Published in final edited form as:

J Parasitol. 2011 April ; 97(2): 305–310. doi:10.1645/GE-2535.1.

Comparison of the ITS1 and ITS2 rDNA in *Eimeria callospermophili* (Apicomplexa: Eimeriidae) from Sciurid Rodents

Dagmara Motriuk-Smith, R Scott Seville, Leah Quealy, and Clinton E. Oliver

Department of Zoology and Physiology, University of Wyoming- Casper College Center, 125 College Drive, Casper, Wyoming 82601

R Scott Seville: sseville@uwyo.edu

Abstract

The taxonomy of the coccidia has historically been morphologically based. The purpose of this study was to establish if conspecificity of isolates of *Eimeria callospermophili* from 4 ground-dwelling squirrel hosts (Rodentia: Sciuridae) is supported by comparison of rDNA sequence data and to examine how this species relates to eimerian species from other sciurid hosts. *Eimeria callospermophili* was isolated from 4 wild caught hosts, i.e., *Uroditellus elegans*, *Cynomys leucurus*, *Marmota flaviventris*, and *Cynomys ludovicianus*. The ITS1 and ITS2 genomic rDNA sequences were PCR generated, sequenced, and analyzed. The highest intraspecific pairwise distance values of 6.0% in ITS1 and 7.1% in ITS2 were observed in *C. leucurus*. Interspecific pairwise distance values greater than 5% do not support *E. callospermophili* conspecificity. Generated *E. callospermophili* sequences were compared to *Eimeria lancasterensis* from *Sciurus niger* and *Sciurus niger cinereus*, and *Eimeria ontarioensis* from *S. niger*. A single well-supported clade was formed by *E. callospermophili* amplicons in Neighbor Joining and Maximum Parsimony analyses. However, within the clade there was little evidence of host or geographic structuring of the species.

The taxonomy of *Eimeria* spp. (Apicomplexa: Eimeriidae) has traditionally been based on the morphology of the infective oocyst and the host range of the isolate under consideration. Unfortunately for eimerian species, the number of morphological characteristics is limited and determination of host ranges via experimental infections for species from wild hosts is rarely attempted. Captive rearing for many wild species is not possible and determining infection history and immune status for wild caught subjects is similarly difficult. When attempted, results of cross transmission experiments have often been equivocal (Todd and Hammond, 1968a, 1968b; Todd et al., 1968). This has resulted in a taxonomy for the genus based on the morphospecies concept, with many untested assumptions regarding host specificity. Thus, morphologically similar species (cryptic species) may be identified as a single species when host specificity is assumed to be broad, or similar species are synonymized when host specificity is thought to be narrow. Recognizing this issue, Wilber et al. (1998) reviewed all published descriptions for species reported from rodents in the sciurid Tribe Marmotini. Because numerous species had similar descriptions and prior studies indicated eimerian host ranges in marmotine rodents are broader than previously recognized, they reduced the number of valid eimerian species from 40 to 26.

The development and decreasing costs of molecular based approaches have provided the opportunity for using molecular characteristics to delineate morphologically similar species and synonymize morphologically variable species from related hosts, and for the development of rigorous phylogenetic hypotheses regarding eimerian evolutionary relationships. At the species level, a number of studies have focused on using the non-

conserved rDNA internal transcribed spacers (ITS) 1 and 2 as genetic characters that could resolve taxonomic difficulties and serve as species bar codes. Hnida and Duszynski (1999) compared ITS1 sequences from morphologically similar eimerians from closely related murid rodents and proposed that similar species may be distinguished when maximum likelihood pairwise distances for ITS1 are greater than 5% between isolates. However, they emphasized that this “yardstick” needs further verification and should not be the only criterion for delineating morphologically similar species, but should be considered along with life history and ecological characteristics, and monophyly of the species under study. *Eimeria callospermophili* Henry, 1932 is a widely distributed species infecting a variety of ground-dwelling sciurids (Rodentia: Sciuridae) from Europe and in North America from Mexico to Alaska (Wilber et al., 1998). To determine if conspecificity of the morphospecies *E. callospermophili* and the assumption of wide host specificity is supported with molecular characters, the ITS1, ITS2, and 5.8S rDNA sequences were amplified and sequenced. The sequences reported in this study were obtained from *E. callospermophili* from 4 host species, including the Wyoming ground squirrel (*Urocitellus elegans*), white-tailed prairie dog (*Cynomys leucurus*), black-tailed prairie dog (*Cynomys ludovicianus*), and yellow-bellied marmot (*Marmota flaviventris*) to examine sequence variation among these hosts over a limited geographic distribution.

