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Eimeria zuernii (Eimeriidae: Coccidia): mitochondrial genome and genetic diversity in the Chinese yak

Xuan Zhou^{1†}, Zhao Wang^{1†}, Pengchen Zhu¹, Xiaobin Gu¹, Ran He¹, Jing Xu¹, Bo Jing¹, Lidan Wang^{1*}, Shun Chen^{2*} and Yue Xie^{1*}

Abstract

Background Coccidiosis caused by *Eimeria zuernii* (Eimeriidae: Coccidia) represents a significant economic threat to the bovine industry. Understanding the evolutionary and genetic biology of *E. zuernii* can assist in new interaction developments for the prevention and control of this protozoosis.

Methods We defined the evolutionary and genetic characteristics of *E. zuernii* by sequencing the complete mitogenome and analyzing the genetic diversity and population structure of 51 isolates collected from eight yak breeding parks in China.

Results The 6176-bp mitogenome of *E. zuernii* was linear and encoded typical mitochondrial contents of apicomplexan parasites, including three protein-coding genes [PCGs; cytochrome c oxidase subunits I and III (*cox1* and *cox3*), and cytochrome b (*cytb*)], seven fragmented small subunit (SSU) and 12 fragmented large subunit (LSU) rRNAs. Genome-wide comparative and evolutionary analyses showed *cytb* and *cox3* to be the most and least conserved *Eimeria* PCGs, respectively, and placed *E. zuernii* more closely related to *Eimeria mephitidis* than other *Eimeria* species. Furthermore, *cox1*-based genetic structure defined 24 haplotypes of *E. zuernii* with high haplotype diversities and low nucleotide diversities across eight geographic populations, supporting a low genetic structure and rapid evolutionary rate as well as a previous expansion event among *E. zuernii* populations.

Conclusions To our knowledge, this is the first study presenting the phylogeny, genetic diversity, and population structure of the yak *E. zuernii*, and such information, together with its mitogenomic data, should contribute to a better understanding of the genetic and evolutionary biological studies of apicomplexan parasites in bovines.

Keywords Yak coccidiosis, *Eimeria zuernii*, MtDNA, Genetic relationships, Population structure

[†]Xuan Zhou and Zhao Wang have contributed equally to this work and share the first authorship.

*Correspondence:

Lidan Wang
wanglidan777@126.com

Shun Chen
shunchen@sicau.edu.cn

Yue Xie
xyue1985@gmail.com

Full list of author information is available at the end of the article



Background

Bovine coccidiosis, caused by *Eimeria* spp., is a widespread and highly pathogenic protozoosis with a significant economic impact on the cattle industry [1]. Similar to that in cattle, coccidiosis is also commonly found in yaks, and there are 17 species of the genus *Eimeria* responsible for this disease, including *E. zurnii*, *E. bovis*, *E. auburnensis*, *E. canadensis*, *E. ellips*, *E. alabamensis*, *E. bareillyi*, *E. brasiliensis*, *E. bukidnonensis*, *E. cylindrica*, *E. kwangsiensis*, *E. mandela*, *E. pellita*, *E. subspherica*, *E. wyomingensis*, *E. illinoisensis*, and *E. stiedai*-like [1, 2]. *Eimeria zuernii* is by far the most pathogenic coccidian species and poses an additional mortality threat to yak calves < 1 year old [2–4]. Infection with *E. zuernii* can cause malabsorption of nutrients, watery or bloody diarrhea, and even death in heavily infected animals [5].

China has the largest population of the yak in the world, with 16 million yaks accounting for > 95% of the global population [6]. Recent epidemiological evidence, however, showed that *E. zuernii* is commonly prevalent in yak farms and is becoming the leading cause of weight reduction in young individuals across the Qinghai-Tibetan Plateau of China [5, 7–10]. Such situations highlight the significance and necessity of the diagnosis and control of *E. zuernii*. Current diagnosis and identification of this protozoan parasite typically rely on morphological characteristics. Unfortunately, *E. zuernii* has similar oocyst morphotypes and overlapping biological features with other coccidian species. In such a context, obtaining an efficient approach to identify *E. zuernii* infections becomes urgent for clinical diagnosis, epidemiological investigation, and control, and achieving this goal is foreseeable only through the utilization of molecular approaches. Although the internal transcribed spacer 1 (ITS-1) region and small subunit ribosomal DNA (SSU) of the nuclear ribosomal DNA (rDNA)-based PCR amplifications were applied to differentiate between bovine coccidian species, including *E. zuernii* [11–13], cumulative studies demonstrated that the mitochondrial DNA (mtDNA) seems to be more powerful in delimiting individual *Eimeria* species because of its rapid evolution rate, absence of recombination, and matrilineal inheritance [14]. Furthermore, Awadi et al. showed that the *Eimeria* mitogenomes have the ability to illustrate their evolution and host adaptations [15]. Therefore, it is reasonable that the mitochondrial (mt) datasets are widely used for species-specific identification in bovine coccidiosis [16–20]. Unfortunately, until now, no complete information on the mitogenome of *E. zuernii* has been characterized. Additionally, a fundamental understanding of the genetic structure and diversity of *E. zuernii* would also assist in the development of a long-lasting control strategy.

Several in-depth studies have proven their significance and effectiveness in controlling protozoan *Plasmodium* spp. and *Toxoplasma* spp. in humans as well as *Eimeria* spp. in chickens by defining their genetic diversity and population architecture [21–23]. Of course, numerous factors, including the effective population size, distance between populations, host dispersal ability, evolutionary history, host population structures, and the complexity of the life cycle, may affect the genetic makeup of *E. zuernii* populations [24–26]. However, little is known about the genetic diversity and population structure of *E. zuernii* so far. Herein, we decoded the complete mitogenome of *E. zuernii* isolated from Chinese yaks and further determined its population genetic diversity by sequencing the *cox1* gene of 51 *E. zuernii* isolates that were collected from different geographical yak breeding parks in Sichuan, China. These results would add novel insights to phylogenetic, population genetic, and molecular epidemiological studies on this yak parasite.

