyeuxiella* remains poorly resolved despite their ecological relevance [9]. Moreover, research on the diversity and host associations within the families Taeniidae and Dipylidiidae in the Western Balkans, particularly in Bosnia and Herzegovina and Croatia, remains sparse. Historical records from the late 19th and early 20th centuries provide morphological observations of several *Taenia* species (*T. saginata*, *T. solium*, *T. hydatigena*, *T. pisiformis*, *T. multiceps*, *T. serialis*, *Hydatigera taeniaeformis*) [10]; however, these findings have never been confirmed by molecular tools, and no new comprehensive studies have been conducted in recent decades. To date, the only *Taenia* species confirmed by molecular tools in the region is *T. crassiceps*, reported from ring-tailed lemurs (*Lemur catta*) in zoos in Zagreb and Sarajevo [11, 12], and a red fox (*Vulpes vulpes*) from Croatia [13]. A recent study from Serbia provided additional insights into small rodent hosts, identifying *T. martis*, *T. crassiceps*, *Hydatigera kamiyai*, and *H. taeniaeformis* s.s. [14], but data from carnivores and domestic animals remain poor.

Given the substantial knowledge gaps, the objective of this study was to provide the first molecular characterization and phylogenetic analysis of Taeniidae and Dipylidiidae cestodes in both final and intermediate hosts from Croatia and Bosnia and Herzegovina. By targeting mitochondrial gene fragments, we aimed to resolve species richness, assess genetic diversity, and reconstruct host-parasite relationships across a wide spectrum of wild and domestic mammals. Special emphasis was placed on identifying potentially cryptic or novel lineages,

elucidating the ecological dynamics of transmission, and establishing a molecular reference framework for future parasitological research in the region.

Materials and Method

2.1. Collection of adult cestodes and larval stages

Tapeworm samples were collected from ten species of definitive hosts and five species of intermediate hosts in Croatia and Bosnia and Herzegovina over a 10-year period (2014–2024). Adult cestodes were recovered from a total of 240 definitive hosts (192 from Croatia and 48 from Bosnia and Herzegovina), including red foxes (*Vulpes vulpes*, n = 70), golden jackals (*Canis aureus*, n = 63), domestic cats (*Felis catus*, n = 23), brown bears (*Ursus arctos*, n = 19), European badgers (*Meles meles*, n = 18), stone martens (*Martes foina*, n = 15), dogs (*Canis lupus familiaris*, n = 14), European wildcats (*Felis silvestris*, n = 13), European hedgehogs (*Erinaceus europaeus*, n = 3), and grey wolves (*Canis lupus*, n = 2).

Larval stages were obtained from 24 intermediate hosts from Croatia, including domestic pigs (*Sus scrofa domesticus*, n = 7), sheep (*Ovis aries*, n = 5), roe deer (*Capreolus capreolus*, n = 5), wild boars (*Sus scrofa*, n = 4), and goats (*Capra aegagrus hircus*, n = 3).

Samples were collected opportunistically through multiple surveillance and diagnostic activities. Wild carnivores were submitted to the Croatian Veterinary Institute within the national rabies surveillance programme or through roadkill monitoring schemes. Domestic animals were submitted as part of routine disease surveillance, post-mortem inspections at slaughterhouses, or clinical submissions. Additional samples were obtained during routine necropsies. Samples collected in Bosnia and Herzegovina were forwarded to Croatia for further analyses.

It should be noted that the sampling was not systematic and was not conducted within a structured epidemiological study design. Consequently, the total number of examined animals, including negative cases, was not recorded, and therefore no reliable estimates of prevalence or infection rates could be calculated. The present study should, therefore, be interpreted as a molecular survey of cestode diversity rather than a quantitative epidemiological assessment.

Furthermore, the present study focused specifically on selected cestode taxa belonging to the families Taeniidae and Dipylidiidae. Other cestode species detected during necropsy were not included in the present analyses.

To collect adult cestodes, the intestines of definitive hosts were opened longitudinally and visually inspected. Detected parasites were examined morphologically using a Zeiss Stereo Discovery V20 and Imager M.2 microscope (Carl Zeiss, Germany), equipped with Axiovision and ZEN 2 Pro software. All specimens were preserved in 96% ethanol until further processing.

2.2. DNA extraction, PCR amplification and sequencing

For molecular analysis, 3–5 mm fragments of the strobila or individual proglottids from each tapeworm specimen were excised. Only material from a single cestode specimen was used per analysis to avoid cross-contamination. Genomic DNA was extracted using a KingFisher™ magnetic particle processor with the MagMAX™ CORE Nucleic Acid Purification Kit (Thermo Fisher Scientific), following the manufacturer's protocol. The extraction procedure included an initial three-hour tissue lysis step with proteinase K and continuous mixing to ensure complete digestion prior to purification. Two mitochondrial gene fragments were targeted for amplification, a partial fragment of the *cytochrome c oxidase subunit 1* gene (*cox1*) using primers JB3 and JB4.5 [15], and a fragment of the *NADH dehydrogenase subunit 1* gene (*nad1*) using primers JB11 and JB12 [5].

Each PCR reaction was performed in a 20 µl volume containing 10 µl of G2 GoTaq® Green Master Mix (Promega, Madison, WI, USA), 0.4 µl of each primer (10 pmol/µl), 7.2 µl of nuclease-free water, and 2 µl of DNA template. Amplification was confirmed by capillary electrophoresis using a QIAxcel Advanced System with a DNA Fast Analysis Kit, QX Alignment Marker 15 bp/3 kb, and QX DNA Size Marker 50–3000 bp (Qiagen, Hilden, Germany).

PCR products were purified using ExoSAP-IT™ (Thermo Fisher Scientific, Waltham, MA, USA) and sequenced in both directions by a commercial sequencing service (Macrogen Europe, Amsterdam, The Netherlands) using the same primer sets. Chromatograms were assembled and edited using SeqMan Pro and EditSeq software (Lasergene v7.1; DNASTAR, Madison, WI, USA), and the resulting sequences were compared with GenBank entries using BLASTn to determine species identity.

