**Hyaloklossia** Labbé, 1896 (Alveolata: Apicomplexa) in frogs: Description of a new species and proposing a new subfamily to accommodate these enigmatic parasites

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**ABSTRACT**

*Hyaloklossia* Labbé, 1896 (Alveolata: Apicomplexa) is a monotypic genus of renal coccidia found in anurans, particularly in the edible frog *Pelophylax* kl. *esculentus* (Amphibia: Anura: Ranidae), distributed in different parts of Europe. Here we propose a new *Hyaloklossia* species from the Tokyo daruma pond frog, *Pelophylax porosus* porosus. The coccidium detected in the renal tissue of *P. p. porosus* shared some morphological characteristics with the type species, *Hyaloklossia lieberkuehni* (Labbé, 1894), reported from *P. kl. esculentus*. However, in addition to size differences in several oocyst and sporocyst features between these parasites, phylogenetic analysis of gene fragments from two nuclear ribosomal loci and the mitochondrial cytochrome oxidase subunit 1, exposed distinct genetic differences between *H. lieberkuehni* and our new species. Although our analysis validated the monophyly of *Hyaloklossia* with some members of the Toxoplasmataine Biocca, 1957, *Cystoisosporinae* Frenkel et al., 1987, and *Eumonosporinae* Chou et al., 2021 (Sarcocystidae Poche, 1913), comparison of genetic differences between *Hyaloklossia* species from *P. p. porosus* and *H. lieberkuehni* revealed the presence of a greater number of polymorphisms than that observed when comparing inter-species (*Heydornia* spp., *Besnoitia* spp.) or inter-genus (*Toxoplasmata* vs *Neospora*, *Neospora* vs *Hammondia*, and *Neospora* vs *Heydornia*) variabilities among members of the Sarcocystidae. This indicates that *Hyaloklossia*, as re-erected and defined by Modrý et al. (2001, Int. J. Syst. Evol. Microbiol. 51, 767–772), with its homoxenous life cycle, requires placement in its own subfamily. Thus, we propose a new subfamily, *Hyaloklossiinae* n. subfam., to accommodate two species, *H. lieberkuehni* from Europe and *Hyaloklossia kasumienesis* n. sp. which we describe here from *P. p. porosus* in Japan.

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1. Introduction

In true numbers of species, the Apicomplexa may be the largest taxonomic group of extant organisms on Earth except, perhaps, for the Fungi (Adl et al., 2007). Many apicomplexans are pathogens to humans and their companion, domestic, wild, and other food animals and all animal species are thought to host at least one apicomplexan species and most likely have several species either unique to them or that they share with congener(s) and/or with sympatric species, or both. We might, therefore, expect that we know a great deal about this group vis-à-vis the species in this group, their biology and, perhaps, even have a clear understanding of their basic phylogenetic relationships (Morrison, 2009). Unfortunately, in terms of biodiversity, the Apicomplexa is probably the least studied and most poorly known group of all parasite lineages, with 6000 to 7000 named species, which is perhaps only 0.1% of the estimated total number of species (Morrison, 2009). They are all unicellular, mostly intracellular, endoparasites that are hard to find and, when found and studied, they have a limited suite of morphological, ultrastructural, cyst-forming, and life history patterns, a combination that makes them among the most difficult and undesirable organisms to work with (Morrison, 2009).

As a case in point, there are ~8120 extant species of amphibians on...
Earth (Frost, 2020), but only 45 amphibian species (0.5%) have been examined for apicomplexans and from them, 52 apicomplexan species and 38 other forms (species incertae sedis, species inquirendae, etc.) have been documented in the literature (Duszynski et al., 2007; Duszynski, 2021). Unfortunately, this demonstrates that our knowledge of apicomplexan species richness is based on very limited, non-exhaustive sampling, which does not allow estimates for species richness at any scale. Similarly, taxa used to construct phylogenetic trees need to represent adequate sampling to provide a conclusive case for determining and understanding phylogenetic relationships, but such taxon sampling for the Apicomplexa is distressing because molecular sequences and molecular data are available for only a trivial subset of known species.