Methods

Sample collection

In November 2022, as part of ongoing surveys of coccidiosis in yaks, 456 *Eimeria* oocyst-positive fecal samples were obtained from eight Yak Modern Industrial Parks in Sichuan, China: 43 in Xinlong, 102 in Hongyuan, 25 in Ya'an, 89 in Ruoergai, 32 in Luhuo, 48 in Litang, 31 in Daofu, and 86 in Ganzi (Fig. 1). For species identification, about 500-g feces from each sampling were mashed and suspended with running water, followed by filtration through a series of sieves (300, 150, and 80 mm; Endecotts, London, UK) and culture in a 5% (w/v) potassium dichromate solution at 28 °C for 72 h under forced aeration. Sporulated oocysts were speciated according to morphometric keys [27]. Of 456 *Eimeria* isolates, 51 were preliminarily identified as pure infections with *E. zuernii* (Additional file 1: Table S1), and their oocysts were further subjected to enrichment with saturated sodium nitrate flotation and molecular identification by PCR amplification using the species-specific primers (forward: 5'-CCCACTACATCCAACCTCCTG-3'; reverse: 5'-GCGTTCGGAAATCTGATGGT-3') [9] and sequence comparisons with the targeted ITS-1 regions. All isolates were determined to share > 99.2% identities with the query sequence of *E. zuernii* in GenBank (Additional file 2: Fig. S1; accession nos. OR351547–OR351597 vs. AB769665).

Sequencing and assembly

After species identification and oocyst quantitation using a McMaster chamber among these *E. zuernii* isolates, one sample from Ganzi was selected for mitogenome sequencing because of its high number of oocysts

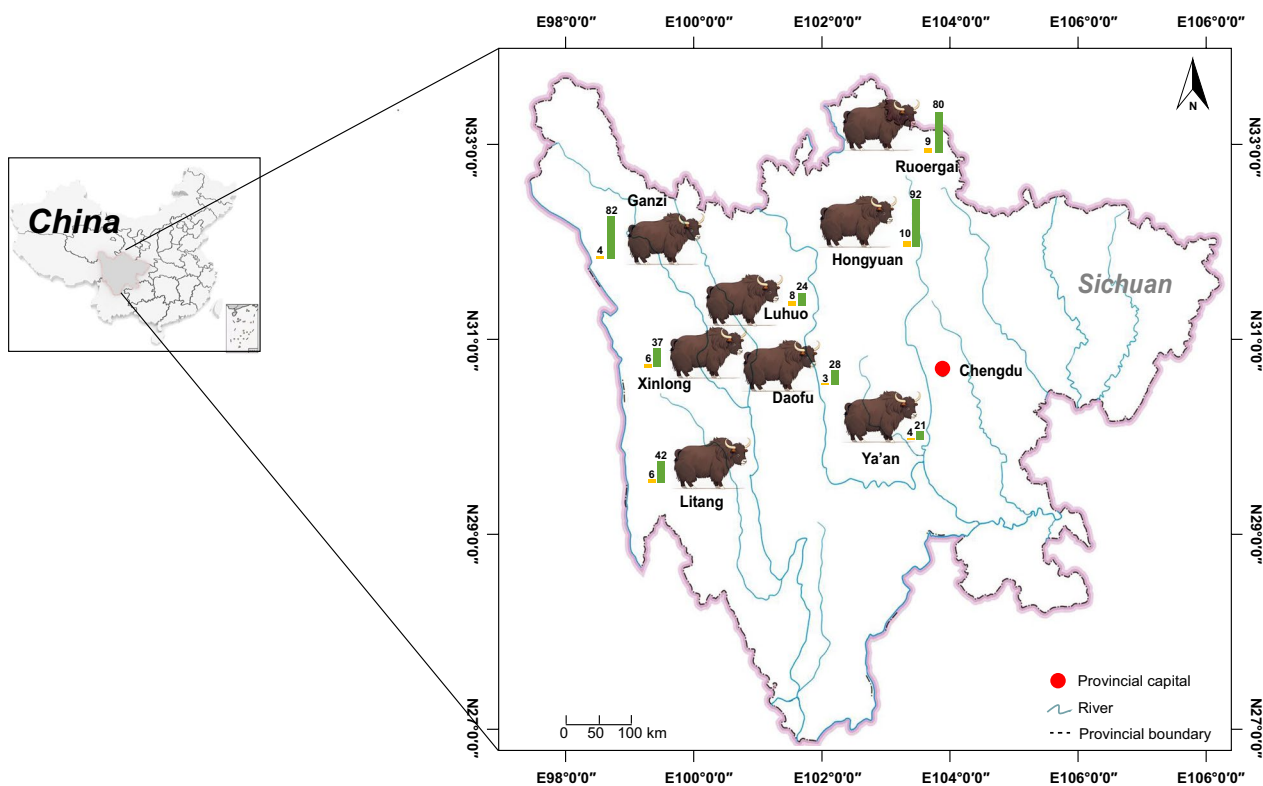


Fig. 1 Map of sampling localities for yak *Eimeria* isolates included in the present study. The number of isolates infected with *E. zuernii* (yellow bars) and other *Eimeria* species (green bars) from each locality are shown, respectively

per gram (OPG = 3.8×10^6). About 2.5 million *E. zuernii* oocysts were harvested and purified by using a combination of saturated sodium nitrate flotation and discontinuous sucrose gradient centrifugation. Following vortex breakdown, the genomic DNA was extracted from oocysts using the Genomic DNA Kit (Tiangen, Beijing, China). Based on the quality and quantity assessment, ~2.5 µg genomic DNA was fragmented to construct a 250-bp paired-end (PE) library, followed by sequencing on an Illumina HiSeq X-TEN platform (BerryGenomics, Beijing, China). The clean reads (~1.8 Gb)

were assembled with MITObim v1.9.1 [28]. The genome was validated by PCR amplifications using four overlapping fragments (Table 1) and then annotated using MITOS2 (<http://mitos2.bioinf.uni-leipzig.de/index.py>) and a whole genome-guided gene alignment against *Eimeria mephitidis* (accession no. KT203398). Based on the *E. zuernii* mitogenome sequenced here, a pair of primers, *cox1*-F (5'-TTGGTTGGACTCTATACC CTC-3') and *cox1*-R (5'-AGATAGTACAAAATGAAA ATGAGC-3'), were designed to amplify the *cox1* gene (776 bp) from the remaining 50 *E. zuernii* isolates. PCR