2.3. Phylogenetic and haplotype analyses

Multiple sequence alignments were generated using the ClustalW algorithm [16] and subsequently refined manually. Maximum likelihood (ML) phylogenetic trees were constructed in MEGA v11.0 [17] using the Hasegawa–Kishino–Yano (HKY + G+I) model, selected based on AICc values. Tree topologies were inferred with the Nearest-Neighbor Interchange (NNI) heuristic method, and nodal support was evaluated with 1,000 bootstrap replicates.

Representative sequences of all confirmed species and haplotypes were submitted to GenBank and are available under the accession numbers: PZ163730- PZ163760, PZ163832, PZ165824-PZ165832, PZ154801- PZ164067 and PZ154817- PZ154823 (supplementary data table 1 and 2).

Results

A total of 264 Taeniidae (n = 233) and Dipylidiidae (n = 21) tapeworm specimens were obtained from definitive or intermediate hosts, and 176 (66%) of them were successfully identified by sequencing. Quality of the remaining sequences was not appropriate for species identification (supplementary data table 1 and 2).

3.1 Taeniidae

Molecular identification of cestodes collected from both definitive and intermediate hosts confirmed the presence of nine taeniid species belonging to two genera, *Taenia* and *Hydatigera*. The identified taxa included *Taenia arctos*, *T. crassiceps*, *T. hydatigena*, *T. krabbei*, *T. martis*, *T. pisiformis*, *T. polyacantha*, *Hydatigera kamiyai*, and *H. taeniaeformis* (Table 1.).

In Croatia, eight species were detected (*T. arctos, T. crassiceps, T. hydatigena, T. krabbei, T. martis, T. pisiformis, H. kamiyai*, and *H. taeniaeformis*), whereas six species were identified in Bosnia and Herzegovina (*T. hydatigena, T. crassiceps, T. krabbei, T. martis, T. polyacantha*, and *H. kamiyai*) (Table 2.). *Taenia polyacantha* was detected only in Bosnia and Herzegovina, whereas *T. arctos*, *T. pisiformis*, and *H. taeniaeformis* were identified only in Croatia, likely reflecting differences in host sampling (Table 2).

Overall, medium-sized canids, particularly the red fox and golden jackal, represented the most important definitive hosts of taeniid cestodes in the study area, harbouring the highest species diversity. In contrast, brown bear hosted only a host-specific taxon.

Taeniid cestodes were recorded in a wide range of carnivorous hosts (Table 2). The highest species diversity was observed in the red fox, which harboured seven species, followed by the golden jackal, which carried six taxa. Domestic dogs and domestic cats each hosted three species.

Species composition differed among host groups. In red foxes, the detected taxa included *T. crassiceps, H. kamiyai, T. krabbei, T. hydatigena, T. martis, T. pisiformis*, and *T. polyacantha*. Golden jackals harboured *T. hydatigena, T. krabbei, H. kamiyai, T. crassiceps, T. martis*, and *T. pisiformis*. Domestic dogs were positive for *T. hydatigena, T. crassiceps*, and *T. krabbei*.

Among felids, domestic cats carried *H. kamiyai, T. martis*, and *T. krabbei*, while European wildcats were infected with *H. kamiyai* and *H. taeniaeformis*.

Several hosts showed strong species associations. The grey wolf harboured exclusively *T. krabbei*, whereas the brown bear was infected solely with the host-specific species *T. arctos*. In mustelids, *T. martis* predominated and was consistently recorded in the stone marten, European badger, and hedgehog.

Larval stages of Taeniidae were detected in several ungulate hosts. The most widespread species was *T. hydatigena*, which was identified in five ungulate species, including wild boar, domestic pigs, sheep, goats, and roe deer, indicating a broad host range.

Other species showed narrower host associations. Larval stages of *T. krabbei* were detected in roe deer, while *T. crassiceps* occurred in wild boar. *T. martis* larvae were identified in domestic pigs, whereas *Hydatigera kamiyai* was recorded in sheep.

All obtained *cox1* and *nad1* sequences of *T. crassiceps* were 100% identical and matched published sequences from Central and Eastern Europe as well as Asia and North America. Phylogenetic analyses placed all sequences within a single monophyletic clade with no detectable geographic structure, indicating high global genetic homogeneity (Figs. 1 and 2).

Genetic analyses of *T. hydatigena* revealed seven nad1 haplotypes (TAHY1–TAHY7) and three cox1 haplotypes (TAHYA–TAHYC). All haplotypes clustered with a reference mitochondrial genome from China (MT784894) with 98.4–100% sequence similarity. The most frequent haplotype was TAHY1, revealed from ten of the analysed sequences. No clustering according to host species or geographic origin was observed.

All *cox1* sequences of *T. krabbei* were identical and matched a reference sequence from the Czech Republic (MT227284). Two *nad1* haplotypes (TAKR1 and TAKR2) were identified, showing 99.39–99.59% similarity to a Finnish reference sequence (EU544625). Phylogenetic analyses revealed minimal intraspecific divergence (Figs. 1 and 2).

Three *T. martis nad1* haplotypes (TAMA1–TAMA3) of and two *cox1* haplotypes (TAMAA–TAMAB) were identified. All sequences showed 99.3–100% similarity to the published mitochondrial genome of *T. martis* (NC020153). Phylogenetic analyses confirmed conspecificity among all analysed isolates (Figs. 1 and 2).

Sequences attributed to *T. pisiformis* formed a distinct lineage showing approximately 90.5% similarity to published reference sequences (MZ287427, NC013844, MW350149), suggesting the possible presence of a cryptic lineage (Fig. 1). Amplification of the *cox1* fragment was unsuccessful, therefore only *nad1* sequences were available.

Both *cox1* and *nad1 T. polyachanta* sequences clustered with a reference sequence from Scotland (EU544635), confirming species identity and indicating limited intraspecific divergence.

