Taxonomy and systematics of some *Eimeria* species of murid rodents as determined by the ITS1 region of the ribosomal gene complex

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SUMMARY

Eimeria arizonensis, *E. albigulae* and *E. onychomysis*, morphologically similar species from closely related murid rodents, were distinguished using nuclear rDNA ITS1 sequences obtained from multiple isolates of each taxon. ITS1 sequences were also obtained from 6 other species parasitizing murid rodents: *E. falciformis*, *E. langebarteli*, *E. nieschulzi*, *E. papillata*, *E. separata* and *E. sevilletensis*, and from *E. reedi*, a parasite of heteromyid rodents. Under parsimony and maximum likelihood analyses, the isolates of *E. arizonensis*, *E. albigulae* and *E. onychomysis* were differentiated as closely related, monophyletic lineages. Maximum likelihood pairwise distances between the latter species ranged from 7 to 12%, and distances within each species ranged from <1 to 5%; thus it is suggested that ITS1 genetic distances may be used to facilitate taxonomic differentiation of *Eimeria* spp. Against expectation, phylogenetic procedures placed *E. reedi* within the phylogeny of the *Eimeria* of murid rodents. ITS1 sequencing appears to provide data that can be used for taxonomic and phylogenetic studies on the speciose genus *Eimeria*, and may be especially useful when samples contain insufficient numbers of oocysts for other molecular-based methods, e.g. RAPD–PCR.

Key words: *Eimeria*, internal transcribed spacer, taxonomy, systematics, phylogeny.

INTRODUCTION

Here we report the use of sequence data from the nuclear rDNA first internal transcribed spacer (ITS1) region to distinguish among 3 species of *Eimeria* whose oocysts are often morphologically indistinguishable: *E. arizonensis* from *Peromyscus* and *Reithrodontomys* spp., *E. albigulae* from *Neotoma* spp., and *E. onychomysis* from *Onychomys* spp. The hosts of these parasites are closely related rodents in the family Muridae (Sullivan, Holsinger & Simon, 1995) and are often sympatric (Hoffmeister, 1986). Consequently, Upton *et al.* (1992) hypothesized that these coccidia might not be 3 distinct species, e.g. they might constitute 1 or 2 highly euryxenous species. The results of their cross-transmission experiments suggested that they were distinct, host-specific forms. Nevertheless, the authors cautioned that their results were preliminary and, thus, the present study was done to determine if there was genetic evidence for distinguishing the 3 putative species. We chose to examine the ITS1 region of these parasites because ITS data have been used to distinguish among other morphologically similar taxa (e.g. Porter & Collins, 1991; Morgan & Blair, 1995; Gasser *et al.* 1996; Felleisen, 1997). In addition, we wanted to develop a method that, unlike RAPD–PCR, would not require millions of oocysts of each species, and that could be used to examine the phylogenetic relationships among *Eimeria* spp. Internal transcribed spacer regions are useful in this regard because small amounts of DNA are required to PCR amplify them (White *et al.* 1990), and ITS sequences have been shown to provide good phylogenetic resolution at the genus or species level in a wide variety of organisms (e.g. Baldwin, 1993; Bowles, Blair & McManus, 1995; Messner *et al.* 1995; Felleisen, 1997; Okamoto *et al.* 1997). Because little work has been done on the phylogenetic relationships among species of *Eimeria* (e.g. Reduker, Duszynski & Yates, 1987; Cere, Licois & Humbert, 1995; Barta *et al.* 1997), we also report the use of ITS1 sequence data to study the phylogenetic relationships among *E. arizonensis*, *E. albigulae*, *E. onychomysis* and 7 other species of *Eimeria* from murid and heteromyid rodents.