Table 1 Primer pairs for PCR amplification and their positions in the *Eimeria zuernii* mitogenome

Primer name	Location based on <i>E. zuernii</i> mitogenome	Primer sequence (5' to 3')
Ez-1	1–20	Forward: GATAAAGTCGCAGCAGTAGC
	1648–1672	Reverse: AGGTTGGAGAGAGTGACATTAGAGA
Ez-2	934–950	Forward: TATTCCAAGTAAAGCTG
	2892–2912	Reverse: AACCCAGCTCACGTATCACAT
Ez-3	2835–2850	Forward: GCTCATCACACCCTTG
	5090–5112	Reverse: CTTAACCCAGCTCACGTATCACA
Ez-4	5039–5060	Forward: AAGACAGAATAGTATTTTTTAA
	511–532	Reverse: AGTAGCACCCCAAGCTCATT

reactions (50 μ l) containing 2 μ l of template genomic DNA, 2 μ l of each primer (10 pmol each), 25 μ l of 2 \times Taq MasterMix (TIANGEN, Beijing, China), and 19 μ l of ddH₂O were performed in a S1000 Thermal Cycler (Bio-Rad, USA) using the following conditions: initial denaturation at 94 °C for 5 min; 35 cycles of 94 °C for 1 min, 53 °C for 30 s, 72 °C for 30 s; followed by a final extension at 72 °C for 8 min. Target bands were isolated by 1.0% agarose-TAE gel electrophoresis and purified using spin columns (Wizard PCR Prep, Promega, USA). The purified amplicons were cloned into the vector pMD19-T (TakaRa, Dalian, China), and each clone was sequenced three times on an automatic DNA sequencer by Sheng-gong Biological Technology Company (Shanghai, China). The complete mitogenome sequence of *E. zuernii* and sequences of *cox1* (776 bp) in 50 *E. zuernii* isolates were deposited in GenBank under accession numbers OQ476205 and OR039219–OR039268.

Sequences analysis

Nucleotide composition and codon usage of the *E. zuernii* mitogenome were measured with MEGA X (www.megasoftware.net). The base skewnesses of different mitogenomic regions of *E. zuernii* were determined using the formulas AT skew = $(A - T)/(A + T)$ and GC skew = $(G - C)/(G + C)$ [29]. The multi-alignments of nucleotide and amino acid sequences of protein-coding genes (PCGs) obtained from the sequenced mtDNA and those of other available *Eimeria* parasites (Additional file 3: Table S2) were achieved using MEGA X. Based on the alignments, the similarities and divergences of PCGs were determined using DNASTar v5.02 (DNASTar Inc., Madison, WI), genetic distances were calculated based on Kimura-2-parameter (K2P) [30] with MEGA X, and the ratio of the nonsynonymous substitution (Ka) and synonymous substitution (Ks) of each PCG was calculated with KaKs_Calculator [31].

Phylogenetic and population structure analyses

The phylogenetic position of *E. zuernii* and relationships between different host-originated eimerian parasites in the genus *Eimeria* were inferred from the complete mitogenomes of *E. zuernii* and other congeneric species (Additional file 3: Table S2), using *Isospora* sp. (accession no. KP658103) as the outgroup. After nucleotide alignments using MAFFT v7.450 [32] and filtration of the ambiguous regions using Gblocks v0.91b (<http://molevol.cmima.csic.es/castresana/Gblocks.html>), a 6552-bp multi-sequence dataset was built to reconstruct the phylogenetic tree with three algorithms, including maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI). For the MP analysis, the tree was constructed by PAUP v4.0b10 [33] using equally weighted

parsimony and heuristic searches and tree-bisection-reconnection (TBR) branch-swapping, and the optimal topology was obtained using the Kishino-Hasegawa method with 1000 replicates. The ML analysis was implemented using PHYML v3.1 [34] under the optimal evolutionary model “TIM+ F+ I+ G4” that was selected with the “Auto” option on the W-IQ-TREE web server (<http://iqtree.cibiv.univie.ac.at>) using an ML+rapid bootstrap algorithm with 1000 replicates. The BI analysis was achieved by MrBayes v3.2.7 [35] using the optimal evolutionary “CAT+GTR+G” model chosen by ModelFinder [36] and four independent Markov chain Monte Carlo (MCMC) chains running for 1,000,000 generations; after sampling a tree every 0.1% generation, a consensus tree was obtained and visualized in Treeview X (<https://www.linuxlinks.com/treeviewx/>). In parallel, 50 *E. zuernii* isolates sampled from eight different geographical yak breeding parks were subjected to an assessment of population diversity using the *cox1*-based mt dataset. Diversity indices for these *E. zuernii* isolates, including the number of haplotypes, haplotype diversity, and nucleotide diversity, were calculated in DnaSP v6.12 (<http://www.ub.edu/dnasp/>). A phylogeny of haplotypes was estimated by MP tree using MEGA X, with the significance of each node estimated using 10,000 bootstrap replicates of the dataset. The neutrality indices, including Fu’s *F_s*, Tajima’s *D*, and the pairwise fixation index (*F_{st}*), were used to estimate the size variation among populations using Arlequin v3.5.2.2 (<http://cmpg.unibe.ch/software/arlequin35/>). The median-joining networks among sequences of populations from eight geographic ranges were drawn using Population Analysis with Reticulate Trees (PopART; <http://popart.otago.ac.nz>) to reflect whether the genetic differentiation between populations was associated with geographical isolations.