Within the coccidia (Apicomplexa: Alveolata), Sarcocystidae Poche, 1913 is one of the better studied families. Currently, it includes four subfamilies: Sarcocystinae Poche, 1913 (Sarcocystis, Flenkedia), Toxoplasmatinae Biocca, 1957 (Toxoplasma, Hammondia, Heydornia, Neospora, Besnoitia), Cystoisosporinae Frenkel et al., 1987 (Cystoisospora), and Eumonosporinae Chou et al., 2021 (Eumonospora), along with the genera Hyaloklossia and Nephropedoncysta (Frenkel et al., 1979; Smith, 1981; Sercundes et al., 2016; Duszynski et al., 2018; Chou et al., 2020; Chou et al., 2021). Hyaloklossia is a monotypic genus that is clearly distinguishable from other genera of Sarcocystidae based on its homogenous (direct) life cycle, gametogony and sporogony in frog renal tissues, and unusual oocyst morphology (Modry et al., 2001; Duszynski et al., 2007; Duszynski, 2021). Based on these observations, Hyaloklossia is an independent lineage from other members of the Sarcocystidae (Modry et al., 2001; Slapeta et al., 2003).

The edible frog, *Pelophylax kl. esculentus* (L., 1758) (Amphibia: Anura: Ranidae), which is widespread in Europe, is the most common definitive host for Sarcocystidae (Laveran and Mesnil, 1902; Nolle, 1923; Kazubski and Grabda-Kazubskia, 1973; Vojtkova, 1976). The northern leopard frog, *Lithobates pipiens* (Schreber, 1782) (Anura: Ranidae), found in the USA, the yellow-bellied toad, *Bombina variegata* (L., 1758) (Anura: Bombinatoridae), found in Bulgaria, the European common frog, *Rana temporaria* L., 1758 (Anura: Ranidae), and the marsh frog, *Pelophylax ridibundus* (Pallas, 1771) (Anura: Ranidae), found in Europe, also have been identified as definitive hosts of *H. lieberkuhnii* (Henry and Leblois, 1911; Walton, 1949; Golemansky and Miceva, 1975; Levine and Nye, 1977; Duszynski et al., 2007). Here, we describe a case of *Hyaloklossia* infection in the kidney of a Tokyo daruma pond frog, *Pelophylax porosus porosus* (Cope, 1868) (Anura: Ranidae), which has not been reported as a host in previous studies. Morphological and phylogenetic analyses were performed to compare the form we studied to *H. lieberkuhnii* and these comparisons allow us to propose a new *Hyaloklossia* species. Furthermore, the taxonomic position of *Hyaloklossia* within the Sarcocystidae is reconsidered, and a new subfamily is proposed to accommodate it.

## 2. Materials and methods

### 2.1. Sample collection and examination

Nine Tokyo daruma pond frogs captured in July 2020 in Lake Kasumigaura, Ibaraki, Japan (35°58′43.3″ N, 140°34′49.0″ E) were examined. These frogs were sacrificed by overdosing with pentobarbital sodium and provided to us after being used in practical training for veterinary students All experiments were performed in accordance with the Institutional Animal Ethical Committee of the College of Agriculture and Use of Laboratory Animals at Nippon Veterinary and Life Science university: 2019-j8). All frogs examined appeared to be 3-year-old adults (6 males and 3 females), with body length 5.8–7.2 cm and weight 19–31 g.

### 2.2. Morphological examination

After harvesting the left kidney from each frog, half of the tissue was homogenized using BioMasher (Nippi, Japan) and the other half was dissected under observation with an Olympus SZX16 stereomicroscope (Olympus, Japan). The intestinal contents were smeared directly on to glass slides. Tissue samples from the liver, lung, spleen, right kidney, and digestive tracts were fixed in 10% neutral buffered formalin and processed into blocks by paraffinization for routine histopathological examination. Serial sections (5 μm) were stained using hematoxylin and eosin (H&E). Blood specimens collected from the heart were smeared and stained using Diff-Quick stain (Sysmex, Japan). These specimens were examined for apicomplexan parasites with an Olympus BX53 optical microscope (Olympus, Japan) using differential interference contrast. Photomicrographs were captured using a DP27 photomicroscope (Olympus) or NanoZoomer-SQ Digital slide scanner (Hamamatsu Photonics, Japan). For measurements, the ImageJ software (Rueden et al., 2017) was used to analyze pre-recorded images captured at 1000×. All values are reported in micrometres and given as a range followed by the mean and standard deviation in parentheses. Abbreviations used throughout are standardized (Wilber et al., 1998); oocyst characters: length (L), width (W), their ratio (L/W), micropyle (M), microple cap (MC), oocyst residuum (OR); sporocyst characters: length (L), width (W), their ratio (L/W), Stieda body (SB), substieda body (SSB), sporocyst residuum (SR), sporozoites (SZ), and nucleus (N) in SZ.