Nine *H. kamiyai nad1* haplotypes (HYKA1–HYKA9) and four *cox1* haplotypes (HYKAA–HYKAD) were identified. The most common haplotype, HYKA1, occurred in twelve analysed samples. Phylogenetic reconstruction clearly separated *H. kamiyai* from *H. taeniaeformis* (Figs. 1 and 2).

The obtained *H. taeniaeformis nad1* sequence showed > 99% similarity to reference sequences from Turkey (KC876043) and China (MT784894). Phylogenetic analysis confirmed its position within the *H. taeniaeformis sensu stricto* clade.

Sequences obtained from *Taenia arctos* were genetically identical and formed a distinct, strongly supported clade. Phylogenetic reconstruction based on both *cox1* and *nad1* markers revealed no detectable intraspecific variation among analysed samples (Figs. 1 and 2).

3.2 Dipylidiidae

At least three species from the family Dipylidiidae were identified in the present study, including representatives of the genera *Joyeuxiella* and *Dipylidium* (Table 3).

Species of *Joyeuxiella* were detected in several carnivore hosts, including domestic cats, red foxes, golden jackals and stone martens, indicating a broad host spectrum among felids, canids and mustelids. *Dipylidium caninum* was recorded in domestic cats and a domestic dog (Table 3).

Phylogenetic analyses based on mitochondrial *cox1* and *nad1* gene sequences revealed clear genetic structuring within the family Dipylidiidae, with distinct and well-supported clades corresponding to different lineages of *Joyeuxiella* and *Dipylidium* (Figs. 3 and 4).

Five different *cox1* haplotypes of *Joyeuxiella* spp. were identified (JOAPAA–JOAPAE). Haplotype JOAPAD showed complete identity with *Joyeuxiella pasqualei sensu stricto*, displaying 100% identity with a sequence from the United Arab Emirates (OR081744) and 99.6% identity with an Italian reference sequence (ON981095). Haplotype JOAPAB grouped within a genetically distinct clade designated as *Joyeuxiella aff. pasqualei*, showing 96.78% similarity to sequence OR081744 and 94.06% similarity to ON981095. Haplotype JOAPAA also clustered within this group, sharing 92.93% similarity with sequence ON981095 and 94.26% similarity with sequence PP028783 from the United Arab Emirates (Fig. 3).

The remaining haplotypes (JOAPAE and JOAPAC) showed only 86–88% similarity to sequences from South Africa and Ethiopia and formed separate, highly divergent clades, suggesting the presence of undescribed or geographically distinct *Joyeuxiella* lineages (Fig. 3). These findings were supported by *nad1*-based phylogenetic analyses, which revealed a similar clustering pattern. The obtained sequences grouped with *Joyeuxiella sp.* and *J. aff. pasqualei* clades and aligned with reference sequences previously obtained from the African wildcat (*Felis silvestris lybica*) and rusty-spotted genet (*Genetta maculata*) (Fig. 4.).

The phylogenetic structure of the *nad1* tree confirmed the presence of at least two divergent *Joyeuxiella* lineages circulating in the study area, consistent with the results obtained from the cox1 marker.

Two major haplotypes of *Dipylidium caninum* were identified using both mitochondrial markers, corresponding to previously described feline-associated and canine-associated lineages. The *cox1* haplotype DICA B was detected primarily in cats and showed 99.6% similarity to the feline genotype (MG587892). In phylogenetic reconstructions, this haplotype consistently clustered with feline-associated sequences (Figs. 3 and 4). In contrast, haplotype DICA A was detected exclusively in dogs and showed 99.6–99.76% similarity to the canine genotype (OK523384), but only approximately 90% similarity to the feline genotype. Phylogenetic analyses confirmed its placement within the canine-associated lineage of *D. caninum*.

Discussion

This study provides the most comprehensive molecular and phylogenetic overview to date of taeniid and dipylidiid cestodes circulating in wild and domestic animals in Croatia and Bosnia and Herzegovina. By analysing both definitive and intermediate hosts using mitochondrial markers, we confirmed the presence of nine *Taenia* species along with representatives of *Hydatigera*, *Joyeuxiella*, and *Dipylidium*. The results expand the known biodiversity of cestodes in the Western Balkans and reveal complex, overlapping transmission networks operating across wildlife, livestock, and peridomestic interfaces.

Taeniidae

Red foxes and golden jackals harboured the highest diversity of taeniid species in the present study, confirming their role as key generalist definitive hosts in the region. Their opportunistic feeding ecology and high habitat plasticity expose them to a wide spectrum of potential intermediate hosts, particularly small mammals and ungulates [18, 19]. Although the occurrence of species such as *Taenia hydatigena*, *T. krabbei*, *T. pisiformis*, and *Hydatigera kamiyai* in these hosts could be expected based on their known ecological cycles [20], these parasites have not previously been molecularly confirmed in Croatia and Bosnia and Herzegovina, apart from *T. crassiceps* [13]. Particularly noteworthy is the frequent detection of *T. martis*, a species traditionally considered a parasite of mustelids, in both red foxes and golden jackals. This finding represents a significant expansion of the currently recognized definitive host diversity of this species and provides the first molecular evidence that canids may play a more substantial role in its transmission ecology than previously assumed. Both foxes and jackals frequently prey on small rodents, which have recently been confirmed as intermediate hosts for *T. martis* and other taeniids in neighbouring Serbia [14]. Such trophic interactions likely facilitate transmission and may explain the repeated detection of this parasite in non-mustelid carnivores in our study. These findings suggest that *T. martis* may not be restricted to mustelid–rodent transmission cycles but may circulate within broader carnivore–rodent systems involving multiple generalist definitive hosts. The unexpected detection of fully developed *T. martis* cysticercus in domestic pigs may indicate environmental contamination associated with the movement of wild reservoirs into rural landscapes, highlighting increasing overlap between sylvatic and peridomestic transmission cycles. The relatively high haplotype diversity observed for *T. martis*, together with the absence of host-associated phylogenetic segregation, suggests ongoing transmission and possible microevolutionary diversification within the region.