Results and discussion

Eimeria zuernii mitogenome feature

The *E. zuernii* linear mitogenome was 6176 bp in size and encoded three PCGs, *cytb*, *cox1*, and *cox3*, as well as seven interspersed small subunit (SSU) and twelve interspersed large subunit (LSU) rDNA fragments (Fig. 2). No transfer RNAs (tRNAs) were found in the *E. zuernii* mitogenome, similar to other *Eimeria* spp. [17, 37–43]. Likewise, the *E. zuernii* mitogenome was biased towards AT (64.52–67.37%) with T as the most favored base and G the least favored. Across the mitogenome, there were three overlapping regions located between *cytb* and *cox1* genes (4 bp), between LSUF and LSUG rRNA genes (7 bp), and between LSUA rRNA and *cox3* genes (6 bp), respectively. Moreover, 18 intergenic spacers were also observed, ranging in size from 9 to 221 bp, in the *E. zuernii* mitogenome (Additional file 4: Table S3).

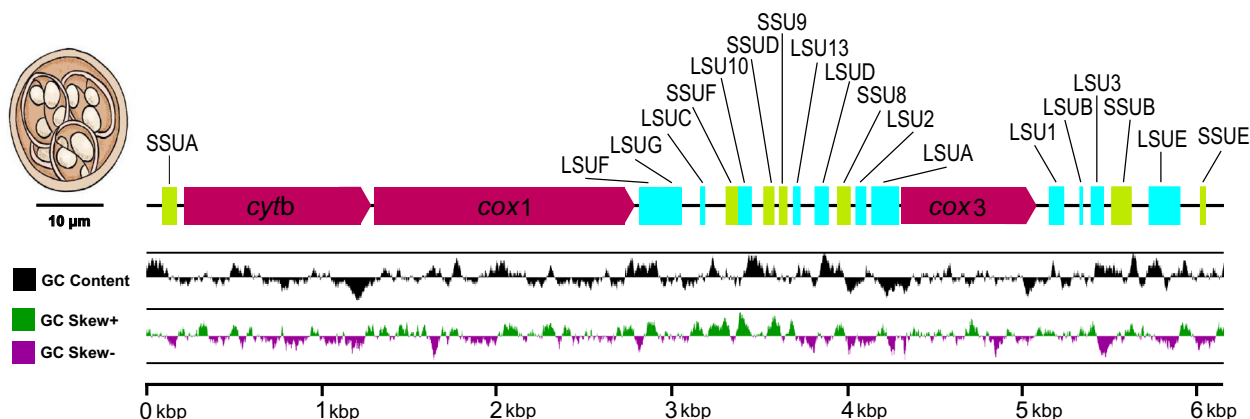


Fig. 2 Linear organization of *E. zuernii* mtDNA. The order and transcriptional direction of three coding regions for *cytb*, *cox1*, and *cox3* are indicated by purple arrows and fragments of seven SSU (green) and 12 LSU (light blue) rDNAs are detected between the protein-coding regions and identical to those of other congeneric species. The nomenclature of these ribosomal fragments follows the convention of Feagin et al. (2012) [40]. Positive and negative GC-skew and GC content are indicated in dark green, purple, and black, respectively, across the whole genome

Within three PCGs, *cox1* was presented between *cytb* and LSUF, *cox3* between LSUA and LSU1, and *cytb* between SSUA and *cox1*, respectively, with obvious AT skewness (AT-skew = -0.17; Additional file 5: Table S4). Such AT skewness was also reflected in their codon usage patterns and relative synonymous codon usage (RSCU) of PCGs. As shown in Fig. 3, the most frequently used codon of the PCGs was AGA (RSCU=4.71), followed by UUA (RSCU=2.35), CCA (RSCU=2.34), and GGU (RSCU=2.15), corresponding to the most frequently

used amino acid Leu ($n=166$), followed by Phe ($n=104$) and Ser ($n=103$), similar to other *Eimeria* species. Furthermore, among the start codon choices, except for the *cox3* gene, which was deduced to use a non-standard start codon TTG, the *cox1* and *cytb* genes used ATA and ATG as the start codons, respectively. TAA was used as the stop codon to terminate the *cytb*, *cox1*, and *cox3* genes. For SSU rDNAs, seven gene fragments included SSUA, SSUB, SSUD, SSUE, SSUF, SSU8, and SSU9 and ranged in size from 37 (SSUF) to 116 bp (SSUB). Within

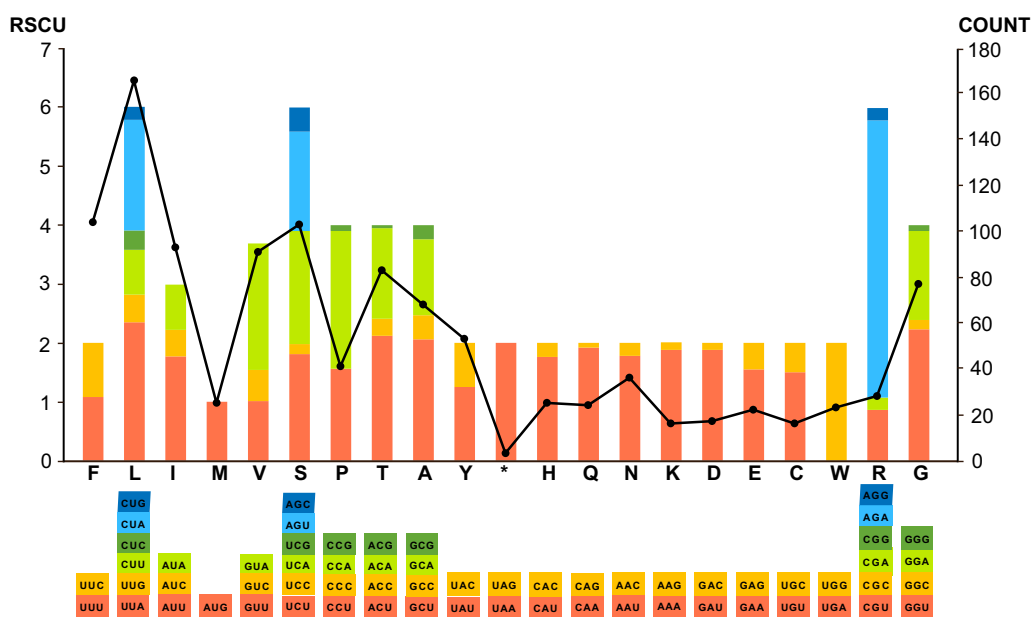


Fig. 3 Codon usage in PCGs of *E. zuernii* mtDNA. The numbers on the left and right Y-axis scales referred to the RSCU value and the total number of codons, respectively. Codon families were plotted under the X-axis scale and represented by different colors. The codon counts were depicted by the black line graph