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MATERIALS AND METHODS

Parasite materials

Eimeria spp. were obtained from either naturally infected wild-caught hosts, animals housed in breeding facilities, or were laboratory-maintained isolates originating from various regions of the USA or

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Table 1. Species list indicating host, location and date of origin of isolates and abbreviation codes for species with multiple isolates

	Collection			
Eimeria spp.	Host	Locality	Date	Isolate code
E. albigulae	*Neotoma albigula*	Rio Salado, Sevilleta LTER*, NM†	1992	ARNM1
E. albigulae	*N. albigula*	Rio Salado, Sevilleta LTER, NM	1995	ARNM2
E. albigulae	*N. albigula*	Two-22, Sevilleta LTER, NM	1995	A22NM
E. albigulae	*N. albigula*	Sandia Mountains, NM	1995	ASNM
E. arizonensis	*Peromyscus eremicus*	Portal, AZ†	1996	ZPAZ
E. arizonensis	*P. leucopus*	Rio Grande Bosque, Albuquerque, NM	1995	ZBNM
E. arizonensis	*P. maniculatus*	Corvallis, OR†	1995	ZOR
E. arizonensis	*P. truei*	Goat Draw, Sevilleta LTER, NM	1996	ZGNM
E. arizonensis	*P. truei*	Rio Salado, Sevilleta LTER, NM	1996	ZRNM
E. falciformis	*Mus musculus*	Tijeras, NM	1997	FNM
E. falciformis	*M. musculus*	Wuppertal, Germany	1961	FGER
E. langebarteli	*P. leucopus*	Two-22, Sevilleta LTER, NM	1996	—
E. nieschulzi	*Rattus norvegicus*	Tempe, AZ	1961	—
E. onychomysis	*Onychomys leucogaster*	Rio Salado, Sevilleta LTER, NM	1992	ORNM1
E. onychomysis	*O. leucogaster*	Rio Salado, Sevilleta LTER, NM	1993	ORNM2
E. onychomysis	*O. torridus*	Portal, AZ	1996	OPAZ
E. papillata	*Mus musculus*	Michigan	1976	—
E. reedi	*Perognathus flavus*‡	Five Points Larrea, Sevilleta LTER, NM	1997	—
E. separata	*R. norvegicus*	Auburn, AL†	1968	—
E. sevilletensis	*O. leucogaster*	Rio Salado, Sevilleta LTER, NM	1993	—

* LTER, Long Term Ecological Research site.
† NM, New Mexico; AZ, Arizona; OR, Oregon; AL, Alabama.
‡ This host is in the family Heteromyidae; all other host species are in the Muridae.

Germany (Table 1). Faeces or intestinal contents from hosts were processed in 2·5% (w/v) $K_2Cr_2O_7$ to allow oocyst sporulation as described by Duszynski & Wilber (1997), and *Eimeria* spp. were identified using cover-slip flotation with Sheather's sucrose solution (Barnard & Upton, 1994). Some samples contained few oocysts, so additional oocysts of these isolates were obtained by inoculating *ca* 20–100 sporulated oocysts into laboratory-reared, coccidia-free hosts (*Mus musculus*, *Neotoma albigula*, *Onychomys leucogaster*, *Peromyscus maniculatus*, *P. truei*) using methods described by Upton *et al.* (1992). All isolates were concentrated and purified from large faecal debris by centrifugation in Sheather's solution (Dubey, 1996) and stored in 2·5% $K_2Cr_2O_7$ at *ca* 4 °C until used for DNA extraction.

DNA extraction, PCR amplification and sequencing

For each isolate, *ca* 10000–500000 oocyts were cleaned of $K_2Cr_2O_7$ by 2–3 washes in sterile distilled H_2O, incubated on ice in 20% NaOCl (10–13% active chlorine) for 1 h to purify the oocysts of fine faecal debris (Hosek, Todd & Kuhlenschmidt, 1988; Cere *et al.* 1995), rinsed 3 times in sterile distilled H_2O, and suspended in 0·5 ml of TE buffer (10 mM Tris, 1 mM EDTA, pH 8·0). The suspended oocysts and their sporocysts were ruptured by vortexing them with glass beads using the procedure described by MacPherson & Gajadhar (1993) but, for most samples, the procedure was carried out using 2 or 3 sterile 4 mm glass beads in a 1·5 ml microcentrifuge tube. After vortexing the oocysts for 10 min the suspension containing the freed sporozoites was added to 1·0 ml of CTAB buffer (2% CTAB, 1·4 M NaCl, 0·2% β-mercaptoethanol, 20 mM EDTA, 100 mM Tris–HCl) containing proteinase K (100 μg/ml), and incubated at 65 °C for 1 h. After digestion, the DNA was extracted using phenol/chloroform/isoamyl alchohol (25:24:1), precipitated by ethanol, air-dried and redissolved in TE buffer.

