Molecular characterization of *Fasciola jacksoni* from wild elephants (*Elephas maximus maximus*) of Sri Lanka: a taxonomic evaluation

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Abstract

Fasciola jacksoni is a significant contributor to the health and mortality of Asian elephants, particularly those in Sri Lanka. Despite the impact of fascioliasis on elephant populations it is a neglected veterinary disease with limited taxonomic understanding. Molecular characterization and phylogenetic analysis of *F. jacksoni* was carried out to evaluate its suggested basal position in the Fasciolidae. Adult worms were collected during post mortem of elephants, and eggs were collected from living elephants in National parks across Sri Lanka. Using the mitochondrial genes nicotinamide dehydrogenase subunit 1 (*nad*1) and cytochrome oxidase subunit 1 (*cox*1), and a partial 28S ribosomal DNA (28S rDNA), DNA sequences were generated from the *F. jacksoni* adult and egg material. Maximum likelihood (ML) phylogenetic analyses did not resolve *F. jacksoni* to be basal to the Fasciolidae. Furthermore, the ML analyses showed that the genus *Fasciola* was not monophyletic and that *F. jacksoni* was a sister species to the deer liver fluke *Fascioloides magna.* A clear framework is required to determine the taxonomic status of *F. jacksoni* and this current study provides the first detailed application of molecular techniques from multiple hosts across Sri Lanka with the production of reference DNA sequences for this important parasite.

Keywords: Asian elephants, *Fasciola jacksoni*, Fasciolidae, mitochondrial genes, phylogenetic analysis, rDNA sequences, Sri Lanka.

Introduction

Fasciola jacksoni (Cobbold, 1869) is one of four recognized species within the genus *Fasciola* Linnaeus, 1758 (Cobbold, 1882; Mas-Coma et al., 2009) and a major health problem for elephants in Sri Lanka, causing fatal fascioliasis in wild elephant populations. The size of the natural populations of Sri Lankan Asian elephants (*Elephas maximus maximus*) are estimated to be 2,500–3,000 individuals; approximately 15% of the total number of elephants found across Asia. Sri Lankan elephant herds vary in size with some made up of a few adults, juveniles and calves through to those composed of several hundred individuals in the Forest Reserves, Sanctuaries, National Parks and Orphanages distributed across the country. However, elephants are one of the most endangered large animal species in the world, in part associated with their continued long-term conflict with people for land. In Sri Lanka there is intense human-wild elephant conflict (HEC), resulting in the death of an elephant every two days and a human death every week (Fernando et al., 2005). Each year elephants destroy human properties and crops valued at millions of rupees leaving thousands of people living in financial uncertainty and insecurity (Perera, Rajapakse, 2009; Lorimer, 2010).

Owing to continued HEC elephant numbers have dramatically decreased due to an ever increasing lack of space, as well as infectious diseases (Fernando, 2000; Lorimer, 2010; Perera et al., 2015). Consequently, emerging parasitic diseases, including *F. jacksoni* are now major factors contributed to the dramatic decline of elephant populations (Bhalerao, 1933; Alahakoon, 1994; Perera et al., 2015). Such flukes contribute to the high burden of gastro-intestinal parasitism in elephants, which has led to high mortality and morbidity of calves and adults alike across Sri Lanka (Alahakoon, 1994). Despite *F. jacksoni* being one of the major risk factors contributing to the decrease of wild Asian elephants, the parasite is completely neglected, regardless of continued reports of infection in India, Sri Lanka and Malaysia causing severe pathology (Windsor and Scott, 1976; Caple et al., 1978; Alahakoon, 1994; Islam, 1997; Perera and Rajapakse, 2009; Hing et al., 2013).

The systematics of *Fasciola* and the Fasciolidae has been fiercely debated over the past decade with the majority of studies depending on tenuous and plastic morphological characteristics of both adult flukes and eggs, which has ultimately affected accuracy of identification and diagnosis. Molecular phylogenetic approaches have been employed to resolve the relationships between members of the Fasciolidae illustrating the complex taxonomic associations between members of the genus *Fasciola* and their association with the genus *Fascioloides* (Nolan and Cribb, 2005; Wey-Fabrizius et al., 2013; Tkatch et al.,

2016; Le et al., 2017;). Recently, such approaches have suggested that *F. jacksoni* should be moved to the genus *Fascioloides* and thus renamed as *Fascioloides jacksoni* (Lotfy et al., 2008; Heneberg, 2013). Phylogenetic analyses of the Fasciolidae family have been dependant on the mitochondrial markers cytochrome oxidase subunit 1 gene (*cox1*) and the NADH dehydrogenase subunit 1 gene (*nad1*) as well as the nuclear ribosomal transcription units particularly ITS1, ITS2, 18S rDNA and partial 28S rDNA. These genetic markers have greatly contributed to molecular identification, diagnostic, epidemiological, phylogenetic and evolutionary studies of the Fasciolidae, especially the highly pathogenic *Fasciola hepatica* and *Fasciola gigantica*, the invasive *Fascioloides magna*, and the neglected but re-emerging *Fasciolopsis buski* (Králová-Hromadová et al., 2008; Lotfy et al., 2008; Heneberg, 2013; Mucheka et al., 2015). Yet, despite their economic importance and the impact of fascioliasis on the health of wild elephant populations to date, molecular data associated with *Fasciola jacksoni* is limited (Lotfy et al., 2008; Singh et al., 1994; Perera and Rajapakse, 2009; Heneberg, 2013).