LSU rRNAs, 12 gene fragments included LSUA, LUSB, LUSC, LSUD, LSUE, LSUF, LUSG, LSU1, LSU2, LSU3, LSU10, and LSU13 and ranged in size from 16 (LUSC) to 188 bp (LSUE). It appeared that these fragmented rRNAs were more conserved than PCGs, possibly related to their functional constraints in the former [39].

Comparative mitogenomics among Eimeria

To understand the evolutionary divergence of *Eimeria* spp., the interspecific variations between and among *E. zuernii* and congeneric species were determined using the nucleotide and amino acid sequences of single and concatenated mt PCGs. The interspecific variations for each PCG ranged from 11.4% to 29.4% at the nucleotide level and 6.4% to 23.5% at the amino acid level. Furthermore, on the basis of the concatenated PCGs, the interspecific variations ranged from 14.0% to 23.2% for nucleotide sequences and 9.0% to 16.3% for amino acid sequences (Additional file 6: Table S5). It was obvious that the highest sequence variation was found in the *cox3* gene while the lowest sequence divergence was found in

the *cytb* gene, suggesting *cytb* was the most conserved PCG among *Eimeria* mitogenomes, which is consistent with previous findings in species of *Babesia*, *Theileria*, and *Plasmodium* [44, 45]. Moreover, it also seemed noteworthy that regardless of nucleotide or amino acid levels, *E. zuernii* always shared the lowest sequence variation with *Eimeria mephitidis*, to some extent, suggesting their closer genetic similarity than other *Eimeria* species.

Evolutionary and phylogenetic analyses

To measure the interspecific genetic similarity between *E. zuernii* and other *Eimeria* spp., single or concatenated mt PCGs were also used to calculate genetic distances among *Eimeria* based on the K2P model [30]. As shown in Fig. 4a, based on either single or concatenated mt PCG datasets, it was clear that the minimum K2P values were always present between *E. zuernii* and *E. mephitidis* (0.114 and 0.139) in contrast with the maximum values present between *E. zuernii* and *Eimeria maxima* (0.210 and 0.173), supporting that *E. zuernii* was closely related to *E. mephitidis* but diverged from