Materials and Methods

Sample collection and identification

Fecal samples were collected from Wyoming ground squirrels and white-tailed prairie dogs (collected in Natrona Co., Wyoming), yellow-bellied marmots (Teton Co., Wyoming) and black-tailed prairie dogs (Larimer Co., Colorado) during 2006-2008. Samples were preserved in 2% aqueous (w/v) $K_2Cr_2O_7$, stored in Petri dishes and allowed to sporulate at 23-25 C for 14 days. A significant portion of each fecal sample was examined prior to DNA extraction. Images of *E. callospermophili* were captured at 1,000 $\times$ magnification, standard morphologic parameters were identified, measured, and compared to published data. Samples containing oocysts of only *E. callospermophili* were selected and transferred to 15-ml conical tubes, washed with distilled water, and centrifuged at 2,000 RPM. This procedure was repeated 5 times. After washing, oocysts were suspended in 1.9 M sucrose solution and centrifuged again at 2,000 RPM. The lids of the tubes were removed and 18 $\times$ 18-mm cover slides were placed on the top of the positive meniscus for 5 min. Cover slides with oocysts attached on the bottom were repeatedly rinsed with distilled water into a new 50-ml conical tube until there were no oocysts present on the cover slide. Oocysts in distilled water were centrifuged at 2,000 RPM for 5 min and 2 ml of distilled water were removed carefully from the top of the tube so that the oocyst pellet on the bottom was not disturbed. This process was repeated until the volume of the sample was 1.5 ml. Samples were transferred to 1.6 ml-tubes and centrifuged at 4,000 RPM for 5 min. One ml of the sample was carefully removed and the oocyst pellets on the bottom were gently suspended in the remaining 0.5 ml of distilled water from which 2 μ l was transferred to a slide and examined using a microscope. Samples representing 10,000-20,000 *E. callospermophili* oocysts from the four hosts were selected for genomic DNA extraction.

Genomic DNA isolation, PCR, cloning, and sequencing

Oocyst pellets were washed and centrifuged 4 $\times$ with high-salinity PBS. They were treated with 200 μ l of 6% NaClO on ice for 30 min. The oocysts were rinsed with 1 ml of sterile H_2O and washed 3 $\times$ with high-salinity PBS (Zhao et al., 2001). DNA was isolated using the DNeasy® Tissue kit (Qiagen, Germantown, Maryland) and precipitated using 3M $NaCH_3COO$ and 95% C_2H_5OH . The DNA pellet was re-suspended in 20 μ l of sterile H_2O . Five μ l of DNA was used as a template in PCR reaction. PCR amplification was carried out

using Platinum® Taq DNA Polymerase (Invitrogen, Carlsbad, California). The final concentration of each of the PCR reaction components (total volume 20 µl) was: 1X PCR buffer, 0.2 mM each of the nucleotides, 1.5 mM MgCl₂, 0.2 µM each of the primers, and 0.5 unit of Taq. Each PCR reaction contained a negative control which was lacking the genomic DNA, but included all other reaction components. Amplification conditions were: initial denaturation at 95 C for 10 min, repeated 35 cycles of denaturation at 95 C for 1 min, annealing at 50-59 C (depending on species) for 30 sec, and extension at 72 C for 1 min 30 sec, and a final extension at 72 C for 7 min. The ITS1-ITS2 coccidian specific primers used were forward primer 5'-GGATGCAAAAGTCGTAACACG-3', and reverse primer 5'-TCCTCCGCTTAATAATATGC-3' (Schwarz et al., 2009).

PCR products were visualized on 0.8% agarose gel, purified and the amplicons were ligated into the pCR-XL-TOPO vector (Invitrogen). The ligation product was transformed into TOP10 electrocompetent cells (Invitrogen). Plasmid DNA was purified using the QIAprep Spin Miniprep Kit (Qiagen). The presence of inserts was verified by EcoRI restriction digest and gel electrophoresis. The final concentration of plasmid DNA was determined by UV spectroscopy. M13F and M13R primers were used in sequencing reactions using BigDye Terminator v3.1 (Applied Biosystems, Foster City, California). Sequencing was carried out using the Applied Biosystems 3130xl genetic analyzer by the Nucleic Acid Exploration Facility at the University of Wyoming. Chromatograms were edited manually and contiguous sequences were created with ChromasPro v1.34 (Technelysium Pty. Ltd. Tewantin, Queensland, Australia, <http://www.technelysium.com.au/ChromasPro.html>) and used in further analysis. Ambiguous sequences were eliminated from the study.