PCR amplifications were performed using AmpliTaq DNA Polymerase (Perkin–Elmer, USA) according to the manufacturer's instructions. The PCR primers for the ITS1 region were the 'universal' eukaryotic primers ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′), which make use of the conserved regions of 18S and 5·8S rDNA genes to amplify the ITS1 region between them (White *et al.* 1990). The amplification conditions were: denaturation at 94 °C for 1 min (except for the first cycle for 5 min), annealing at 48 °C for 30 sec, and extension at 72 °C for 1 min with the primer extension time increased by 3 sec for each subsequent reaction cycle. After 30 cycles, an additional 7 min extension at 72 °C was performed.

PCR products from *E. nieschulzi*, *E. albigulae* (isolate ARNM1), and *E. onychomysis* (isolate

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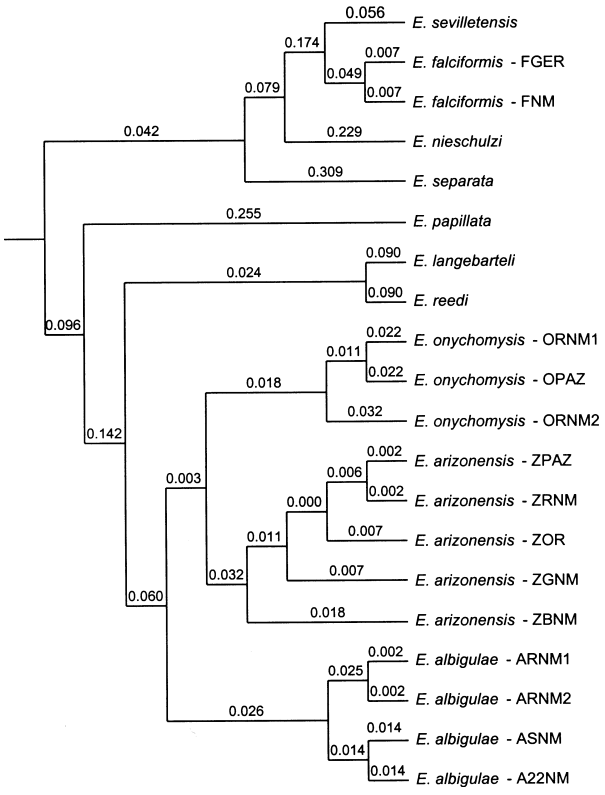

Fig. 1. Maximum likelihood (ML) tree with molecular clock enforced for the ITS1 data set from 20 taxa of *Eimeria* species from rodents. TI : TV ratio and rate parameters same as indicated in caption for Table 2. Isolate acronyms are listed in Table 1.

ORNM1) were cloned using the pCRII vector TA cloning kit (Invitrogen); sequencing of clones was done with Sp6 and T7 primers. The PCR products from all other samples were purified with Centricon-100 columns (Millipore) and directly sequenced using the PCR primers as sequencing primers. Cycle sequencing was done using ABI Prism dye terminator cycle sequencing (Perkin–Elmer) and analysed on an ABI Model 377 DNA sequencer (Perkin–Elmer). For all isolates, sequencing was performed on both strands until sequences from the 2 ends overlapped for the boundaries of the ITS1 region.

Analysis of sequences

The boundaries separating the 18S and 5·8S ribosomal gene sequences from the ITS1 sequences were determined by comparisons with the rDNA sequences of *Toxoplasma gondii* (GenBank acc. no. X75453) and 2 eimerian parasites of chickens: *E. tenella* (GenBank acc. no. AF026388) and *E. maxima* (GenBank acc. no. AF027723). Sequences were aligned by eye and gaps were introduced into the alignment to adjust for differences in sequence length and areas that were hypothesized to be non-homologous. For outgroup comparisons, the sequence of the ITS1 region of *E. maxima* (GenBank acc. no. AF027723) was aligned by eye against a subset of the original alignment. The subset included a representative of each of the 10 spp. of *Eimeria* from rodents; gaps were introduced as described above. All sequence alignments used in this paper are available from the first author in NEXUS format (see Swofford, 1996).