Thus, we present one of the first detailed applications of molecular approaches for the identification and phylogenetic analysis of *F. jacksoni* from wild elephants in Sri Lanka. This study was part of a larger epidemiological survey of *F. jacksoni* infection in National Parks in Sri Lanka and aimed to address the taxonomic position of *F. jacksoni* species in the Fasciolidae and ultimately the Trematoda.

Materials and Methods

Post-mortem pathological examination was performed on a total of 47 elephants during an large scale epidemiological study carried out from January 2000 to April 2003 (39 months) across six districts of Sri Lanka (**Fig. 1; Table 1**) (Perera and Rajapakse, 2009). The liver was opened and separated with scissors, and the bile ducts were examined for the presence of adult *Fasciola*. Flukes were collected from the infected animals, washed with saline several times and fixed with 70% ethanol. Morphological species identification was performed by microscopy after adult flukes were stained with carmine (**Fig. 2**). Total worm burden within the liver was used to assess intensity of the parasitic infestation in the affected animals (Perera and Rajapakse, 2009). In addition, faecal samples from 48 living elephants were also collected randomly from the Maduruoya, Minneriya and Wasgamuwa National Parks (see map in **Fig. 1**), and *Fasciola* eggs were harvested for identification by filtration and examined using standard microscopy and faecal egg count techniques. Eggs were washed and centrifuged several times in normal saline (0.9% NaCl), then a further three times in

phosphate buffered saline (PBS) before storage at –20 °C until use. A total of three to four adult worms from each post-mortem elephant from each locality were individually fixed in 70% molecular grade ethanol. One ethanol-fixed worm and eggs samples from the selected localities were separately subjected to genomic DNA extraction and molecular analysis (**Table 1**).

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from approximately 10 mg lateral tissue from individual adult flukes or pooled eggs (about 1000 eggs) using the GeneJET™ Genomic DNA Purification Kit (Thermo Fisher Scientific Inc., MA, USA), according to the manufacturer's instructions. Genomic DNA was eluted into 50 µl of the elution buffer provided in the kit and stored at –20 °C. Complete gene fragments of both the mitochondrial markers *cox1*, 1.9 kb, and *nad1*, 1.2 kb, where amplified using novel primers designed during this current study (**Table 2**). These were coupled with the amplification of a 1.4 kb portion of 28S ribosomal DNA and additional internal primers were designed and used for sequencing (see Le et al., 2017) (**Table 2**). The PCR reactions were performed in a 50 µl volume containing 25 µl of DreamTaq PCR Master Mix (2×) (Thermo Fisher Scientific Inc., MA, USA) and 2 µl DNA template (50 ng/µl), 2 µl of each primer (10 pmol/µl), 2 µl DMSO (dimethyl sulfoxide) and 17 µl H_2O. All PCRs were performed in a MJ PTC-100 thermal cycler with the following cycling conditions: initiation at 94 °C for 5 min, followed by 35 cycles consisting of denaturation for 30 s at 94 °C, annealing for 30 s [at 52 °C for *nad1* and *cox1*; at 56 °C for 28S rDNA], extension at 72 °C for 3 min; and a final extension at 72 °C for 7 min. The PCR products (10 µl of each) were examined on a 1% agarose gel, stained with ethidium bromide, and visualized under UV light (Wealtec, Sparks, NV, USA). All the purified or gel-extracted PCR amplicons were sent for sequencing (Macrogen Inc., South Korea) using amplifying/flanking and internal primers (**Table 2**) in both directions. All sequences obtained from adult or eggs of *F. jacksoni* samples were identical, regardless of the adult or eggs stage or locality.

Sequence analyses and phylogenetic reconstruction

An alignment of 21 concatenated *nad1* and *cox1* DNA sequences, approximately 2,439–2,457 nucleotides in length, containing representations of trematode isolates from the Fasciolidae including *F. jacksoni* from three localities, the Echinostomatidae and

Schistosoma haematobium as the outgroup (**Supplementary Table S1**) was constructed using GENEDOC 2.7 (available at: http://iubio.bio.indiana.edu/soft/molbio/ibmpc/genedoc-readme.html). The final alignment was 2,470 nucleotide (nt) long of which 21 nt positions were trimmed from either end, leaving 2,449 characters for analyses. Edited alignments were used to perform phylogenetic reconstructions using the maximum likelihood (ML) method in MEGA 7 (Kumar et al., 2016). Pairwise distance analysis, was also performed on the concatenated *nad1* and *cox1* alignment as a measure of genetic distances (*p*-distance) between 12 representative species across 3 families of the Echinostomatoidea (Fasciolidae, Echinochamidae, Echinostomatidae) (**Supplementary Table S1**). DNA sequences of 28S rRNA genes (listed in **Supplementary Table S2**) were also aligned using GENEDOC 2.7. The resultant alignment of 1,349 nucleotide long was edited by the removing 282 nt positions from both ends leaving to a total 1,067 characters for analyses. The final 28S rDNA alignment was composed of 43 species/isolates and MEGA 7 was again used to perform ML phylogenetic reconstruction construction (Kumar et al., 2016). For both alignments MEGA 7 identified the general time reversible GTR + G + I model (gamma rate heterogeneity and a proportion of invariant sites) as the most appropriate model for phylogenetic reconstruction based on the lowest Bayesian information criterion score. Thus phylogenetic analyses for both alignments were performed under the conditions of the above mentioned model with nodal support values calculated using 1000 bootstrap replicates (Kumar et al., 2016).