Sequence analysis

There were 36 sequences identified (GenBank accession numbers: HM241616-HM241651), i.e., 12 from white-tailed prairie dogs (amplicons Cleu1-Cleu12), 9 from black-tailed prairie dogs (Clud1-Clud9), 4 from yellow-bellied marmots (Mfla1-Mfla4), and 11 from Wyoming ground squirrels (Uele1-Uele11). Each sequence was divided into segments ITS1, 5.8S or ITS2 rDNA region only. BLAST searches of the individual segments were performed using a nucleotide blast program optimized for highly similar sequences. Sequences were imported to MEGA v4 (Tamura et al., 2007) and aligned. Constant and variable sites were determined. Pairwise distances values were calculated using the Maximum Composite Likelihood method. ITS1 and ITS2 sequences from *Eimeria ontarioensis* (EU302682-EU302686) and *Eimeria lancasterensis* (EU302672- EU302681) from fox squirrels (*Sciurus niger*) were included in the Maximum Parsimony (MP) and Neighbor Joining (NJ) analyses, as well as *Eimeria tenella* (AF446074.1, AM922247.1) and *Eimeria necatrix* (AF446068.1, AM922243.1) from chickens as outgroups. Sequences alignments were created using ClustalW with default settings. Duplicate sequences were eliminated. The alignments were examined by eye, obvious misalignments were manually adjusted, and sequences were truncated if necessary to improve the alignment appearance. Gap only sites were eliminated from the alignment. MEGA v4 (Tamura et al., 2007) was used to create NJ and MP trees. The overall distance mean (d=0.369) was computed using the Maximum Composite Likelihood model (Nei and Kumar, 2000). NJ trees (Saitou and Nei, 1987; Tamura et al., 2004) were created using the Maximum Composite Likelihood method. A bootstrap test of phylogeny was computed based on 1,000 replicates (Felsenstein, 1985). The tree was rooted with *E. tenella* and *E. necatrix*. MP analyses were performed using close-neighbor-interchange method with the search level set to 3. The consensus tree was computed, bootstrapped with 1,000 replicates, and rooted with *E. tenella* and *E. necatrix* sequences. The 5.8S rDNA sequences had insufficient variation for this analysis.

Results

BLAST searches of 5.8S resulted in the best match to *Eimeria papillata*, *E. lancasterensis*, and *E. ontarioensis*, thus confirming the identity of the *Eimeria* genus. BLAST searches of ITS1 and ITS2 regions resulted in no significant homologies. ITS1 and ITS2 *E. callospermophili* sequence diversity is summarized in Table I. ITS1 sequence size ranged from 296 to 302 nucleotides in Clud2 and Cleu12, respectively. Sequence identity within an individual host infection ranged from a low of 91.1% in Cleu to a high of 99.3% in Mfla. A and T nucleotides were predominant in each sequence with the highest average (61.6%) in Cleu. ITS1 pairwise distance values within a single host infection were the highest in Cleu with a high of 6.0% between Cleu4 and Cleu12. Three pairs of identical ITS1 sequences were identified, i.e., Cleu1 and Cleu8, Mfla3 and Mfla4, and Uele5 and Uele7. ITS1 comparison of sequences from separate infections showed that Cleu1, Cleu8, and Uele4 also shared 100% identity. The highest maximum likelihood pairwise distance (8.3%) was between Clud6 and Uele10. The alignment of all ITS1 sequences was 304 nucleotides long and showed 81% identity. There were 58 variable sites noted with 26 positions designated as singletons (Fig. 1). The NJ analysis for ITS1 placed all *E. callospermophili* sequences in a monophyletic branch with 100% bootstrap support. Within the group all Mfla sequences formed a distinct clade with 62% bootstrap support. Likewise, Cleu2 and Cleu12 formed a single clade with 73% support. The NJ analysis was not able to resolve relationships among other *E. callospermophili* ITS1 sequences and, in particular, Cleu and Uele sequences were scattered throughout the phylogeny. A second branch was comprised entirely of tree squirrel sequences with *E. ontarioensis* and *E. lancasterensis* forming weakly supported sister groups (Fig. 2).