The occurrence of *T. krabbei* in all examined canid species, with roe deer serving as intermediate hosts, reflects the classical predator–prey transmission cycle widely described across Europe [21]. In contrast, *T. hydatigena* displayed the broadest host spectrum, infecting several definitive hosts and multiple ungulate intermediate hosts. The detection of seven distinct *nad1* haplotypes without host-associated clustering supports its ecological plasticity and continuous circulation across domestic and sylvatic cycles. Phylogenetic reconstruction showed a well-supported monophyletic lineage, with Croatian and Bosnian isolates interspersed within the broader European population, suggesting ongoing gene flow.

Of particular epidemiological relevance is also the finding of *T. crassiceps* in foxes, jackals, domestic dogs, and wild boar. Although the species is typically maintained in rodent–canid cycles, its presence in wild boar indicates broader ecological adaptability and may reflect spillover events linked to complex trophic interactions. The significance of this observation is further emphasized by the zoonotic relevance of *T. crassiceps* [22, 23]. Phylogenetic analyses showed that all isolates clustered within the established European lineage and did not exhibit host-associated structuring, supporting the existence of active cross-host transmission within shared ecological networks.

The identification of *T. arctos* in brown bears, with identical sequences among isolates, confirms previous observations of strong host specificity within this lineage [24, 25, 26]. In contrast to the broader haplotype networks observed in generalist *Taenia* species, *T. arctos* formed a tight and genetically homogeneous cluster, consistent with a host-restricted evolutionary trajectory associated with ursids. The detection of this species in the Western Balkans substantially expands its known geographic distribution and demonstrates that the parasite occurs across a much broader latitudinal range than previously documented.

Importantly, the occurrence of *T. arctos* in this region raises questions regarding its transmission ecology, as the classical intermediate host moose (*Alces alces*) is absent in the study area. The persistence of this parasite in brown bear populations therefore strongly suggests the involvement of alternative intermediate hosts. This hypothesis is further supported by recent findings from Romania [27], indicating that the life cycle of *T. arctos* may not be strictly linked to moose but could involve other cervids or ungulates present in southeastern Europe. Given the phylogenetic proximity within the subfamily Capreolinae, roe deer (*Capreolus capreolus*), which is widespread in the region, represents a plausible candidate intermediate host. Consequently, the key ecological question is no longer whether alternative intermediate hosts exist, but rather which species fulfil this role in maintaining the life cycle of *T. arctos* in regions where moose are absent. The observed genetic homogeneity of the analysed isolates further supports the existence of a stable and locally maintained transmission cycle rather than sporadic introductions.

The detection of *T. polyacantha* and *T. pisiformis* in the present study highlights previously underappreciated diversity of taeniid cestodes in southeastern Europe and reflects the ecological complexity of trophic transmission cycles operating across the region. These species are typically associated with predator–prey interactions involving small mammals and lagomorphs as intermediate hosts, indicating that local carnivore communities exploit a wide spectrum of prey species capable of sustaining multiple taeniid life cycles [21, 28].

The occurrence of these parasites in sympatric carnivore hosts therefore reflects not only the diversity of definitive hosts but also the structure and connectivity of underlying prey communities. *Taenia pisiformis* is classically maintained in carnivore–lagomorph cycles, with canids as definitive hosts and hares or rabbits as intermediate hosts, whereas *T. polyacantha* is primarily associated with rodent intermediate hosts [29, 30]. Together, these patterns illustrate how multiple prey guilds contribute to the maintenance of taeniid transmission within natural ecosystems [28].

The relatively low similarity of *T. pisiformis* sequences to available references may indicate the presence of a genetically divergent lineage, suggesting potential cryptic diversity within this complex. Such findings underscore the importance of molecular surveys in historically understudied regions, where parasite diversity remains insufficiently characterized despite complex and heterogeneous ecological settings.

Felids harboured a narrower spectrum of taeniid species, likely reflecting ecological specialization and the smaller sample size available in this study. Wild cats and domestic cats were infected with *Hydatigera kamiyai* and *Taenia martis*, while *Hydatigera taeniaeformis* was detected exclusively in wildcats, consistent with rodent-based transmission cycles [14, 31, 32].

The detection of *T. krabbei* in felids was unexpected and may reflect occasional scavenging behaviour [33] or overlap in prey communities with sympatric canids, facilitating incidental host switching.

Phylogenetic analyses based on *cox1* and *nad1* sequences revealed largely congruent tree topologies, with strong support for species-level clades and only limited intraspecific geographic structuring. Instead, the occurrence of identical or closely related haplotypes across both countries suggests a continuous regional transmission system maintained by shared wildlife populations, landscape connectivity, and overlapping ecological niches of definitive and intermediate hosts.

Dipylidiidae

Within Dipylidiidae, *Joyeuxiella* spp. displayed notable genetic diversity, supporting previous evidence of cryptic diversity and the limitations of morphology-based identification [34] Although three species are currently recognized *(J. pasqualei, J. gervaisi* [syn. *J. fuhrmanni*], and *J. echinorhyncoides*) [35], the divergent haplotypes identified in this study, particularly among wild carnivores, expand the known diversity in southeastern Europe and suggest that regional lineages may be underrecognized [36]. Phylogenetic analyses based on *cox1* revealed multiple distinct lineages (JOAPA A–D), with some clustering within *J. pasqualei*, while others formed separate subclades associated with wild carnivores. Notably, haplotypes from canids grouped separately from typical felid-associated isolates, indicating potential host-associated structuring and suggesting that different carnivore communities may maintain partially independent transmission cycles [34].