Phylogenetic analyses were carried out using maximum likelihood (ML), maximum parsimony (MP), and distance procedures in prerelease test versions d63 and d64 of PAUP* 4.0 (Swofford, 1996) with gaps coded as unknown characters (Barta *et al.* 1997). To estimate transition : transversion (TI : TV) ratios and among-site heterogeneity parameters (Swofford *et al.* 1996), initial MP trees were obtained for all data sets using the branch-and-bound search option with all characters treated as unordered and equally weighted (Hershkovitz & Lewis, 1996). The MP trees were used as topological constraints for ML analyses with settings fixed to estimate TI : TV ratios and rate parameters; these were used to generate ML trees, which served as constraints for re-estimating the TI : TV ratios and rate parameters. The re-estimated values were used for subsequent ML and distance analyses (Hershkovitz & Lewis, 1996; Swofford *et al.* 1996). All subsequent parsimony analyses were done using either heuristic or branch-and-bound search options, and weighting step matrices that incorporated the TI : TV ratios that were estimated as described above. Heuristic searches were done with random stepwise addition of taxa and TBR. Maximum likelihood estimation was used to calculate pairwise distances between taxa (Hershkovitz & Lewis, 1996). For the *Eimeria* of rodents, ML predictions with and without the assumption of a molecular clock were calculated to test the hypothesis of a molecular clock (Felsenstein, 1988). The rooted tree that was produced under the molecular clock model was used to determine species that were used as an outgroup for analyses of the clade that included *E. arizonensis*, the *E. arizonensis*-like taxa, and several other species. The amount of phylogenetic signal in each alignment was assessed using the random trees analysis in PAUP* (Hillis & Huelsenbeck, 1992). Bootstrap values (Felsenstein, 1985) were obtained with heuristic searches; 1000 replicates were used for MP and 100 replicates for ML bootstrap analyses. Because excessive processing time would be needed to do a ML bootstrap of the data set from the 20 isolates from rodents, a subset comprised of the same 10 spp. analysed with *E. maxima* (see above), was bootstrapped.

RESULTS

Sequences

The ITS1 regions of the 10 *Eimeria* spp. from rodents ranged from 251 (*E. reedi*) to 320 (*E. sevilletensis*) bp in length. Within species, variation