ITS2 sequence sizes varied from 616 nucleotides in Cleu1 and Cleu8 and Uele to 633 in Cleu12 and Clud9. Sequence identity within an individual infection ranged from 87.7% in Cleu to 100% in Mfla. A and T frequencies varied from a low of 59.3% in Uele to a high 61.6% in Cleu (Table I). ITS2 sequences designated as Cleu5 and Cleu8 as well as Uele3 and Uele4 had pairwise distance values equal to 0. All 4 Mfla ITS2 sequences also had distance values equal to 0. The highest value for ITS2 pairwise distances within an individual host was 7.1% between Cleu1 and Cleu12. The highest pairwise distance value overall (8.1%) was between Clud1 and Uele9. Overall, the ITS2 alignment for all sequences was 669 nucleotides with 79% identity. There were 137 variable sites and 54 singletons (Fig. 3). Maximum Parsimony (MP) analysis of ITS2 sequences resulted in a monophyletic clade comprised of all *E. callospermophili* sequences from rodents with 99% bootstrap support. Within this clade, all Clud sequences formed a single lineage with 88% bootstrap support, while other ITS2 sequences from other hosts were not well resolved. *E. ontarioensis* ITS2 sequences formed a sister group to *E. callospermophili* and *E. lancasterensis* formed a monophyletic clade with 99% bootstrap support (Fig. 4). The alignment of all 5.8S sequences from all hosts resulted in 157 nucleotides with only 7 variable sites and 6 singletons.

Discussion

The rapidly evolving ITS1 variable region has been used in numerous studies of coccidian parasites (Marsh et al., 1999; Šlapeta et al., 2002; Gondim et al., 2004; Sreekumar et al., 2004) to serve as molecular marker to distinguish closely related species and strains. Intraspecific variation was not detected in different isolates of *Toxoplasma gondii* (Homan et al., 1997), *Besnoitia* sp. found in cattle, wildebeests, and goats (Ellis et al., 2000) and *Isoospora* sp. infecting camels in Kenya and Dubai (Bornstein et al., 2008). In contrast, *Eimeria* spp. parasitizing chickens were shown to possess sufficient variability within ITS1 and ITS2 regions to construct phylogenies separating species and strains although high

sequence variation within species and strains was reported. *Eimeria maxima* ITS1 and ITS2 were found to possess long and short variants and phylogenetic analyses showed that each of the variants formed a distinct clade (Barta et al., 1998; Lew et al., 2003; Cantacessi et al., 2008; Schwarz et al., 2009). Results of comparisons of the ITS1 and ITS2 rDNA sequences presented here suggest that isolates of the morphospecies *E. callospermophili* from a variety of ground dwelling sciurid hosts form a distinct clade separate from isolates of *E. lancasterensis* and *E. ontarioensis* from fox squirrels. However, within the ground dwelling sciurid host clade, MP and NJ analyses were unable to resolve isolates based on host species except the ITS2 sequence that resolved isolates from black-tailed prairie dogs collected in northern Colorado.

Previous studies (Motriuk-Smith et al., 2009) have supported the hypothesis proposed by Hnida and Duszynski (1999) that pairwise distance values for ITS1 sequences less than, or equal to, 5% support conspecificity of similar morphotypes from closely related hosts, and values greater than 5% may be used to resolve separate species. In general, our results do not support the 5% criterion. For *E. callospermophili* from marmotine rodents across a limited geographic range, there is considerable variation in ITS1 and ITS2 sequences within isolates from a single infected host and among isolates from different hosts of different species and geographic locations. We observed pairwise distance values greater than 5% for both ITS1 and ITS2 sequences from *E. callospermophili* clones from infections within a single host and values as high as 8.3% between isolates from a black-tailed prairie dog and a Wyoming ground squirrel. Results suggest some geographic structuring of the isolates south to north and provide limited support for the recognition of the morphotype from black-tailed prairie dogs from northern Colorado as a sub- or distinct species. This isolate formed fairly distinct clades for both ITS1 and ITS2 and, considering the Hnida and Duszynski (1999) criterion, the pairwise distance values were less than 5% for ITS1 only when compared to Cleu2, 10, and 12, and for ITS2 only with Cleu2. However, given the questionability of the 5% guideline, specific recognition is not justified until additional molecular markers provide support.