The divergent JOAPA-E haplotype from red fox formed a distinct lineage closely related to sequences from wild canids, further supporting the presence of unrecognized diversity within the genus. Such patterns may reflect ecological structuring of transmission systems or incomplete taxonomic resolution, highlighting the need for multilocus approaches and broader geographic sampling. The observed phylogenetic structure, including relatively long branches and moderate support values, suggests that currently available reference data capture only part of the diversity circulating in wildlife populations.

In contrast, *Dipylidium caninum* showed clear separation into canine and feline genotypes, both present in the study area [8, 37, 38]. The detection of the feline genotype in a dog indicates that these lineages are not strictly host-specific and may be influenced more strongly by the ecology of flea vectors, particularly *Ctenocephalides felis* and *C. canis*, than by definitive host identity. This supports the occurrence of cross-host transmission in environments where domestic animals and wildlife interact [39].

Taken together, these findings indicate that dipylidiid cestodes circulate within interconnected transmission systems involving domestic and wild carnivores, with wildlife likely contributing to the maintenance and diversification of these parasites in southeastern Europe. Such patterns are consistent with broader trends observed in other carnivore-associated cestodes, where generalist hosts and shared ecological niches facilitate parasite circulation across sylvatic and anthropogenic environments.

Conclusion

Beyond *Echinococcus granulosus* and *E. multilocularis*, previously documented in the region [40, 41, 42, 43], the detection of zoonotic *T. martis* and *T. crassiceps* carries direct public health relevance [22, 44]. Both species have been associated with human cysticercosis and

neurocysticercosis in Europe [23, 45]. The presence of these parasites in wildlife, domestic carnivores, and livestock clearly demonstrates overlapping transmission interfaces and highlights the necessity of molecular diagnostics in suspected human cases. Together with the recent detection of *Spirometra mansoni* in golden jackals from Croatia [46], the findings indicate that southeastern Europe represents an active and dynamic interface for cestode transmission. Molecular characterization of human cysticercosis cases is therefore essential, as historical attribution to *T. solium* may mask involvement of other zoonotic *Taenia* species.

The concurrent occurrence of multiple zoonotic cestodes within the same ecosystems further underscores the role of generalist carnivores as key ecological links between transmission cycles. Rather than discrete, host-restricted systems, our results support a model of interconnected transmission networks maintained through ecological overlap, host plasticity, and landscape connectivity. In this context, the Western Balkans emerge as a region of continuous parasite circulation shaped by cross-border gene flow and complex host interactions. To our knowledge, this study provides the first integrative molecular overview of taeniid and dipylidiid cestodes across wildlife, domestic animals, and livestock in the region, establishing a robust baseline for future ecological and One Health–oriented investigations.

Declarations

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Author contributions

RB designed the study. ŠN, AA and JD performed necropsies of studied animals. EG, DJŽ, AŠ, BR, MS performed the laboratory work, dana analysis and draft the manuscript. AH assisted with data analysis and contributed to the drafting and editing of the manuscript. RB was responsible for providing resources and supervision. All authors were involved in writing the manuscript and read and approved the final manuscript.

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Data availability

Data supporting the main conclusions of this study are included in the manuscript.

Ethics approval and consent to participate

All animals collected were killed legally during regular hunting events and under the respective hunting laws. Ethical approval number of the Ethical Committee of the Croatian Veterinary Institute: Z-VI-4-4185-6/23.

Consent for publication

Not applicable.

Competing interests

Adnan Hodžić is a Subject Editor of the journal Parasites & Vectors (‘Parasites of veterinary importance’), but the peer review process and final decision for publication were handled independently by another editor.

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Tables

Table 1. Overview of Taeniidae species diversity and number of analysed parasite specimens across definitive and intermediate hosts. Data do not represent prevalence due to opportunistic sampling.

	Taenia arctos	*Taenia crassiceps*	*Taenia hydatigena*	*Taenia krabbei*	*Taenia martis*	*Taenia pisiformis*	*Taenia polyacantha*	*Hydatigera kamiyai*	*Hydatigera taeniaformis*	Total
Golden jackal *(Canis aureus)*		3	4	17	2	1		3		30
Grey wolf (*Canis lupu)*				2						2
Red fox (*Vulpes vulpes)*		17	1	2	1	1	1	3		36
Dog (*Canis familiaris)*		1	2	1						4
Stone marten (*Martes foina)*					20					20
Hedgehog (*Erinaceus europaeus)*					1					1
European badger (*Meles meles)*					7	1				8
Brown bear *(Ursus arctos)*	19									19
Wild cat *(Felis silvestris)*								11	1	12
Dometic cat *(Felis catus)*				1	2			8		11
Wild boar *(Sus scrofa)*		1	2							3
Goat *(Capra hircus)*			3							3
Sheep (*Ovis aries)*			5					1		6
Roe deer *(Capreolus capreolus)*			2	1						3
Domestic pig *(Sus scrofa domesticus)*			6		1					6
Total	19	22	25	24	34	3	1	26	1	155

Table 2. Host–parasite associations of Taeniidae detected in definitive and intermediate hosts in Croatia and Bosnia and Herzegovina.

	Detected cestodes in final hosts	
Host	**Bosnia & Herzegovina**	**Croatia**
Golden jackal	*T. hydatigena*	*T. krabbei, , T. hydatigena, H. kamiyai, T. crassiceps, T. martis, T. pisiformis*
Red fox	*H. kamiyai, T. polycantha, T. crassiceps,*	*T. crassiceps, H. kamiyai, T. krabbei, T. hydatigena, T. martis, T. pisiformis,*
Dog	*T. hydatigena*	*T. hydatigena, T. crassiceps, T. krabbei*
Grey wolf	*T. krabbei*	
Cat		*H. kamiyai, T. martis, T. krabbei*
Wildcat	*H. kamiyai, H. taeniformis*	*H. kamiyai*
European badger		*T. martis, T. pisiformis*
Stone marten		*T. martis*
Brown bear		*T. arctos*
Hedgehog		*T. martis*
	Intermediate host	
Dog		*T. crassiceps*
Goat		*T. hydatigena*
Roe deer		*T. hydatigena, T. krabbei*
Sheep		*T. hydatigena, H. kamyiai*
Wild boar		*T. hydatigena, T. crassiceps*
Domestic pig		*T. hydatigena, T. martis*