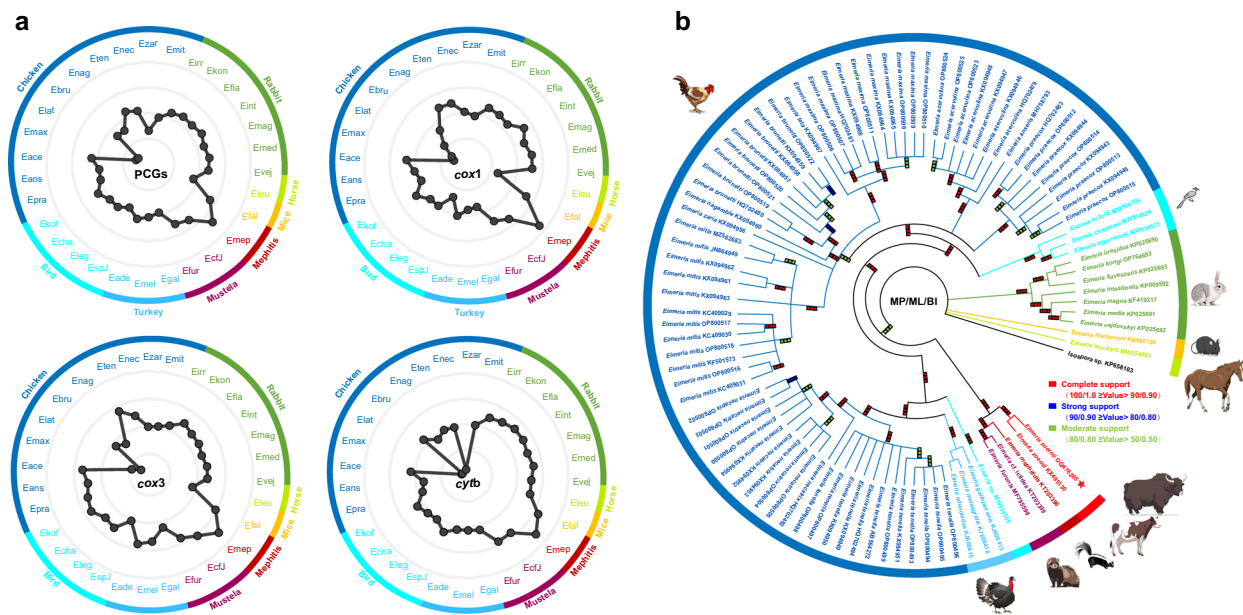


Fig. 4 Evolutionary and phylogenetic relationships between *E. zuernii* and other congeneric species. **a** Patterns of K2P distance between *Eimeria zuernii* and other 30 *Eimeria* species determined on the basis of single and concatenated mt PCGs. Gray lines indicate the same K2P distance from the center. Black dots show the relative K2P distances between *E. zuernii* and other eimerian parasites. Dots closer to the edge of the patterns indicate a smaller K2P distance between *E. zuernii* and the corresponding species. Species abbreviations: *E. anseris*, Eans; *E. chapmani*, Echa; *E. kofoidi*, Ekof; *E. legionensis*, ELEG; *Eimeria* sp._JRBarta-2021b, Espj; *E. acervuline*, Eace; *E. brunetti*, Ebru; *E. lata*, Elat; *E. maxima*, Emax; *E. mitis*, Emit; *E. nagambie*, Enag; *E. necatrix*, Enec; *E. praecox*, Epra; *E. tenella*, Eten; *E. zaria*, Ezar; *Eimeria* cf_ictidea_JRB-2016, Ech; *E. falciformis*, Efal; *E. furonis*, Efur; *E. leuckarti*, Eleu; *E. mephitidis*, Emep; *E. flavescens*, Efla; *E. intestinalis*, Eint; *E. irridua*, Eirr; *E. kongi*, Ekon; *E. magna*, Emag; *E. media*, Emed; *E. vejdvoskyi*, Evej; *E. adenoeides*, Eade; *E. gallopavonis*, Egal; *E. meleagridis*, Emel. **b** Phylogenetic tree inferred by MP, ML, and BI algorithms using complete mitogenome datasets of *E. zuernii* and other congeneric species. *Eimeria* species, together with their branches, are marked with different colors according to host origins as follows: bird in light blue, chicken in dark blue, horse in light green, mephitis in dark red, mouse in yellow, mustela in purple, rabbit in green, turkey in blue, and yak/cattle in red. *Eimeria zuernii* sequenced in this study is indicated in bold font along with a star. The color boxes at each node showed bootstrap values for MP/ML/BI

E. maxima. Furthermore, among genetic distance structures, the *cox3*-based K2P values were all obviously larger than those of the *cytb*, *cox1*, and concatenated PCGs, in agreement with the aforementioned result in which *cox3* was determined to be the most divergent gene among *Eimeria* PCGs. In parallel with genetic distance analysis, the selective pressure placed on mt PCGs of *Eimeria* during evolution was determined by qualification of the K_a , K_s , and K_a/K_s ratios (Additional file 7: Table S6). Our results showed that all K_a/K_s values were < 1 , suggesting that three PCGs were subjected to negative or purifying selection, to a certain extent, in agreement on the conservation and validity of the genus *Eimeria* in the course of evolution.

The phylogenetic relationships among eimerian parasites were inferred based on the complete mitogenomic dataset (containing 4315 conserved and 2237 variable sites) of *Eimeria* spp. available in GenBank (Additional file 3: Table S2). As shown in Fig. 4b, three phylogenetic trees (MP/ML/BI) consistently supported a paraphyletic relationship among species of *Eimeria*, and this relationship can be marked according to their host origins, except for bird-infecting species, consistent with previously conducted phylogenetic studies [46–49]. Notably, *E. zuernii* was placed close to *E. mephitidis* and together grouped with *Eimeria* cf. *ictidea* and *Eimeria furonis* as a clade (Clade I), with high statistical support (all statistical values ≥ 99 or 0.99). Furthermore, this clade was more closely related to *Eimeria* species of birds (Clade II) than to *Eimeria* species of rabbits (Clade III), in accordance with recent mtDNA-based phylogenetic conclusions [18, 19, 41, 50]. It appeared that these three clades occupied the main part of the phylogenetic tree, although their topological relationships with *Eimeria* species of mice and horses remained to be determined because of the poor bootstrap supports observed here (Fig. 4b). The previous mt *cox1*-based phylogenetic analysis showed that the *E. zuernii*-contained clade was more closely related to mouse *Eimeria* spp. than to bird *Eimeria* spp.; however,

the nuclear 18S-based phylogeny indicated a closer relationship between *E. zuernii*-contained clade and rabbit *Eimeria* spp. than between *E. zuernii*-contained clade and bird *Eimeria* spp. [51]. This discordance in the mt and nuclear phylogenies is surprising and might be due to a greater rate of nucleotide change in the mitogenomes of eimerian parasites than that seen in nuclear-encoded sequences, as reported in helminths [52]. Therefore, future studies using mitogenomic data from more widespread species or isolates of *Eimeria* from herbivorous and omnivorous animals worldwide are required to determine the evolutionary relationships within the entire genus *Eimeria*.

Population structure

The number of haplotypes, haplotype diversity value H_d , and nucleotide diversity π of each *E. zuernii* population were calculated and are shown in Table 2. The Xinlong population had the highest H_d value (0.867) and π (0.00326), while the Ya'an population had the lowest H_d value (0.500) and π (0.00064). Ganzi and Litang populations had the same H_d value of 0.800, whereas the Litang population had a lower π value than the Ganzi population (0.00163 vs. 0.00232). Likewise, Daofu and Ruorgai populations had the same H_d value of 0.667, whereas the Daofu population had a lower π value than of the Ruorgai population (0.00086 vs. 0.00143). For the Luhuo population, the respective H_d value was 0.750 and π was 0.00138, and for the Hongyuan population, the H_d value was 0.644 and π was 0.00097. Combined, these results showed that these eight *E. zuernii* populations had high haplotype diversities and low nucleotide diversities. Similar phenomena were seen in other protozoan parasites with large standing population sizes and extremely high fecundities, including *Trichomonas vaginalis*, *Giardia duodenalis*, and *Theileria annulata* [53–55], which might reflect a high matrilineal effective population size for *E. zuernii* and also signify the occurrence of expansion of a low effective population size of *E. zuernii* after a period

Table 2 Summary of the genetic diversity of eight geographic populations of *E. zuernii* on the basis of the *cox1* dataset

Population	No. of individuals	No. of haplotypes	Haplotype diversity (H_d)	Nucleotide diversity (π)	Fu's F_s	Tajima's D
Hongyuan	10	3	0.644	0.00097	-0.046	0.222
Xinlong	6	4	0.867	0.00326	-0.071	0.878
Ya'an	4	2	0.500	0.00064	0.172	-0.612
Ganzi	5	3	0.800	0.00232	0.469	1.573
Daofu	3	2	0.667	0.00086	0.172	-0.612
Luhuo	8	3	0.750	0.00138	0.330	1.449
Ruorgai	9	3	0.667	0.00143	0.551	1.754
Litang	6	4	0.800	0.00163	-1.350	-0.185

because of the rapid population growth enhancing the retention of new regions.