### 2.3. DNA extraction, PCR amplification, and sequencing

A sporocyst mass detected in the kidney tissue was collected using a glass Pasteur pipette under observation with an SZX16 stereomicroscope (Olympus) and used for DNA extraction. Genomic DNA was extracted from the mass using a QIAGEN Power Soil DNA Isolation Kit (Qiagen, Germany) according to the specified procedure with a prolonged vortex time of 20 min. The specimen produced was used as PCR template. Double-distilled water was included as the negative control.

Three genetic loci, nuclear small subunit ribosomal RNA (18S), nuclear large subunit ribosomal RNA (28S), and mitochondrial cytochrome c oxidase subunit I (cox1), were amplified using specific primers and amplification conditions (Table 1). PCR was performed using 20 μl reaction volumes, each containing 0.2 μl of Takara ExTaq polymerase (Takara, Japan), 2 μl of 10 × buffer, 1.6 μl of dNTPs (2.5 mM each), 0.2 μl of each primer (50 μM), 1.0 μl of the template, and 14.8 μl of double-distilled water. The thermocycling conditions were as follows: initial denaturation at 95 °C for 5 min, followed by 30–40 cycles of denaturation at 94 °C for 30 s, annealing at 45–60 °C for 30 s, and extension at 72 °C for 1 min. Final extension was performed at 72 °C for 10 min (for 18S) or 5 min (for 28S and cox1), followed by a hold step at 4 °C.

The PCR products were separated by electrophoresis in 1.5% agarose gels and visualized under an LED transilluminator after staining with 0.5% ER Green (Bio Craft, Japan). The size of the PCR products was estimated by comparison with a 100-bp DNA plus DNA ladder (Maestrogen, Taiwan). The PCR products were purified using ExoSAF-IT (Applied Biosystems, USA) and dispatched to a sequencing service provider (Macrogen Corp., Japan) and analyzed using an ABI 3730xl DNA Analyzer (Thermo Fisher Scientific, USA) with the same PCR primers.

### 2.4. Genetic analysis

Sequence similarity was studied separately using the BLASTN program available in the website of the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/Blast.cgi). To determine whether the genetic variation detected between *Hyaloklossia* species from *P. p. porosus* and *H. lieberkuhnii* corresponded to intraspecific and/or interspecific variation, the 18S, 28S, and cox1 sequences of closely related or sister taxa were examined from the International Nucleotide Sequence Databases (INSD) (Table 2), and divergence was analyzed using the MEGA X software (Kumar et al., 2018). The sequences were aligned by using MAFFT with Q–INS–I (Katoh and
Standley, 2013), with application of the same algorithm in all cases. The gaps and missing data were eliminated. The percentage of sequence gaps and missing data were determined to be the Tamura-Nei model with the maximum likelihood (ML) methods using MEGA X with 18S, 28S, cox1 sequences differed. The average distance was also calculated to compare species and sequence used for comparing 18S and 28S distances.

### Table 2

| Subfamily                   | Genus            | Species       | Accession nos. |
|-----------------------------|------------------|---------------|----------------|
| Toxoplasmatinae             | Toxoplasma       | T. gondii     | L24381 L25635  |
|                            | Hammondia        | H. hammondii  | AI008381 AF101077 |
|                            | Heydornia        | H. heydornii  | KT184370 AF159240 |
|                            | Neospora         | N. caninum    | AJ271354 AF001946 |
|                            | Besnoitina       | B. besnoiti   | AFI09678 AF076866 |
|                            |                  | B. bennetti    | AY665399 AY778965 |
|                            |                  | B. tharandi    | MH217579 AY161614 |
|                            |                  | B. darling     | GU479631 - |
|                            |                  | B. jeelioni    | AY294126 AF076868 |
| Cystoisporinae              | Cystoispora      | C. canis      | KT184368 - |
|                            |                  | C. bellii      | DQ006683 - |
|                            |                  | C. choemias    | GU292305 - |
|                            |                  | C. felis       | KT184364 UI85705 |
|                            |                  | C. nos         | RF852452 - |
| Eumonosporinae              | Eumonospora      | E. bennae     | LC595644 LC595645 |
|                            |                  | E. neofalcius  | KTO37081 - |
|                            |                  | E. dancele     | KJ634019 - |
| Incertae subfamiliae        | Hyaloklossia     | H. ibertaeusni | AY29623 AF513499 |

### 3.1. Description of Hyaloklossia kasumiensis n. sp

#### 3.1.1. Type host

Amphibia (Anura: Ranidae), Pelophylax porosus porosus (Cope, 1886), Tokyo daruma pond frog.