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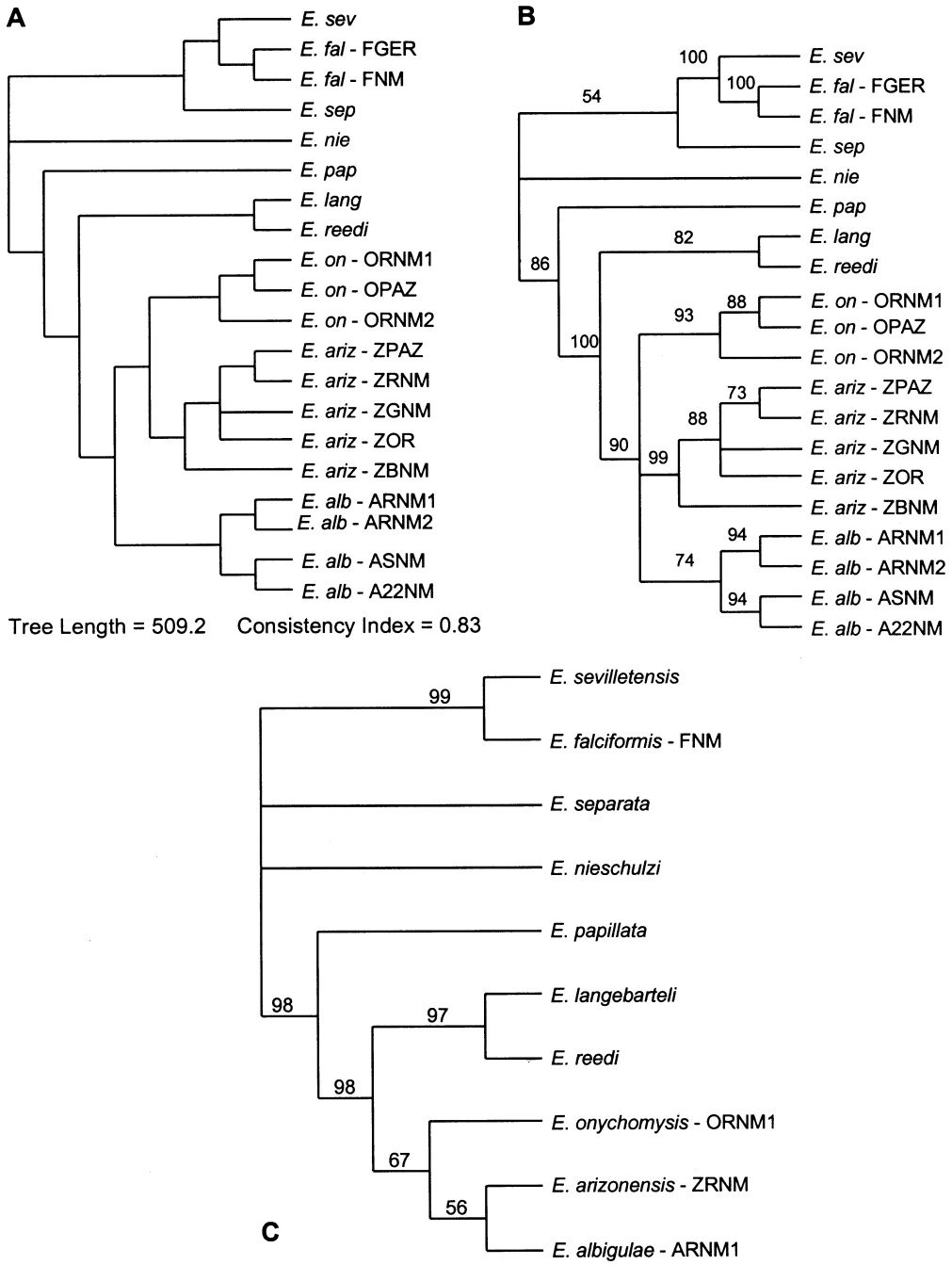

Fig. 2. Trees for ITS1 data set from *Eimeria* species from rodents. (A) Consensus of 3 equally parsimonious trees. Isolate acronyms listed in same order as in Fig. 1. (B) Maximum parsimony (MP) bootstrap tree with bootstrap values above internodes. (C) ML bootstrap tree for subset of 10 species from the 20 taxon data set. TI:TV ratio and rate parameters same as listed in caption for Table 2.

in the length of this region was 0–5 bp. In comparison, 2 *Eimeria* spp. from the domestic fowl had ITS1 regions that were *ca* 320 bp (*E. maxima*; GenBank acc. nos AF027722–AF027726) to 562 (*E. tenella*; see above) bp in length, and the variation among strains of *E. maxima* was 0–1 bp. Base composition was biased towards a preponderence of Ts (*ca* 33%) for the *Eimeria* of rodents; similarly, the ITS1 region of *E. maxima* strains was T-biased (*ca* 35%). Using the alignment method described above, the overall length of the 20 aligned sequences of the *Eimeria* from rodents was 455 bases, including gaps, of which 135 were variable and 132 were phylogenetically informative in the parsimony analyses. The sequences from *E. maxima* and the subset of 10 spp. of *Eimeria* from rodents was 473 bases when aligned; of these, 152 characters were variable and 142 parsimony-informative.

Phylogenetic analysis and differentiation of species

Using the random trees procedure, significant phylogenetic signal (Hillis & Huelsenbeck, 1992) was

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Table 2. Pairwise distance values, calculated by maximum likelihood with empirically estimated parameters. TI : TV = 1·54; proportion of invariable sites = 0·039; gamma distribution shape = 2·22
(See Table 1 for information on codes and geographical origin of isolates.)