The observation of marked molecular sequence variation for clones from single infections does raise several important issues. Previous studies of eimerian ITS genetic sequence data from wild hosts typically report a single sequence for each eimerian morphotype from each host and do not report variation among clones from individual hosts. Hnida and Duszynski (1999) reported single ITS1 sequences from multiple species and/or isolates of *Eimeria*. If these authors sequenced multiple clones from single isolates following standard practice they did not report it, and thus there is no discussion of how much, if any, variation was observed. While a small portion of the variation we observed is likely attributable to amplification errors (Arezi et al., 2003), there must be other sources. One plausible source of sequence variation would be that host infections are the result of ingestion of more than one, and possibly, many oocysts. Many marmotine rodent species, including Wyoming ground squirrels and black- and white-tailed prairie dogs, live in high density populations and often the different host species overlap in distribution. In addition, *E. callospermophili* typically occurs at high prevalence in these host populations. Seville (1997) reported that 71% of white-tailed (N = 61) and 64% of black-tailed prairie dogs (39) in central Wyoming and Shults et al. (1990) found 52% of Wyoming ground squirrels (69) in southeast Wyoming infected with this species. Thus, there are likely numerous oocysts in the environment, particularly in the shaded and damp burrows ideal for oocyst survival, in which hosts can ingest multiple oocysts with variable molecular markers causing increased genetic heterogeneity. Second, ribosomal rDNA genes in the Apicomplexa do not always form typical tandem arrays of repeats. Le Blancq et al. (1997) found that *Cryptosporidium* spp. rDNA units are dispersed on 3 chromosomes and 2 distinct types of rDNA units, each with noticeable differences in ITS regions. Wellems et al. (1987) and Li et al. (1994) reported

that *Plasmodium* species contain 4-7 rDNA genes dispersed on different chromosomes, each gene has a unique structure, and their expression varies during different life cycle stages. Thus, the observed ITS variation could be due to amplification of different copies of rDNA genes. Even though we observed relatively high ITS sequence diversity among *E. callospermophili* isolates, it is important to mention that ITS1 sequences showed remarkable similarity between 2 clones from Wyoming ground squirrels and 1 from white-tailed prairie dogs and intraspecific identity was observed among all 4 ITS2 sequences from the yellow bellied marmot.

Results of this study and others question the utility of the ITS1 and ITS2 sequences for taxonomic analyses and for differentiating morphologically similar eimerian species from some host groups. Our results indicate that for eimerians from ground-dwelling sciurids, these markers should be used with caution and suggest that additional markers for distinguishing *Eimeria* species should be identified and utilized (Hnida and Duszynski, 1999; Barta, 2001; Tenter et al., 2002). We also concur with Hnida and Duszynski (1999) that the use of genetic distances to discriminate among species with structurally similar oocysts should also include consideration of monophyly, as well as life history and ecology of hosts and parasites. Further researcher reporting sequence data from eimerians from other host groups should provide data on amplicon sequence variation within, and between, isolates from hosts from which oocysts are obtained. This will allow those studying eimerians to identify more generally suitable sequence targets for future studies of taxonomy and systematics of these parasites in wild hosts.

Acknowledgments

This work was made possible in part by NIH Grants # P20RR016474 from the INBRE Program of the National Center for Research Resources. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of NIH. Additional support was provided by Wyoming NSF EPSCoR Grant, NSF Award No. EPS – 0447681. We thank T. Clifford and M. Foust for fecal sample collection from white- and black-tailed prairie dogs.