Results

Cause of mortality of elephants, description and incidence of *Fasciola jacksoni*

The survey was performed on a total of 47 post mortems of elephants across all age groups over the duration of the four-year study period. There were a variety of causes of fatalities of these elephants which included septicaemia associated with gunshot wounds (n=23), gunshot leading to severe damage of the brain and lungs (n=7), electrocution (n=9), drowning (n=1), suffocation through airway obstruction (n=1), severe pneumonia (n=2), old age (n=1), unknown causes (n=1) and fatal infection with *F. jacksoni* (n=2) (Perera and Rajapakse, 2009).

The parasitological investigation revealed that 27 out of the 47 elephant post mortems were infected with *F. jacksoni*, with worm burdens varying from 7 to 325 flukes (**Fig. 2A**). Livers of the affected animals showed cholangitis and fibrous tissue proliferation on the bile duct wall. The liver flukes present in the elephants were identified as typical for *F. jacksoni* species, according to the morphological characters described by Bhalerao (1933). The

average size of each *F. jacksoni* fluke was 12 – 14 mm x 9 – 12.5 mm (**Fig. 3**) and the severity of infection varied greatly between individual elephants. There were 2 elephants with heavy worm burdens (>100 flukes), 4 with moderate (51 – 100 flukes), 7 with mild (10 – 50 flukes), and 14 with low burden (<10 flukes). It was clear that the severity of the *Fasciola* infection was higher in the weaker animals compared to those considered to be healthy. Faecal examination of 48 live animals revealed that 60% of the dung samples harboured eggs of *F. jacksoni* and faecal egg counts in the infected animals were varied from 6 to 30 EPG (eggs per gram).

Phylogenetic analyses of *Fasciola jacksoni* isolates

Four of each representative *F. jacksoni* adult flukes from three localities (Maduruoya, Minneriya and Wasgamuwa National Parks) and faecal eggs from live elephants were subjected to molecular characterization. The *p*-distance calculations of divergence for the concatenated *nad1+cox1* nucleotide sequences of *F. jacksoni* showed the parasites to have the lowest level of divergence with 12.9% *Fa. magna* relative to the 15.0% to 15.6% with other members of the *Fasciola* genus including *F. gigantica, F. hepatica* and an "intermediate" *Fasciola* sp. Within the Fasciolidae *F. jacksoni* had the highest rate of divergence with *Fasciolopsis buski* at 21.9%, however, this rate of divergence increased substantial to 26.2%–27.9% among echinostomids; and at highest rate of 28.2% with *Echinochasmus japonicus*, within the superfamily Echinostomoidea (**Table 3**).

To examine the phylogenetic position of *F. jacksoni* in the family Fasciolidae and in the superfamily Echinostomatoidea, an ML tree was constructed from 21 complete *nad1* and *cox1* nucleotide sequences from 13 trematode species belonging to 4 families including Fasciolidae, Echinochasmidae, Echinostomatidae and *Schistosoma haematobium* of Schistosomatidae as the outgroup (**Fig. 3; Supplementary Table S1**). Sequences of *nad1* and *cox1* from all *F. jacksoni* samples were identical regardless of being generated from adults or eggs and no geographical differentiation was shown. The ML analyses clustered *F. jacksoni* firmly within the Fasciolidae and closely associated with trematodes of ruminants. It was placed in a monophyletic clade as a sister taxon to the European isolate of *Fa. magna* from the Czech Republic and Hungary; while *Fasciola* species and *Fasciolopsis buski* clustered into separate groups, respectively, with strong nodal support of 98–100% (**Fig. 3**). This rendered the genus *Fasciola* polyphyletic as all other species within the genus formed a *Fasciola* specific clade, which contained *F. gigantica*, *F. hepatica* and the intermediate form of *Fasciola* (**Fig. 3**).

The 28S rDNA alignment produced a well-supported phylogeny of 4 families in Echinostomatoidea produced topology for 43 sequences of 33 species, with *S. haematobium* as an outgroup (**Fig. 4; Supplementary Table S2**). Sequences of 16 fasciolid species/isolates were consistently grouped together, forming a discrete Fascioloidae clade, distinct from Philophthalmidae, Echinostomatidae and Echinochasmidae (**Fig. 4**). *Fasciola jacksoni* sequences generated in this study clustered into a single group with a published reference sequence of the same species (Acc: EU025871) (Lotfy et al., 2008). However, the topology of the 28S rDNA placed *F. jacksoni* basal to the *Fasciola* genus, which also included *Fa. magna* as paraphyletic sister taxon to a distinct *F. gigantica*/*F. hepatica* clade, again illustrating a lack of monophyly in the genus *Fasciola* (**Fig. 4**). In Echinostomatoidea, broadly, the family Echinochasmidae clearly is distinct from the cluster formed by Echinostomatidae; Fasciolidae and Philophthalmidae. The *sensu lato Philophthalmus gralli* is closer to Fasciolidae than other echinostomids and echinochasmids with a high nodal support (**Fig. 4**).