#### 3.1.2. Type locality

Japan: Ibaraki, Lake Kasumigaura (35°58’43.3”N, 140°34’49.0”E).

#### 3.1.3. Other hosts

None to date.

#### 3.1.4. Geographic distribution

Known only from the type locality.

#### 3.1.5. Unsporulated and sporulated oocysts

Immature oocysts (Fig. 3B–D) were elongated-ovoidal or bean-shaped; in tissue sections they measured L × W (n = 3): 25–30 × 15–25. The oocyst wall was thin, single-layered; M, MC, OR: all absent. Prior to sporulation, the sporont was spheroidal with granular cytoplasm and an elongate N that was usually at the margin (Fig. 3B–C) and one of them showed cytoplasmic cleavage (Fig. 3C). Two sporoblasts formed within the oocyst and were spheroidal with granular cytoplasm and an elongate nucleus that was usually at its margin (Fig. 3D).

#### 3.1.6. Sporocysts and sporozoites

Sporocysts (Fig. 4A–C) were broadly spindle-shaped with a smooth, single-layered wall, and measured L × W (n = 20): 24.6–29.1 (27.1 ± 1.4) × 14.0–16.6 (15.5 ± 0.8); L/W ratio: 1.5–2.0 (1.8 ± 0.1); SB, SSB, PSB: all absent; sporozoite with four SZ that measured L × W (n = 34): 16.4–23.3 (20.7 ± 2.0) × 3.3–4.9 (4.1 ± 0.4); L/W ratio: 3.9–6.8 (5.1 ± 0.8); each SZ had a rounded end enclosing the N and a tapered end; SR: present as a round, granular body and measured, L × W (n = 38): 7–10 (8.4 ± 1.1) × 6–8 (7.3 ± 0.6).

#### 3.1.7. Prevalence

This species was recovered from one of nine (11%) specimens of the type species; the type host was an adult female (body length 6.0 cm, weight 20.0 g).

#### 3.1.8. Sporulation

Endogenous.

#### 3.1.9. Prepatent and patent periods

Unknown.

#### 3.1.10. Site of infection

Mature sporocysts (Fig. 1) were found in the kidney. Most of the sporocysts formed agglomerates in the interstitium (Fig. 1A and B, 2, 3A), and some were detected within renal endothelial cells (Figs. 1C and 2). A few immature and mature oocysts were observed in the kidney tissues (Figs. 1D, 3B–E). No coccidial stages were detected in the other tissues examined (or in specimens from other frogs).

#### 3.1.11. Cross-transmission

None to date.

#### 3.1.12. Material deposited

A holotype slide (kidney tissue, H&E) with oocysts and sporocysts from one P. p. porosus was deposited in the Meguro Parasitological Museum, Meguro, Tokyo, Japan, accession number, MPM Col. No. 21755. Representative sequences were deposited in the DNA Data Bank of Japan, accession numbers LC602188 (18S), LC602187 (28S) and LC602189 (cox 1).

#### 3.1.13. Zoobank registration number

urn:lsid:zoobank.org:pub:E2BC99ED-CEA2-4EA5-914F-2E79A5C265A2.
Fig. 1. Light microscopy of Hyaloklossia sporocysts in the kidney of Pelophylax porosus porosus. (A) Mature sporocysts within a cyst-like structure are visible in the renal interstitium. (B) Squash preparation of kidney showing numerous immature sporocysts. Note the granular cytoplasm of the sporoblasts (sporonts) and the barely visible membrane surrounding them. (C) A sporocyst (arrowhead) in the renal endothelial cell. (D) Mature oocyst (arrowhead) with two sporocysts in the renal endothelial cell. Asterisk indicates renal tubules. Bars = 25 μm.