Table 3. Host–parasite associations of Dipylidiidae and genotypes detected in definitive hosts in Croatia and Bosnia and Herzegovina

	Detected cestodes in final hosts	
Host	**Bosnia & Herzegovina**	**Croatia**
Golden jackal		*Joyeuxiella sp. JOAPA3*
Red fox		*Joyeuxiella sp. JOAPA5*
Dog	*Dipylidium caninum 1*	*Dipylidium caninum 1, Dipylidium caninum 2*
Cat		*Joyeuxiella aff. pasqualei JOAPA1, JOAPA2, JOAPA4 , Dipylidium caninum 2*
Stone marten		*Joyeuxiella aff. pasqualei JOAPA1*

Figures

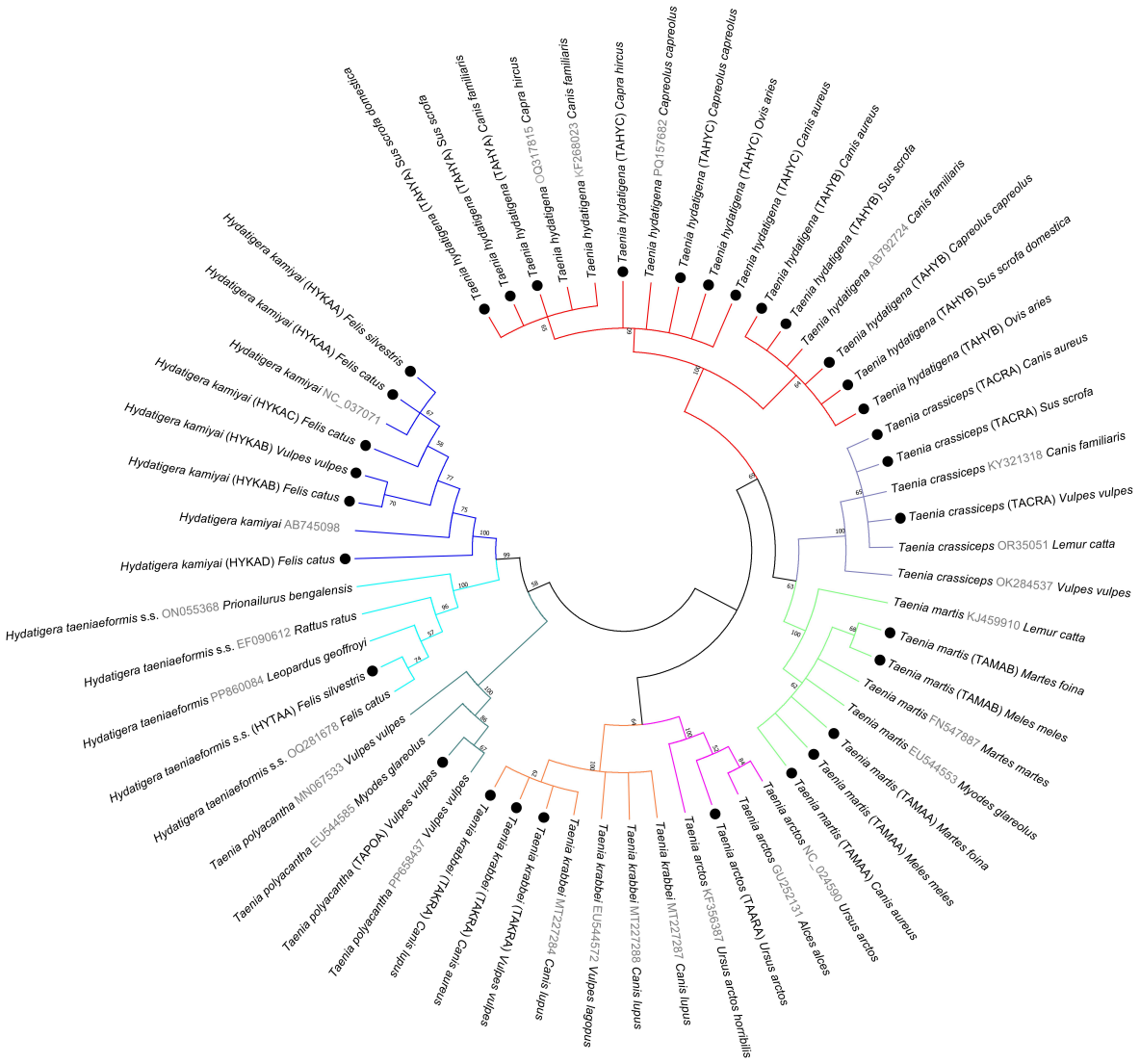


Figure 1

A ML tree constructed from partial mitochondrial *cox1*sequences showing the phylogenetic relationships among Taeniidae species and their haplotypes.. Bootstrap values based on 1,000 replicates are indicated at the nodes (only values over 50% are included). Sequences obtained in this study are indicated by black circles.