Discussion

To date, *F. jacksoni* has been found to be highly host specific residing in the bile ducts of the liver and causing severe fascioliasis only in Asian elephants (*Elephas maximus maximus* and *Elephas maximus indicus*) (Cobbold, 1882; Islam, 1997; Heneberg, 2013; Perera et al., 2015). In this current study, among the 27 infected dead elephants examined, there were 22.2% of individuals with more than 50 flukes (6/27) and only 50% with low worm burdens with less than 10 flukes (14/27). There was a high rate of infection of *F. jacksoni* in living elephants with 60% of the 48 elephants examined, although egg shedding seemed to be moderate, with 6 to 30 EPG. Despite the high burden of infection, generally *F. jacksoni* has low egg counts and has also been shown to have reduced numbers of rediae and cercariae relative to those of *Fasciola* spp infecting ruminants, indicating a low rate of transmission (Islam, 1997; Perera and Rajapakse, 2009). Such low levels of transmission could possibly be related to the infrequent consumption of food contaminated with metacercariae owing to the wide-ranging habit of the elephant, with herds taking advantage of large territories and transmission sites being visited infrequently (Lorimer, 2010; Hing et al., 2016). However, it could be argued that even with low infection and transmission rates, *F. jacksoni* has evolved to by highly virulent with infections of elephants being highly pathogenic causing lesions not only in the liver parenchyma and bile ductules, but also in

other organs such as lungs and kidneys leading to fatalities, particularly in calves (Singh et al., 1994; Islam, 1997; Perera and Rajapakse, 2009; Heneberg, 2013; Perera et al., 2015).

The family Fasciolidae Railliet, 1895 comprises of nine recognized species of which only three are found in the genus *Fasciola* including *F. jacksoni, F. hepatica* and *F. gigantica* which are considered to be taxonomically valid (Mas-Coma et al., 2009). Although listed in the genus *Fasciola*, taxonomic consideration of *F. jacksoni* is limited and its phylogenetic position within the Fasciolidae continues to be debated (Lotfy et al., 2008; Heneberg, 2013). In both molecular phylogenetic reconstructions in this study the members of the genus *Fasciola* collected from ruminants and humans always clustered together in one monophyletic clade that did not include *F. jacksoni,* placing it with other fasciolid species (Lotfy et al., 2008; Le et al., 2008; Nguyen et al., 2009; Mas-Coma et al., 2009; Heneberg, 2013; Tkach et al., 2016). Using three ribosomal markers, 28S rDNA, ITS1, ITS2 and a single mitochondrial *nad1* marker Heneberg (2013) indicated the high similarity of *F. jacksoni* to *Fa. magna* rather than to the other ruminant *Fasciola* spp. group and suggested re-classification of *F. jacksoni* as *Fascioloides jacksoni* comb. nov. In this current study mitochondrial and ribosomal 28S DNA analyses based on increased numbers and longer sequences of the *nad1* and 28S, as well as representations of *cox1*, once again illustrated the close evolutionary relationships of *F. jacksoni* to *Fa. magna* (**Figs 3** and **4**). Similarly, evolutionary distance analyses (shown in **Table 3**) in this study found the lowest rate of divergence (12.9%) between *F. jacksoni* and *Fa. magna,* rather than 15.0–15.6% among the ruminant *Fasciola* spp. and other echinostomatids (around 27%) in Echinostomatoidea. Interestingly, the close phylogenetic relationship between *F. jacksoni* and *Fa. magna* is also supported by several shared morphological characteristics such as a thick body, lack of distinct cephalic cone and have long extensive median intestinal branches as highlighted by Lofty et al. (2008).

The close evolutionary relationship between *F. jacksoni* and *Fa. magna* makes it therefore challenging to disentangle the origins of these two species. *Fascioloides magna* is a parasite of North American cervids and it has been argued that proboscideans brought liver fluke with them into the Neoarctic as they moved from Asia into North America (Lofty et al., 2008). Eventually, the parasite would have possibly underwent a host shift into North Amercian cervids and persisted in deer after the North American elephants became extinct (Lofty et al., 2008). However, only by sampling liver flukes from elephants, antelope and deer across Asia and cervids in the Americas will provide a deeper understanding of the evolutionary relationships of *F. jacksoni* and *Fa. magna* and ultimately resolve the taxonomic

relationship between *F. jacksoni*, the ruminant *Fasciola* spp. and monotypic *Fa. magna* of the genus *Fascioloides.*

The present study revealed the utility of molecular taxonomic approaches for the accurate identification of highly pathogenic liver flukes in Asian elephants from different localities in Sri Lanka. However, despite the accuracy of the species identification it was challenging to resolve the taxonomic relationships of *F. jacksoni* within the Fasciolidae. Molecular data analysed in this study suggested the reappraisal of the family Fasciolidae and recognition of the right taxonomic position of *F. jacksoni* in the superfamily Echinostomatoidea. Such molecular approaches not only provide vital DNA sequence reference data to develop tools but also provide a foundation for a detailed reconciliation of the taxonomy of these parasites, which is crucial to aid in accurate diagnostics and monitoring of the spread of such an important, but neglected disease of wild and domestic elephants in Asia.

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Conflict of interest. None.

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Authors' contributions

Rajapakse, R.P.V.J, Thanh Hoa Le and Scott P. Lawton conceived the study, analyses of final data and wrote the manuscript. K. J. K. Karunathilake, Nga Thi Bich Nguyen conducted laboratory work and preliminary sequence analyses. B.V.P. Perera provided specimens and collected field data. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval

The study had ethical approval from the Sri Lankan Government. Appropriate permission was obtained from the commune authorities before the collection of parasite specimens from elephants.

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Table 1. Information of geographical localities with estimated living elephants for *Fasciola jacksoni* infection survey

Geographical localities	Provinces/areas	Estimated Living Elephants	Geographical Coordinates
Kaudulla National Park	North Central	++	8.161111 N, 80.905 E
Maduru Ova National Park[a]	Eastern/Uva	+++	7.575833 N, 81.142778 E
Minneriva National Park[a]	North Central	+++	7.978889 N, 80.848889 E
Somawathiva National Park	North Central	+++	8.120833 N, 81.168889 E
Udawalawe National Park	Sabaragamuwa/Uva	+++	6.438333 N, 80.888333 E
Wasgamuwa National Park[a]	North Central/Central	++	7.716667 N, 80.933333 E
Yala National Park	Southern /Uva	++	6.372778 N, 81.516944 E
Lunugamwehera National Park	Southern	++	6.383333 N, 81.233333 E

Note: [a]Geolocalities where *Fasciola jacksoni* samples (adult and eggs) were collected; [b]Scientific name: *Elephas m. maximus*: *Elephas maximus maximus*; [c]Estimated numbers of living elephants in the area: +: <50; ++: 50-250; +++: >250 elephants based on the information in public media and statistics (see map of living areas for elephants in **Fig. 1**), geographical coordinates provided in latitude and longitude.