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222234455 5778001111 2223456778 8899901223 3334445566 77789000
8036783967 8094272569 2680272581 5916745592 3463453916 12621014
Clea1 CCAAGTATCA AGGCGCGTAT CTACTCCTAT ACTACT-AGA CTTCTTCTTA AGCGACCA
Clea2 G..... .A..T.C.. .T...G. .T.C...T A..G...G. GA.....
Clea3 ..... .A..... .T...T.C..T A..G...G. ....C...
Clea4 .....C.. .A..... .C...T... .G.C..T. G..G.....AT.
Clea5 .....T..... ..-..... ..-..... ..-.....
Clea6 .....A..... ..CGT. G..G.....AT.
Clea7 .....C.T. G..G.....AT.
Clea8 .....-..... ..-..... ..-.....
Clea9 ..G.....G .....G.C..T A..G.....T..AT.
Clea10 .....C... A..G.....
Clea11 .....G.C... A.....
Clea12 .....A..T.C.. .T...G. .T.C...T A..G...G. GA.....
Clud1 G..... .A..T.CC. .GT...G. .T..... A..GC...G. G-.C...
Clud2 G..... .A..T.CC. A..T...G. .T.....G A..GC...G. G-.CC...
Clud3 G..... .A..T.CC. .T...G. .T..... A..GC...G. G-.C...
Clud4 G..... .A..T.CC. .T...G. .T..... A..GC...G. G-.C...
Clud5 G..... .A..T.CC. .T...G. .T.....G A..GC...G. G-.A..G
Clud6 G..... .A..T.CC. .TG...G. .T...C...G A..GC...G. G-.A...
Clud7 G...CG... .A..T.C.. .T...G. .T..... AC.G...G. G-.C...
Clud8 G.....G. .AA.T.CC. .T...G. .T..... A..GC...G. G-.C...
Clud9 G.....G. .A..T.AC. .T...G. .T..... A..G...GG. G-.C...
Mfla1 .....C.....C.....GC.....-.....
Mfla2 .T.....C.....C.....G.....-.....
Mfla3 .....C.....C.....G.....-.....
Mfla4 .....C.....C.....G.....-.....
Uele1 .....T.....-.....-.....
Uele2 .....C.....-.....C.....
Uele3 .....C.....G.....-.....
Uele4 .....-.....-.....
Uele5 .....T.....A..C... A.....
Uele6 .....A.....T.....G.T... A.....G
Uele7 .....T.....A..C... A.....
Uele8 .....T.....C..A..C... A..G.....
Uele9 .....G.....-.....
Uele10 .....A..C.....G...G.T... A..C...G
Uele11 ...TA.....C.T. A..G.....

```

FIGURE 1.

Alignment of the variable sites of the ITS1 sequences derived from *E. callospermophili*. Identical sequences are indicated with a ".", gaps with "-". The numbers above each of the columns represent the position of a given nucleotide; the star symbols above indicate that this column is a singleton.

FIGURE 2.

Phylogenetic tree generated using Neighbor Joining method from the ITS1 nucleotide alignment from *E. lancasterensis* and *E. ontarioensis* from fox squirrels, and *E. callospermophili* from marmotine (ground) squirrels. The evolutionary distances were computed using the Maximum Composite Likelihood method. Bootstrap test of phylogeny was performed using 1,000 replicates. Only values $\geq 50\%$ are shown. The tree was rooted with the *E. tenella* and *E. necatrix* sequences.

[illegible]

FIGURE 3.
Alignment of the variable sites of the ITS2 sequences derived from *E. callospermophili*. Identical sequences are indicated with a “,” gaps with “-“. The numbers above each of the columns represent the position of a given nucleotide; the star symbols above indicate that this column is a singleton.

FIGURE 4.

Maximum Parsimony bootstrap consensus tree based on the ITS2 nucleotide alignment from *E. lancasterensis* and *E. ontarioensis* from fox squirrels, and *E. callospermophili* from marmotine (ground) squirrels. The tree was computed using the close-neighbor-interchange algorithm. Bootstrap test of phylogeny was performed using 1,000 replicates. Only values $\geq 50\%$ are shown. *E. tenella* and *E. necatrix* sequences was used as an outgroup.

Summary of *E. callospermophili* ITS1 and ITS2 sequence types, names, analyses, and GenBank accession numbers from white-tailed prairie dogs (Cleu), black-tailed prairie dogs (Clud), yellow-bellied marmots (Mfla), and Wyoming ground squirrels (Uele).

TABLE 1

Sequence type	Sequence name	Size (in nucleotides)	Identity (%)	A+T frequencies (%)	Pairwise distance values (%)	GenBank accession numbers
ITS1	Cleu1- Cleu12	298-302	91.1	61.1-62.6	0.0-6.0	HM241636-HM241647
ITS2	Cleu1- Cleu12	616-633	87.7	58.7-59.8	0.0-7.1	HM241636-HM241647
ITS1	Clud1- Clud 9	296-298	93.6	59.9-61.8	0.3-3.5	HM241616-HM241624
ITS2	Clud1- Clud 9	620-633	91.6	59.1-60.5	0.8-4.1	HM241616-HM241624
ITS1	Mfla1- Mfla14	298	99.3	60.0-60.7	0.0-0.7	HM241648- HM241651
ITS2	Mfla1- Mfla14	617	100	59.5	0.0	HM241648- HM241651
ITS1	Uele1- Uele 11	298-301	94.0	60.5-62.1	0.0-3.8	HM241625- HM241635
ITS2	Uele1- Uele 11	616-627	93.7	58.8-60.3	0.0-4.1	HM241625- HM241635