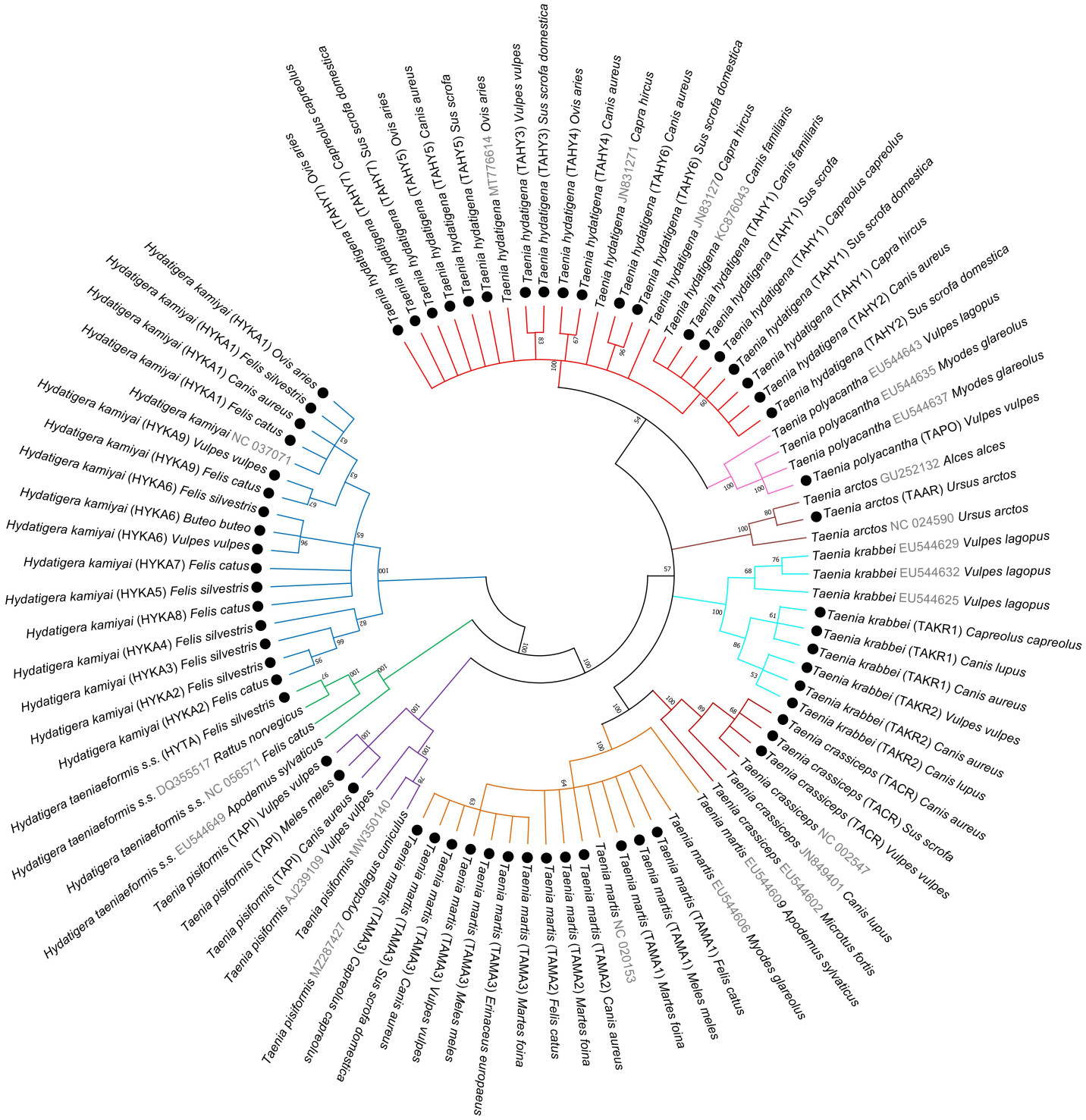


Figure 2

A ML tree constructed from partial mitochondrial *nad1* sequences showing the phylogenetic relatioships among Taeniidae species and their haplotypes. Bootstrap values based on 1,000 replicates are indicated at the nodes (only values over 50% are included). Sequences obtained in this study indicated by black circles.