Table 2. Primers for amplification and sequencing of the mitochondrial protein-coding and nuclear ribosomal genes used in this study

Primer name	Sequence (5'–3')	Target gene	Amplicon by PCR	Length of Sequence used	Reference
FJND1F	CATTGCGAGGACGGTGTAGT	*nad*1	1.2 kb	903 bp	This study
FJND1R	AATACCGTACACGGGCAACA				
FJCO1F	CGGGGGTATGTTCGTTGGAG	*cox*1	1.9 kb	1,554 bp	This study
FJCO1R2*	AAGTGAGCCACCACAAACCA				
FJCO1R	ATCAGTATCCTTCGGATACCCC				
U28SF	CTAACAAGGATTCCCTTAGTAAC	28S	1.3 kb	~1,100 bp	Le et al., 2017
U28SR	GTCTTTCGCCCCTATACTCAC				

Abbreviations: F, forward; R, reverse; *Internal primer used for sequencing.

Table 3. Estimation of pairwise genetic distances (%) between *Fasciola jacksoni* and the published or GenBank deposited representative species of the superfamily Echinostomatoidea (Fasciolidae, Echinochasmidae, Echinostomatidae) inferred from the concatenated nucleotide sequence of mitochondrial *nad1* and *cox1*

Nucleotide sequences[a]		No. of base substitutions/site in sequences[b]													
		Accession No	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Fjac-Maduru-LK	KX787886		0.007	0.010	0.009	0.009	0.008	0.012	0.012	0.013	0.014	0.015	0.015	0.015	0.015
2	Fmag-Kokorinsko-CZ	KU060148	0.129		0.010	0.008	0.008	0.008	0.013	0.013	0.012	0.015	0.016	0.016	0.016	0.016
3	Fbus-Jiangxi-CN	KX169163	0.221	0.224		0.010	0.011	0.009	0.012	0.013	0.012	0.015	0.016	0.016	0.016	0.016
4	Fgig-Guangxi-CN	KF543342	0.152	0.150	0.205		0.003	0.006	0.011	0.012	0.011	0.014	0.014	0.015	0.015	0.015
5	Fsp-GHL-CN	KF543343	0.151	0.147	0.210	0.025		0.006	0.011	0.012	0.012	0.014	0.014	0.015	0.015	0.015
6	Fhep-Geelong-AU	AF216697	0.157	0.153	0.197	0.096	0.100		0.011	0.012	0.013	0.015	0.015	0.016	0.015	0.016
7	Ecap-SAMEA-EG	AP017706	0.265	0.263	0.270	0.250	0.252	0.259		0.009	0.013	0.014	0.016	0.015	0.016	0.015
8	Epar-UNM-MX	KT008005	0.269	0.286	0.279	0.252	0.252	0.257	0.144		0.013	0.015	0.016	0.016	0.017	0.016
9	Hcon-Hubei-CN	KM111525	0.257	0.261	0.265	0.246	0.245	0.236	0.257	0.250		0.015	0.017	0.016	0.016	0.016
10	Cmic-Hunan-CN	KR337555	0.347	0.365	0.346	0.339	0.340	0.332	0.369	0.371	0.367		0.008	0.008	0.008	0.007
11	Eexp-Hunan-CN	KT198989	0.352	0.358	0.362	0.334	0.332	0.340	0.372	0.383	0.392	0.134		0.009	0.007	0.008
12	Ostr-Tianmen-CN	KM659177	0.367	0.371	0.354	0.343	0.347	0.351	0.389	0.387	0.396	0.152	0.153		0.008	0.009
13	Pcer-Qinghai-CN	KF475773	0.333	0.339	0.350	0.331	0.330	0.324	0.364	0.376	0.371	0.125	0.131	0.154		0.008
14	Pley-Nimu-CN	KP341657	0.344	0.346	0.350	0.330	0.332	0.337	0.373	0.379	0.380	0.142	0.130	0.152	0.129	

Note: [a]Sequence abbreviation is listed in **Supplementary Table S1**; [b]The percentage representing number of base substitutions per site from all sequence pairs between species is shown. Genetic distances were inferred by the analysis of 2,439-2,457 nucleotides of the *nad1* and *cox1* genes, conducted using the Maximum Likelihood method in MEGA 7 (Kumar et al., 2016). Nodal values were obtained by a bootstrap procedure (1000 replicates).