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	*E. albigulae*–ARNM1	—																		
2	*E. albigulae*–ARNM2	0·004	—																	
3	*E. albigulae*–ASNM	0·048	0·053	—																
4	*E. albigulae*–A22NM	0·048	0·052	0·024	—															
5	*E. arizonensis*–ZPAZ	0·092	0·087	0·084	0·083	—														
6	*E. arizonensis*–ZBNM	0·088	0·088	0·081	0·079	0·036	—													
7	*E. arizonensis*–ZOR	0·083	0·078	0·076	0·074	0·012	0·032	—												
8	*E. arizonensis*–ZRGD	0·087	0·083	0·076	0·079	0·015	0·032	0·012	—											
9	*E. arizoensis*–ZRNM	0·087	0·083	0·089	0·088	0·004	0·032	0·015	0·019	—										
10	*E. onychomysis*–ORNM1	0·118	0·113	0·110	0·104	0·119	0·115	0·109	0·119	0·114	—									
11	*E. onychomysis*–ORNM2	0·071	0·066	0·068	0·071	0·076	0·072	0·067	0·067	0·071	0·048	—								
12	*E. onychomysis*–OPAZ	0·094	0·090	0·092	0·095	0·100	0·096	0·090	0·095	0·095	0·052	0·040	—							
13	*E. reedi*	0·188	0·181	0·181	0·166	0·183	0·173	0·170	0·182	0·190	0·204	0·166	0·198	—						
14	*E. langebarteli*	0·242	0·233	0·214	0·249	0·221	0·207	0·205	0·219	0·228	0·285	0·227	0·275	0·180	—					
15	*E. papillata*	0·344	0·342	0·364	0·359	0·394	0·380	0·403	0·396	0·385	0·371	0·375	0·379	0·485	0·540	—				
16	*E. nieschulzi*	0·489	0·475	0·491	0·508	0·494	0·474	0·482	0·475	0·494	0·490	0·456	0·487	0·488	0·581	0·438	—			
17	*E. separata*	0·576	0·576	0·559	0·602	0·623	0·561	0·606	0·604	0·623	0·556	0·526	0·566	0·592	0·657	0·557	0·500	—		
18	*E. sevilletensis*	0·437	0·425	0·451	0·461	0·463	0·432	0·453	0·436	0·463	0·524	0·435	0·501	0·495	0·589	0·524	0·390	0·543	—	
19	*E. falciformis*–FGER	0·499	0·486	0·482	0·529	0·480	0·448	0·469	0·473	0·480	0·542	0·474	0·530	0·489	0·588	0·580	0·363	0·553	0·106	—
20	*E. falciformis*–FNM	0·489	0·476	0·473	0·518	0·471	0·440	0·460	0·464	0·471	0·521	0·456	0·509	0·495	0·615	0·608	0·376	0·563	0·110	0·013

found in both of the alignments with g1 = −1·37 ($P<0·01$) for the alignment of the 20 taxa from rodents, and g1 = −0·84 ($P<0·01$) for the alignment comprised of the 10 *Eimeria* spp. from rodents and *E. maxima* from the chicken.

Using the likelihood ratio test, the molecular clock hypothesis was not rejected for the data set comprised of 20 *Eimeria* taxa from rodents ($\chi^2=17·8$, D.F. = 18, $0·25<P<0·50$). The ML tree that was obtained under the assumption of a molecular clock (−ln likelihood = 2332·1542) was rooted such that 2 major lineages were distinguished, and the 3 species with morphologically similar oocysts (*E. arizonensis*, *E. albigulae* and *E. onychomysis*) were found to be closely related, but distinct, clades (Fig. 1). When the lineage containing *E. sevilletensis*, *E. falciformis*, *E. separata* and *E. nieschulzi* was used as an outgroup for a heuristic MP analysis of the clade containing *E. arizonensis* and the *E. arizonensis*-like species, 3 equally parsimonious trees were produced and the latter 3 species again were differentiated as separate, monophyletic groups; the consensus tree is presented in Fig. 2A. Bootstrap analysis under parsimony produced a consensus tree with the same topology, but the evolutionary relationships of *E. arizonensis*, *E. albigulae* and *E. onychomysis* were not resolved (Fig. 2B). The consensus of trees derived using ML and the same outgroup (−ln likelihood = 2323·2079) had identical topology to the parsimony consensus tree. The bootstrap analysis using ML found a consensus tree that was similar to the MP bootstrap output; however, there was moderate (67%) support for a clade separating *E. arizonensis*, *E. albigulae* and *E. onychomysis* from the lineage containing *E. langebarteli* and *E. reedi* (Fig. 2C).