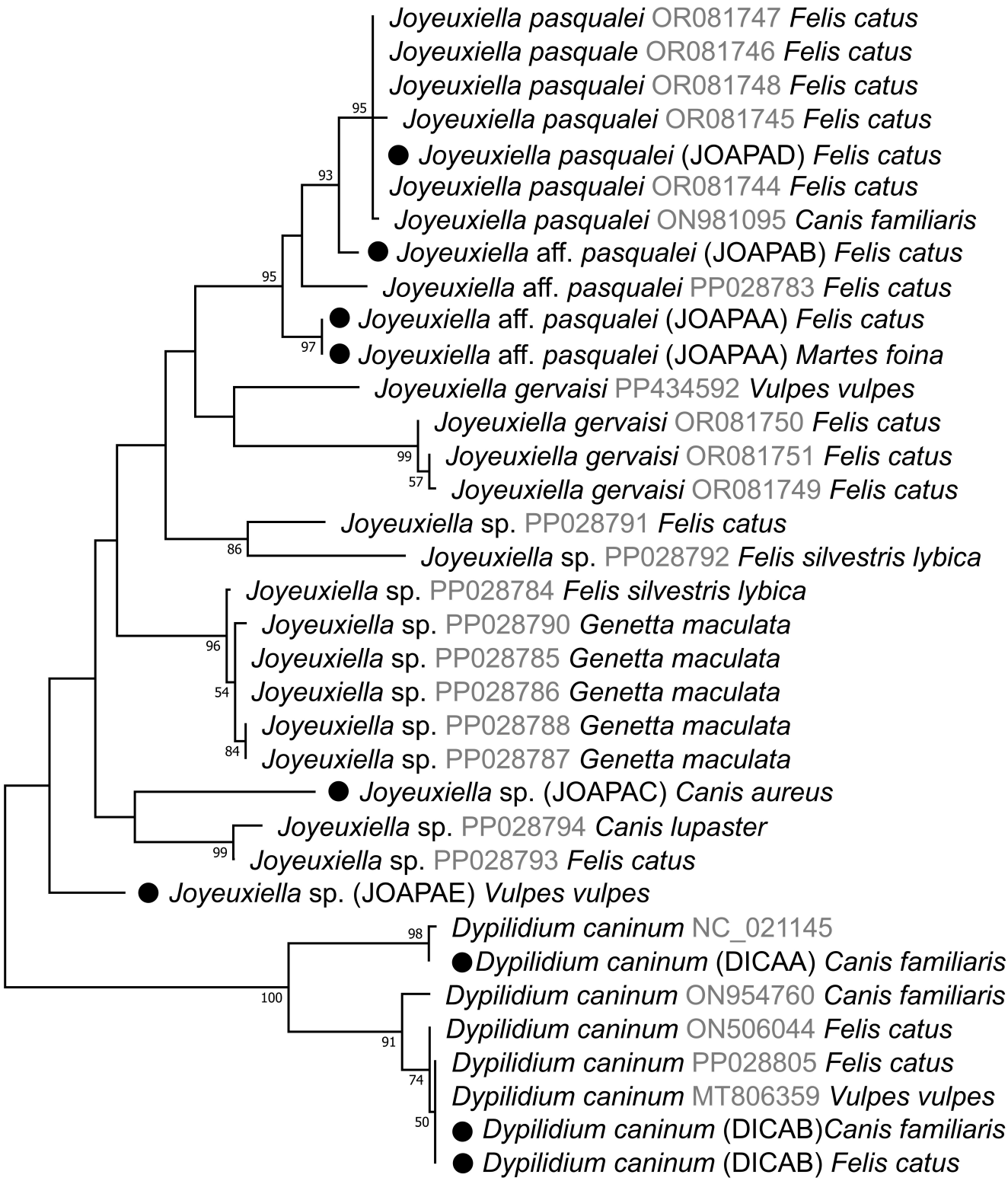


Figure 3

A ML phylogenetic tree constructed from partial mitochondrial *cox1* sequences showing phylogenetic relationships among Dipylidiidae species and their haplotypes. Bootstrap values based on 1,000 replicates are indicated at the nodes (only values over 50% are included). Sequences obtained in this study are indicated by black circles.

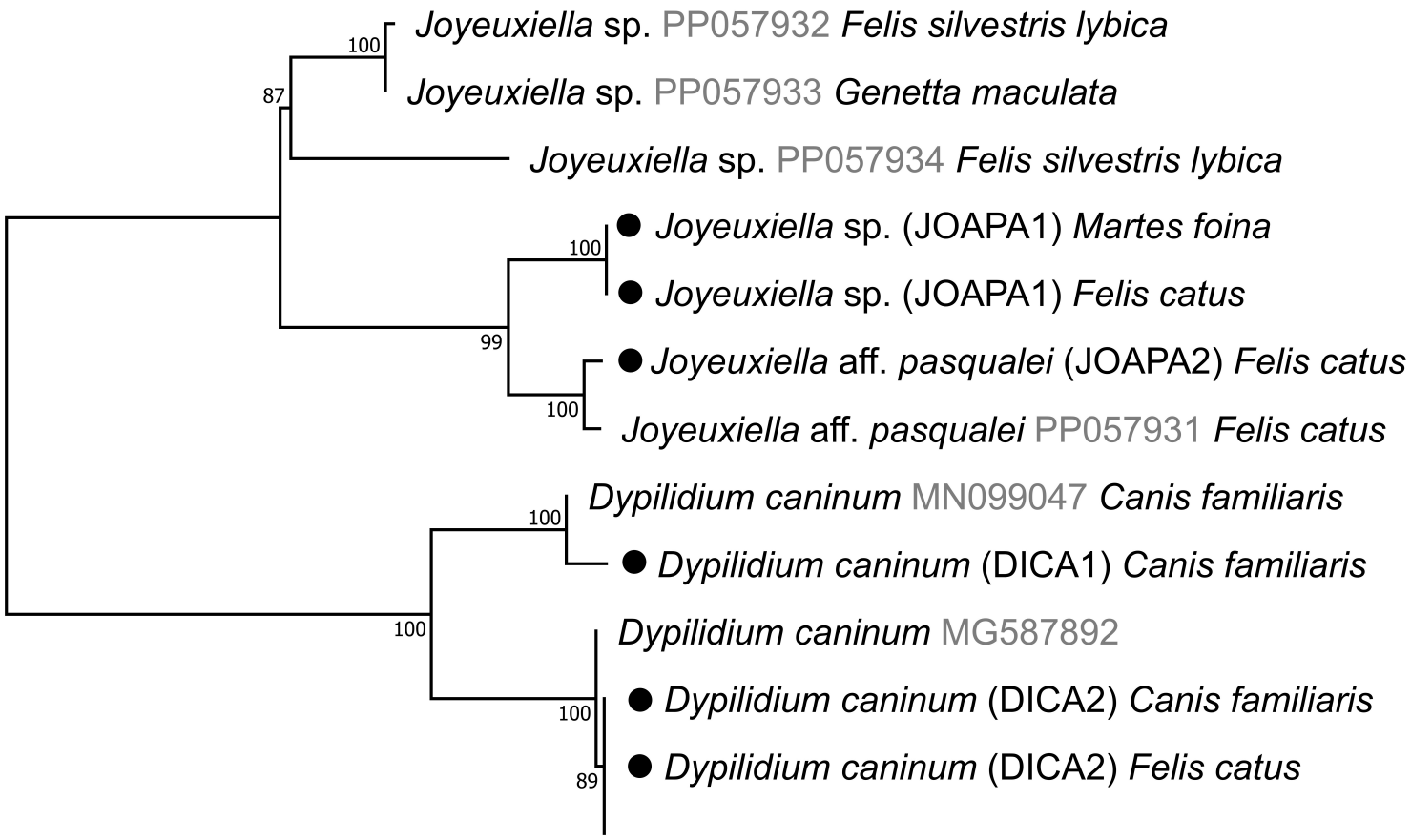


Figure 4

A ML tree constructed from partial mitochondrial *nad1* sequences showing the phylogenetic relationships among Dipylidiidae species and their haplotypes. Bootstrap values based on 1,000 replicates are indicated at the nodes (only values over 50% are included). Sequences obtained in this study are indicated by black circles.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterial1.Table1COI.xlsx
- SupplementaryMaterial.Table2NAD1.xlsx