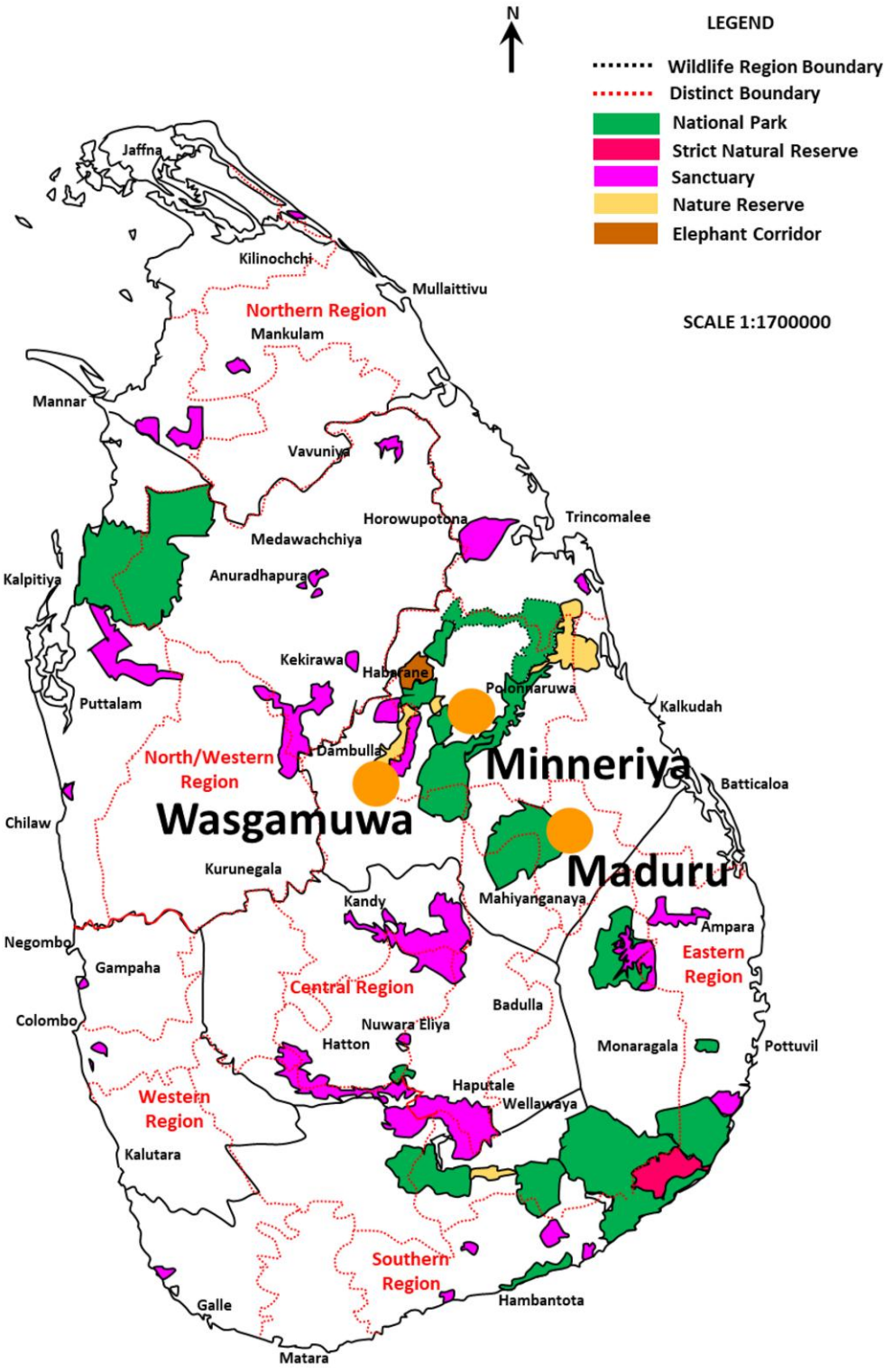

Figure 1. Schematic map of living elephants in Sri Lanka and the study localities of National Parks where *Fasciola jacksoni* samples were collected (indicated by solid circles).