When *E. maxima* was used as an outgroup for MP and ML analyses of the 10 *Eimeria* spp. from rodents, the trees that were obtained had topologies similar to the MP and ML trees described above. However, the bootstrap values were generally lower than those observed for the corresponding nodes in the MP and ML bootstrap trees described above (data not shown).

Thus, *Eimeria arizonensis* and the *E. arizonensis*-like taxa were differentiated and found to be closely related (Figs 1 and 2). Maximum likelihood pairwise distances between these species ranged from 7 to 12% and distances between isolates within each of these species ranged from <1 to 5% (Table 2). In contrast, the pairwise distance values between *E. arizonensis*, *E. albigulae* or *E. onychomysis* and the 7 other species from rodents were *ca* 17%, or greater.

DISCUSSION

Phylogenetic analyses of ITS1 sequence data clearly differentiated *E. arizonensis*, *E. albigulae* and *E. onychomysis* as monophyletic lineages and thus provides support for the hypothesis of Upton *et al.* (1992) that, although their oocysts are often morphologically indistinguishable, each is a valid species, with the host ranges of *E. albigulae* and *E. onychomysis* restricted to murid rodents in the genera *Neotoma* and *Onychomys*, respectively, and the host range of *E. arizonensis* including *Peromyscus* and *Reithrodontomys*, but not rodents from the other 2 genera. In addition, we have demonstrated that ITS1 sequencing provides a method for distinguishing among morphologically similar species of *Eimeria* when samples contained as few as 10000 oocysts. These samples contained too few oocysts for RAPD–PCR analysis, because millions of sporulated oocysts are needed to select taxonomically informative RAPD–PCR primers and then use them to fingerprint *Eimeria* spp. (see MacPherson & Gajadhar, 1993; Shirley & Bumstead, 1994; Cere *et al.* 1995; Johnston & Fernando, 1995). Furthermore, the ITS1 sequences from different isolates of *E. arizonensis*, *E. albigulae* and *E. onychomysis* contained sufficient interspecific variability, and minimal intraspecific variation to be taxonomically informative (see Tang *et al.* 1996), even with isolates that were obtained from hosts that were separated by hundreds or thousands of km (e.g. *E. arizonensis*: isolates from Arizona, New Mexico, and Oregon). Likewise, the ITS1 sequences from the European and North American isolates of *E. falciformis* were very similar (ML distance = 1·3%), indicating the stability of this locus within at least 4 species of *Eimeria*. Therefore, we suggest that ITS1 sequencing can provide data that will be useful for taxonomic work with members of the speciose genus *Eimeria*.

Intraspecific ML distances were less than or equal to *ca* 5%, distances between closely related, morphologically similar species ranged from 7 to 12%, and distances between morphologically distinct species were generally in the range 20–60%. Although increasingly prevalent in the taxonomy of free-living and parasitic organisms, the use of genetic distances to discriminate among coccidian species with structurally similar oocysts might be problematic if they are applied without regard to the monophyly (Lymbery, 1992), or the life-history and ecology (Davis, 1994) of the taxa under consideration. In this regard, we have found that isolates of *E. arizonensis*, *E. albigulae* and *E. onychomysis* form monophyletic lineages that are distinct from each other and cross-transmission experiments have demonstrated the host specificity of these taxa (Upton *et al.* 1992; Hnida & Duszynski, 1999). Thus we hypothesize that, for ITS1 sequence data from eimerian parasites, ML genetic distances that are greater than 5% are indicative of species differentiation. However, we emphasize that this measure should not be the sole means to discriminate among taxa (Lymbery, 1992; Davis, 1994) and that phylo-

genetic analyses of ITS1 sequences from a variety of other *Eimeria* spp. must be done to test the validity of, and perhaps further calibrate, this 'genetic yardstick'.