Fig. 2. Light microscopy of hematoxylin and eosin-stained sections of renal tissue of Pelophylax porosus porosus. Hyaloklossia sporocysts and/or oocysts congregated in a diffused manner in the renal interstitium (circles), and some were found solitarily in renal epithelial cells or in the lumen (arrowheads). Bar = 200 μm. A high-resolution version of this slide for use with the Virtual Microscope is available as eSlide: VM06312.
3.1.14. Etymology

Derived from the ancient name, “kasumi-no-ura” of Lake Kasumi-gaura and -ensis (L., belonging to, from) to reflect where the type host was collected.

3.1.15. Remarks

The morphological features of oocysts and sporocysts are of importance as taxonomic keys that influence the description of coccidian species (Tenter et al., 2002; Berto et al., 2014). Here we compare the morphological characteristics of our species alongside the available data of its closest relative, *H. lieberkuehni* from *P. kl. esculentus* in Europe (Labbé, 1894, 1896; Laveran and Mesnil, 1902), Czechoslovakia (Vojtová, 1976), Poland (Kazubski and Grabda-Kazubska, 1973), the Czech Republic (Modrý et al., 2001), and from *B. variegata*, in Bulgaria (Golemansky and Miceva, 1975) (Table 3). Levine and Nye (1977) also reported *H. lieberkuehni* (which they called *Isospora lieberkuehni*) from *L. pipiens*, in the USA, but they only found “merozoites” in the cytoplasm of kidney tubule epithelial cells, so their data are not included in Table 3. When we look at all structural dimensions available (Table 3) we see that our form has slightly smaller oocysts, in length but not width, than the oocysts described from all *P. kl. esculentus* from Europe. We also note that the *Hyaloklossia* species detected in *B. variegata* might constitute a third *Hyaloklossia* species owing to the significant differences in the dimensions of its oocysts (20.5 × 15.0) and sporocysts (10.5 × 9.0) vs. those of *H. lieberkuehni* in the Czech Republic (35–45 × 20–25 and 25–30 × 14–16, respectively); confirmation of the other stages is necessary to confirm correct identification and gene sequencing would also help resolve this issue. The oocysts of *Hyaloklossia* species are exceptionally thin, irregular, and fragile, which makes them difficult to compare. In contrast, sporocysts are resistant structures. The sporocyst

![Fig. 3. Light microscopy of hematoxylin and eosin-stained sections of renal tissues of *Pelophylax porosus porosus*. (A) Mature sporocysts in the renal interstitium. Arrows indicate the transverse section of sporocysts showing four sporozoites with nuclei and a granular sporocyst residuum. (B and C) Immature oocysts. Note the very thin oocyst wall (arrows), sporonts with granular cytoplasm, and nuclei distributed at the cell margin (arrowheads). (D) Immature oocysts with two sporoblasts each and containing two polar nuclei (arrowheads). (E) Mature oocysts in renal epithelial cell showing two sporocysts with circular nuclei (arrowhead) and a granular sporocyst residuum. Arrows indicate the sporocyst wall. Bars = 50 μm (A) and 10 μm (B–E).](image)

![Fig. 4. Mature sporocysts of *Hyaloklossia* in the kidney of *Pelophylax porosus porosus*. (A) Light microscopy of a mature sporocyst in homogenized kidney tissue. (B) Nomarski interference contrast microscopy of a mature sporocyst in squash preparation of renal tubular tissue showing the presence of four spindle-shaped sporozoites. (C) Composite line drawing. Bar = 10 μm. Asterisk: sporocyst residuum.](image)
of *H. kasumiensis* in *P. p. porosus* was slightly wider than that of *H. lieberkuehni* reported by both Kazubski and Grabda-Kazubska (1973) and Modrý et al. (2001); thus, it has a smaller L/W ratio. Finally, the sporocyst residuum in our form is smaller than that of *H. lieberkuehni* sporocysts reported by Kazubski and Grabda-Kazubska (1973) and Vojtková (1976) (see Table 3). Based on our structural and molecular results, as well as geographical isolation, we consider the coccidian described in *P. p. porosus* as new to science.

### 3.2. Sequence analyses of *Hyaloklossia* spp

#### 3.2.1. Sequence similarity

The partial 18S gene sequence (1449 bp) of *H. kasumiensis* was 99.7% identical (1445/1449 bp) with that of *H. lieberkuehni* (accession no. AF298623) reported from the edible frog in the Czech Republic (Modrý et al., 2001) but similarity with other genera in the Sarcocystidae was less than 97.5%.