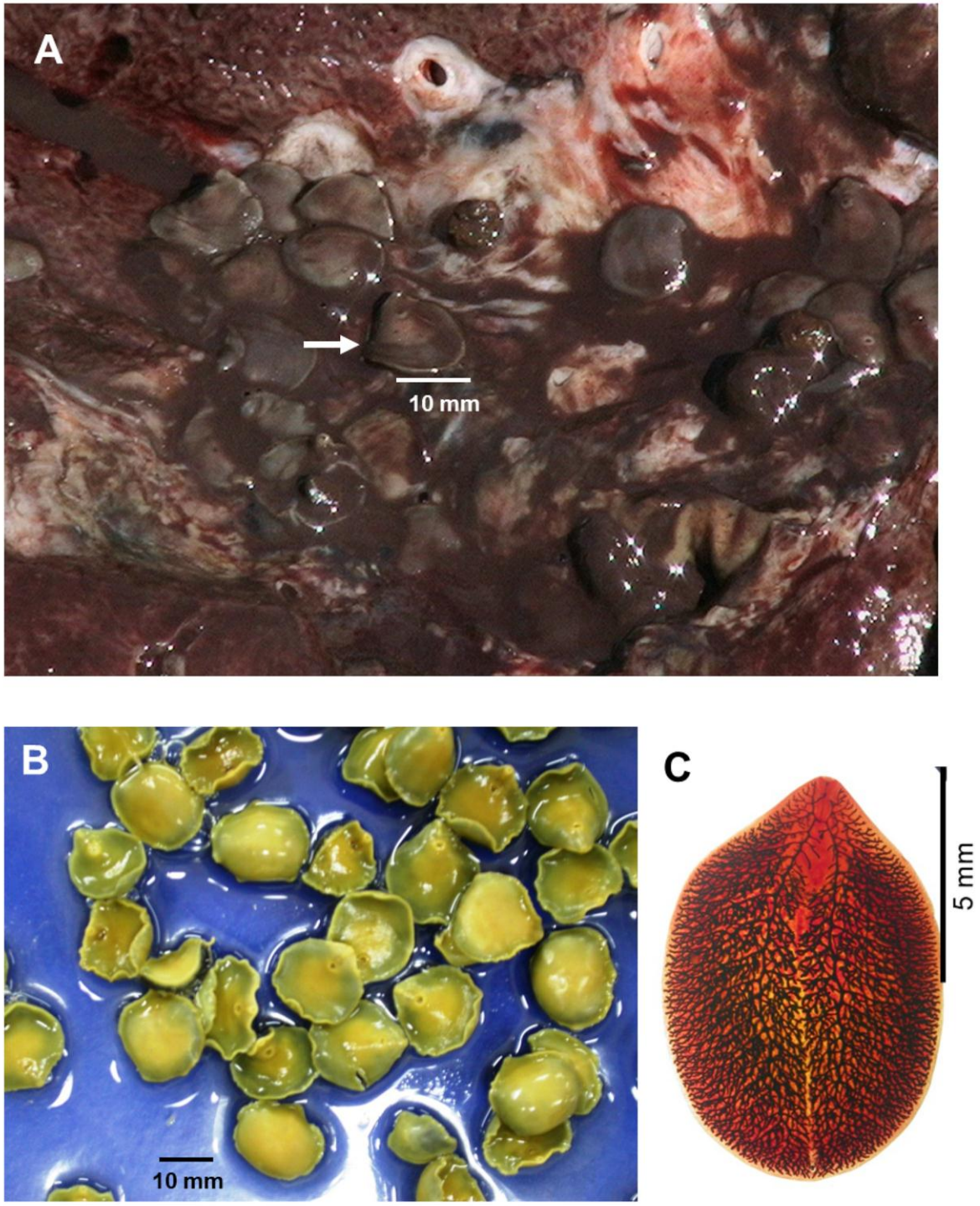

Figure 2. Adult *Fasciola jacksoni* collected from elephants. Where A) are adult *Fasciola jacksoni* flukes (indicated by arrow) in the bile duct of the infected liver in a dead elephant. Bar (10 mm) indicates size of the flukes; B) are adult flukes collected from bile duct of the liver from a post-mortem elephant in Sri Lanka and illustrate gross morphology; c) an adult *F. jacksoni* fluke stained (5X) with carmine. Bars indicate size of the flukes. Bars (100 mm and 5 mm) indicate size of the flukes.

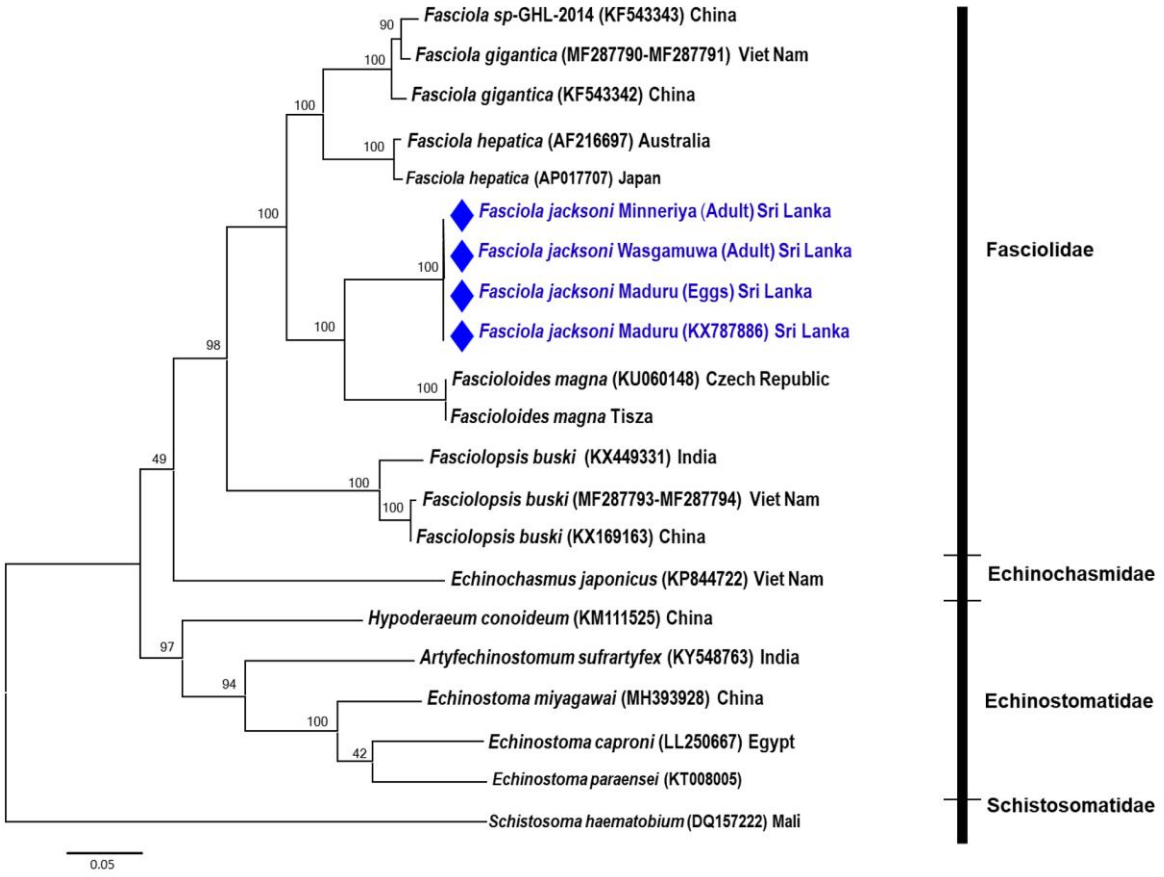

Figure 3. Maximum likelihood phylogenetic reconstruction showing the position of *Fasciola jacksoni* within the Fasciolidae based on concatenated cox1 and nad1 DNA sequences. Three trematode families are represented Fasciolidae, Echinostomatidae, Echinochasmidae, and the position of *F. jacksoni* indicated by a diamond symbol. Nodal support values are shown based on 1000 bootstrap replicates.