In addition, the negative values of Fu's F_s were observed in Hongyuan ($F_s = -0.046$), Xinlong ($F_s = -0.071$), and Litang ($F_s = -1.350$) populations, in contrast with the positive values of Fu's F_s found in Daofu ($F_s = 0.172$), Ya'an ($F_s = 0.172$), Luhuo ($F_s = 0.330$), Ganzi ($F_s = 0.469$), and Ruoergai ($F_s = 0.551$) populations (Table 2). Likewise, the negative value of Tajima's D was also observed in the Litang ($D = -0.185$) population, in contrast with the positive values of Tajima's D found in Luhuo ($D = 1.449$), Ganzi ($D = 1.573$), and Ruoergai ($D = 1.754$) populations (Table 2). Although the differences among populations in both neutrality tests were not significant, the negative values of the two neutrality indices in the Litang population indicated that the population had undergone expansion, while positive values in the Luhuo, Ganzi, and Ruoergai populations suggested these populations experienced a bottleneck [56–58]. Furthermore, significantly high F_{st} values were also observed between populations (Additional file 8: Table S7). The highest F_{st} value was seen between Hongyuan and Daofu populations ($F_{st} = 0.92157$, $P < 0.05$), followed by F_{st} values between Hongyuan and Ya'an populations ($F_{st} = 0.91220$, $P < 0.01$), between Hongyuan and Litang populations ($F_{st} = 0.89787$, $P < 0.05$), between Luhuo and Daofu populations ($F_{st} = 0.88665$, $P < 0.05$), between Ruoergai and Daofu populations ($F_{st} = 0.88406$, $P < 0.05$), between Ya'an and Luhuo populations ($F_{st} = 0.88360$, $P < 0.05$), and between Ya'an and Ruoergai populations ($F_{st} = 0.88066$, $P < 0.01$). Such high F_{st} values indicated a possible geographical isolation of *E. zuernii*. Indeed, when taking the yak's features, including the free-ranging, high-altitude grazing, and self-reproduction model, as well as the captive history of individuals sampled here into account, it is possible that the lack of translocations and/or introductions of these host populations between parks contributes to this population isolation of *E. zuernii*. Interestingly, such high F_{st} values were also observed in other protozoan parasites, such as *G. duodenalis* and *Cryptosporidium* spp., which were sampled from self-breeding dairy farms [55, 57]. To some extent, this high F_{st} value supports the phenomenon that the low gene flow and high genetic diversity of the bovine protozoa could be accelerated in an independent self-service farming mode. Of course, the phenomenon remains further validated when more additional protozoan population genetic data become available, especially those from hosts bred in an independent self-service farming model.

Based on 51 *E. zuernii* isolates (including the isolate for mitogenome sequencing in this study) and three reference isolates from GenBank, the mt *cox1* sequences containing 31 variable sites (3.99%) defined 24 haplotypes

of *E. zuernii*. The ML-based phylogenetic tree revealed that Ganzi population-specific haplotypes Hap_23 and Hap_24 closely clustered with Hap_1, and then three haplotypes together were paraphyletic with haplotypes of Daofu (Hap_21 and Hap_22), Ya'an (Hap_9 and Hap_10), LuHuo (Hap_14–16), and Xinlong plus Hongyuan (Hap_2–8) (Fig. 5a). It was also clear that Litang population-specific haplotypes Hap_17–20 were more closely related to Ruoergai population-specific haplotypes Hap_11–13 than others. These relationships among these haplotypes were further confirmed using the Network analysis. As shown in Fig. 5b, the network map revealed a radial-shaped clustering (RSC) with Hap_0 as the center, which was hypothetically inferred as the ancestral haplotype. It seemed that the haplotypes in each geographic population were highly specific. Although Hap_1 was shared by Ganzi, Daofu, and Yangzhou populations, Hap_12 was shared by Ruoergai and Litang populations, and Hap_7 was shared by Hongyuan and Xinlong populations. Moreover, *E. zuernii* from LuHuo and Ya'an appeared to form their own populations, and the latter was inferred as a possibly unsampled or extinct population because of the presence of a median vector (Fig. 5b). In general, network analysis is regarded as a better approach for representing genealogical relationships at a population level than traditional phylogenetic analysis because this approach is able to consider several factors related to intraspecific gene evolution, including the persistence of ancestral haplotypes, the existence of multiple descendant haplotypes, and low levels of sequence variation [58, 59]. Combined, the phylogenetic and network results suggested a low genetic structure among yak *E. zuernii* populations although they were prevalent in different geographic ranges of China.

Conclusions

In this study, we sequenced the entire mitogenome of *E. zuernii* from the yak and characterized its genetic diversity across eight geographic ranges in China. Comparative mitogenomics determined both *cytb* and *cox3* genes as the most and least conserved PCGs, respectively, within the genus *Eimeria*. Evolutionary and phylogenetic analyses indicated that *E. zuernii* shared the closest relationship with *E. mephitidis*, and all species of *Eimeria* can be grouped into three clades according to their host origins. Further *cox1*-based genetic structure inference revealed 24 haplotypes of *E. zuernii* with high haplotype diversities and low nucleotide diversities, suggesting a low genetic structure and rapid evolutionary rate as well as a previous expansion event among *E. zuernii* populations. Future additional samplings could possibly uncover the geographic structure of *E. zuernii* in more detail.