When different outgroups were used for phylogenetic inference, the MP and ML trees that were produced had similar topologies. However, when *E. maxima* was the outgroup, the MP and ML bootstrap values were generally lower than those obtained when the outgroup consisted of 4 *Eimeria* spp. from rodents. This is understandable, because eimerian parasites tend to be genus specific (Kogut, 1990) and, since ITS regions appear to evolve rapidly (White *et al*. 1990), it seems unlikely that *E. maxima*, a parasite of domestic fowl, would be related closely enough to the ingroup taxa to provide as much phylogenetic signal for outgroup analysis (Swofford *et al*. 1996) as would the *Eimeria* of rodents. In addition, when we limited the phylogenetic analyses to only the *Eimeria* from rodents, we obtained results that were congruent with those of Reduker *et al*. (1987, see below). Consequently, the remaining discussion on phylogenetics will focus on the results obtained from the analyses of the ITS1 data set from the 10 *Eimeria* spp. of rodents.

The topology of the phylogenetic trees from the present study concur, for the most part, with those of Reduker *et al*. (1987, Figs 2 and 4), who used isozyme banding patterns, sporulated oocyst morphology, and life-history traits to examine the evolutionary relationships among 9 species of *Eimeria* from murid rodents (5 of which were included in the present study: *E. nieschulzi*, *E. papillata*, *E. langebarteli*, *E. albigulae* and *E. arizonensis*). Their results differ from ours because they suggested that *E. papillata* is more closely related to *E. arizonensis* and *E. albigulae* than is *E. langebarteli*, whereas the trees we obtained hypothesize the converse. As in the present study, Reduker *et al*. (1987) obtained trees indicating that *E. albigulae* and *E. arizonensis* are closely related, and that *E. papillata* belongs to a lineage that includes the latter 2 parasites as more highly derived taxa. The congruence of these results, which are derived from different types of data, gives strong support for the hypothesized evolutionary relationships of *E. papillata*, *E. arizonensis* and *E. albigulae* (Quicke, 1993).

The MP and ML analyses placed *E. reedi*, which parasitizes heteromyid rodents (Ernst, Oaks & Sampson, 1970; Ford, Duszynski & McAllister, 1990), within a clade that includes *E. arizonensis*, *E. albigulae* and *E. onychomysis* – a result that was strongly supported by bootstrap analyses. This is noteworthy because the latter 3 taxa are parasites of murid rodents (Levine & Ivens, 1990). Because the Muridae and Heteromyidae are within the Sciurognathi (Wilson & Reeder, 1993), and the *Eimeria* of rodents are considered to be, with some exceptions, genus-specific (Levine & Ivens, 1988), we originally thought that the ITS1 data from *E. reedi* could be used for outgroup comparisons to the ITS1 sequences from the 9 species of *Eimeria* from murids. Although unexpected, this result is probably not spurious because restriction enzyme analysis of PCR amplified 18S rDNA (Clark, 1997) from the same 10 *Eimeria* spp. has also indicated that *E. reedi* is closely related to *E. arizonensis* and the *E. arizonensis*-like taxa (J. A. Hnida & D. W. Duszynski, unpublished observations).

Reduker *et al*. (1987) noted the morphological similarity between the sporulated oocysts of some of the eimerian parasites of both heteromyid and murid hosts, and postulated that, because members of these host families often live sympatrically, cross-transmission from heteromyids to murids may have occurred over evolutionary time, followed by speciation within the murid hosts. The results of the present study, and the morphological similarity between *E. reedi*, *E. arizonensis*, *E. albigulae* and *E. onychomysis* (see Ernst *et al*. 1970; Reduker, Hertel & Duszynski, 1985; Levine & Ivens, 1990) provides support for the scenario of Reduker *et al*. (1987) or for cross-transmission having occurred from murid to heteromyid hosts over evolutionary time. Alternatively, a number of lineages of *Eimeria* spp. (see Reduker *et al*. 1987) may have been present in the common ancestor of the heteromyids and murids (and perhaps other rodents), and *E. reedi*, *E. arizonensis*, and the *E. arizonensis*-like taxa are related through mutual descent from one of these lineages. Sequence information from the ITS1 regions of more *Eimeria* from a variety of sympatric rodents would be useful in resolving which of these explanations is more likely. However, a caveat of phylogenetic inferences based on a single sequence is that phylogeny may reflect evolutionary history of the sequence but not that of the species (Avise, 1994). Therefore we suggest that additional sequence information from independent loci (e.g. mitochondrial genes) be included in future work with the eimerian parasites of rodents and other host taxa.

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