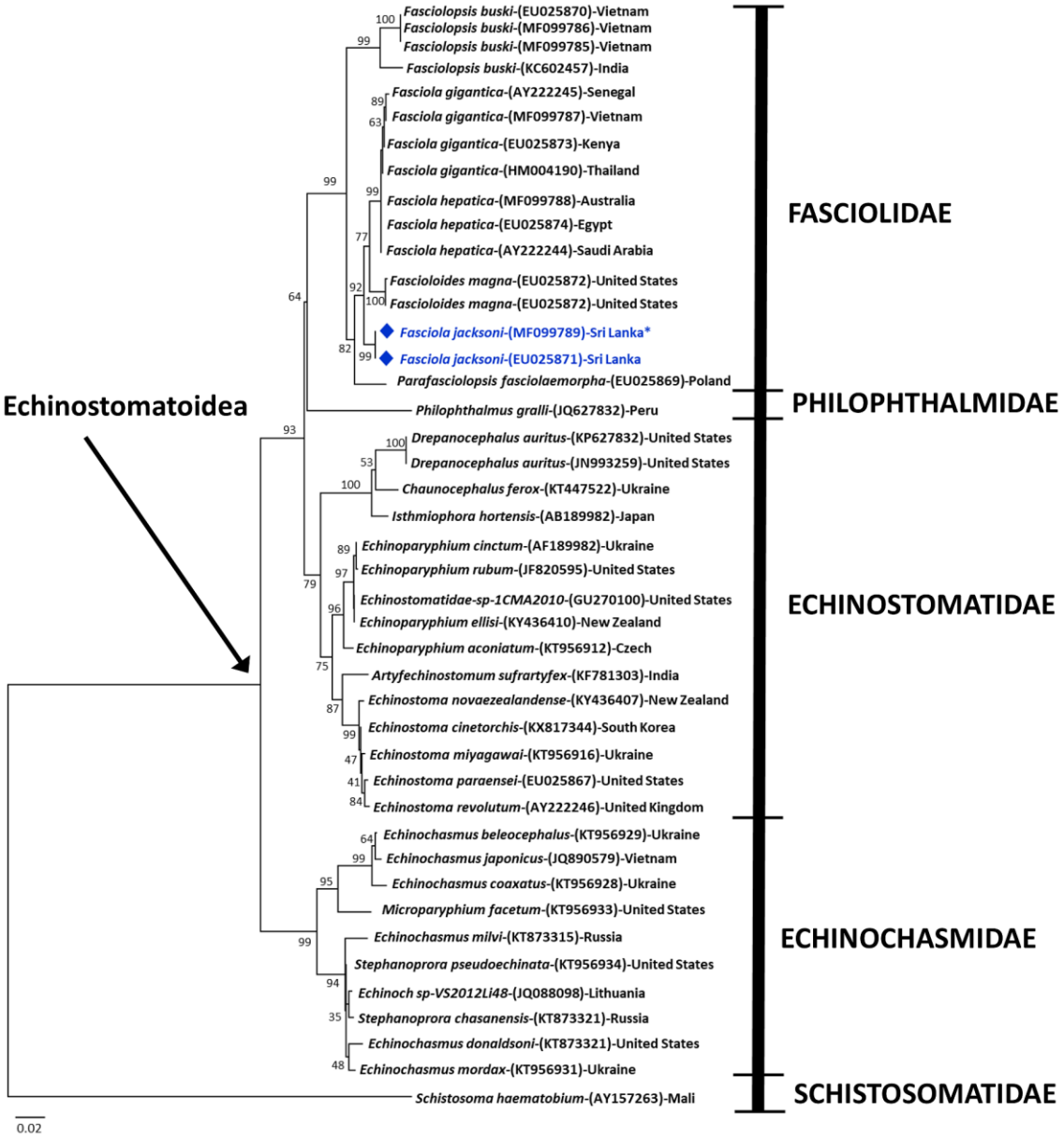

Figure 4. Maximum likelihood phylogenetic reconstruction showing the position of *Fasciola jacksoni* within the Fasciolidae based on partial 28S rDNA sequences. Four families of the superfamily Echinostomatoidea (Fasciolidae, Philophthalmidae, Echinostomatidae, Echinochasmidae) are represented and the position of *Fasciola jacksoni* is indicated by a diamond symbol. Nodal support values are shown based on 1000 bootstrap replicates.

SUPPLEMENTARY INFORMATION

Supplementary Table S1. Source of nicotinamide dehydrogenase subunit 1 (*nad*1) and cytochrome oxidase subunit 1 (*cox*1) sequences to be used for phylogenetic/genetic distance analysis of *Fasciola jacksoni* collected from elephants in Sri Lanka and other trematodes

[a]Sequences species of Echinostomatoidea selected for use for estimation of genetic distance (**Table 3**).
[b]Sequence used as an outgroup.

Supplementary Table S2. Information list of trematodes of the superfamily Echinostomatoidea providing 28S rDNA sequences for phylogenetic analysis and taxonomic relationships of *Faciola jacksoni* and other trematodes