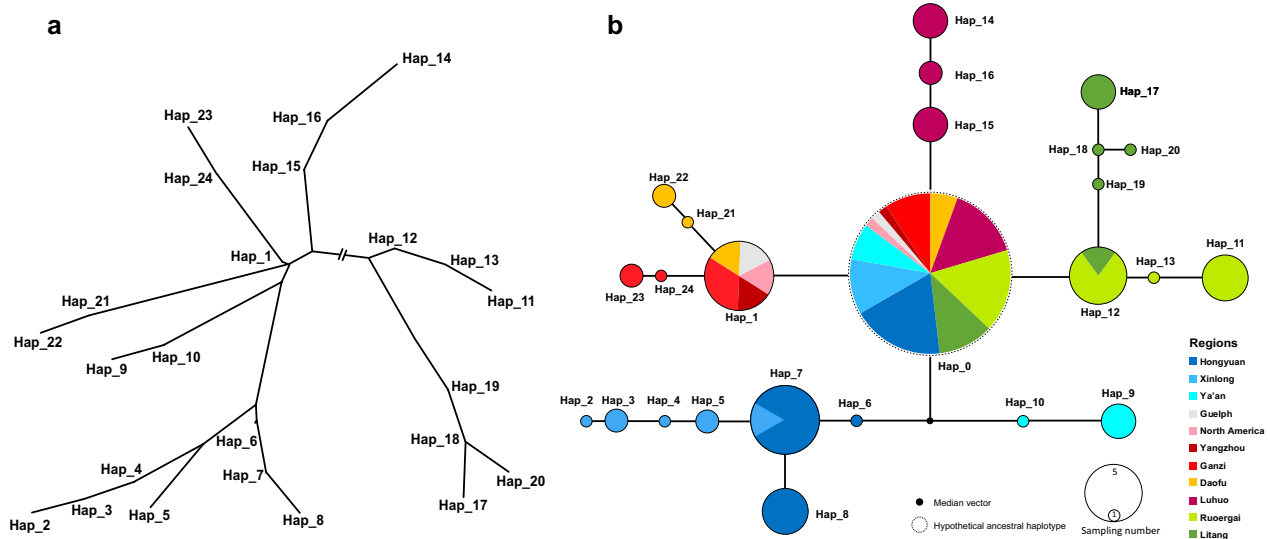


Fig. 5 Phylogeny and network map of *Eimeria zuernii* haplotypes inferred on the basis of 54 *cox1* data. **a** Topology tree of 24 haplotypes of *E. zuernii*. **b** Network map of *E. zuernii* haplotypes. *Eimeria zuernii* isolates from different geographic origins are shown in dark blue (Hongyuan), blue (Xinlong), light blue (Ya'an), gray (Guelph), pink (North America), dark red (Yangzhou), red (Ganzi), yellow (Daofu), purple (Luhuo), green (Ruergai), and dark green (Litang). The small black dot represents the median vector, indicating unsampled or extinct haplotypes. The dotted cycle denotes the hypothetically ancestral haplotype. The proportion of haplotype frequencies is shown with sizable coils. The network branches linking the cycles show the relationships between the haplotypes

Abbreviations

18S	Small-subunit ribosomal RNA
ITS-1	Internal transcribed spacer 1
rDNA	Nuclear ribosomal DNA
mtDNA	Mitogenomic DNA
mt	Mitochondrial
MP	Maximum parsimony
ML	Maximum likelihood
BI	Bayesian inference
PCG	Protein-coding gene
tRNA	Transfer RNA
rRNA	Ribosomal RNA
LSU	Large subunit ribosomal RNA
SSU	Small subunit ribosomal RNA
<i>cox1</i>	Cytochrome c oxidase subunit I
<i>cox3</i>	Cytochrome c oxidase subunit III
<i>cytb</i>	Cytochrome b
Fst	Wright's fixation index
Ka	Non-synonymous substitutions
Ks	Synonymous substitutions
RSCU	Relative synonymous codon usage

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13071-023-05925-8>.

Additional file 1. Table S1. *Eimeria zuernii* isolates sequenced in this study.

Additional file 2. Figure S1. Multi-sequence comparisons of the ribosomal ITS-1 from the yak *Eimeria* and the congeneric *Eimeria zuernii*. 99.2%–100% identities are observed between *E. zuernii* and 51 *Eimeria* isolates identified in this study.

Additional file 3. Table S2. Summary of the mitogenome information of *Eimeria* spp. included in this study.

Additional file 4. Table S3. Organization of the *Eimeria zuernii* mitogenome.

Additional file 5. Table S4. Nucleotide composition of the complete *Eimeria zuernii* mitogenome.

Additional file 6. Table S5. Pairwise genetic divergences of *Eimeria* parasites based on mitogenome datasets.

Additional file 7. Table S6. Ka, Ks, and Ka/Ks ratios of three mt PCGs of *Eimeria zuernii* and congeneric species sequenced so far.

Additional file 8. Table S7. Wrights Fixation Index (Fst) values of eight geographic populations of *Eimeria zuernii* based on *cox1* datasets.

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Author contributions

L.W., S.C., and Y.X. conceived and designed the study. X.Z. and Z.W. performed the experiments, analyzed the data, and wrote the manuscript. P.Z., X.G., and R.H. participated in the implementation of the study. J.X. and B.J. contributed reagents/materials/analysis tools. L.W., S.C., and Y.X. critically revised the manuscript. All authors read and approved the final version of the manuscript.

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Availability of data and materials

Molecular data have been deposited to GenBank with the following accession numbers: OQ476205 for *Eimeria zuernii* mitogenome, OR351547–OR351597 for *E. zuernii* ITS-1 dataset and OR039219–OR039268 for *E. zuernii* *cox1* dataset. Fifty-one *E. zuernii* isolate specimens including the one for mitogenome sequencing in this study have been deposited at the Parasitological Museum of Sichuan Agricultural University (Sichuan, China) under the collection numbers XY2022_28–XY2022_78.

Declarations

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Ethics approval and consent to participate

This study was conducted in strict accordance with the recommendations in the Guide for the Care and Use of Committee of Sichuan Agricultural University, China (approval no. SYXK 2019-187). All animal procedures used in this study were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council) and recommendations of the ARRIVE guidelines (<https://www.nc3rs.org.uk/arrive-guidelines>).

Author details

¹Department of Parasitology, College of Veterinary Medicine, Sichuan Agricultural University, Sichuan 611130, China. ²Institute of Preventive Veterinary Medicine, Sichuan Agricultural University, Sichuan 611130, China.

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