The partial 28S sequence (1422 bp) of *H. kasumiensis* revealed 98.6% identity (995/969 bp) with that of *H. lieberkuehni* (accession no. AF513499), also from the edible frog in the Czech Republic (Modrý et al., 2001). However, *H. kasumiensis* displayed similarity values < 90.8% identity with other genera in the Sarcocystidae.

A partial *cox1* sequence (910 bp) also was identified from *H. kasumiensis* and it had 87.9%–87.3% identity with the sequence from *Cystoisospora* spp. (nos. MF774038, MF774037, KT184385, LC377843, KU184384). The *cox1* sequence of *H. lieberkuehni* was not reported in the INSID, and therefore, was unavailable for comparison.

Accumulated nucleotide substitutions in the 18S and 28S genes between *H. kasumiensis* from *P. p. porosus* in Japan and *H. lieberkuehni* from the edible frog in the Czech Republic were 0.23% and 1.76%, respectively (Table 4). Some pairs of congers among the other sarcocystids showed up to 1.20% divergence in the 18S sequence and 3.35% divergence in the 28S sequence. However, several pairs showed divergence values lower than or equal to the divergences observed between *H. kasumiensis* and *H. lieberkuehni*. In particular, the inter-specific divergence for both 18S and 28S genes between our species and *H. lieberkuehni* was higher or equivalent to that observed for *H. heydorni* vs. *H. heydorni trifftiae* (18S: 0%, 28S: 0.35%) and *Besnoitia* spp. vs. *Besnoitia* spp. (18S: 0.23%, 28S: 0.35%) (Table 4). Furthermore, the inter-genus divergence between *Toxoplasma gondii* vs. *Neospora caninum* (18S: 0.23%), *N. caninum* vs. *Hammondia hammondi* (18S: 0.23%, 28S: 1.41%), and *N. caninum* vs. *H. heydorni* (18S: 0.23%) was lower than that observed between *H. kasumiensis* and *H. lieberkuehni*.

#### 3.2.2. Phylogenetic analysis

In our phylogenetic trees, members of the Toxoplasmatinae formed a monophyletic clade (clade I) that divided into two clades (Fig. 5A–C). Clade Ia included *Toxoplasma, Hammondia, Heydornia,* and *Neospora*
with high bootstrap values [18S: 95 (NJB)/91 (MLB); 28S: 100 (NJB)/96 (MLB)]. The Eumonosporinae clade (clade II) was monophyletic, whereas clade Ib composed of Besnoitia spp. had moderate to high bootstrap values [18S: 70 (NJB)/72 (MLB); 28S: 95 (NJB)/91 (MLB)]. The Hyaloklossia kasumiensis (clade III) and Cystoisosporinae (clade IV) were monophyletic groups, with low to moderate bootstrap values [18S: 55 (NJB)/51 (MLB); 28S: 97 (NJB)/88 (MLB)]. The Toxoplasmatinae, Eumonosporinae, and Cystoisosporinae and related taxa collectively formed a monophyletic clade (III + IV) with moderate bootstrap values (above 50%), whereas with respect to the 28S sequence, clade III showed the formation of an early branch at the monophyletic clade comprising Ia + Ib + I + II + IV. The phylogenetic positions of Nephroisospora were unstable, and it was placed in clade I (based on 18S sequence) or clade IV (based on cox1 sequence).
other taxa of Eimeriorina as well, such as in members of Pseudoklossia (Aggregatidae), several Eimeria species (e.g., E. truncta, E. fraterculae, and E. gaviae) and Margoliouthia Desser & Bower, 1977 in the Eimeridae, and in Nephroisospora epsici from the Sarcocystidae (Montgomery et al., 1978; Leighton and Gajadhar, 1986; Friedman et al., 1995; Desser and Bower, 1997; Wünschmann et al., 2010). The kidney receives a high blood flow, and renal endothelial cells could serve as the primary target for these coccidians that invade the bloodstream. Therefore, the occurrence of coccidia in the kidney is not unique to the genus Hyaloklossia, but might be a case of plesiomorphy. As indicated in phylogenetic trees, Hyaloklossia appears abruptly among genera within the Sarcocystidae, most of which have developed a heteroxenous, predator-prey life cycle involving final (carnivore) and intermediate/paratenic (herbivore/omnivore) hosts. Meanwhile, Hyaloklossia species exhibit less diversity for definitive host specificity than other sarcocystids and do not require an intermediate host during their life cycle. It is reasonable to suppose that Hyaloklossia species have a recent common ancestor with sarcocystids that parasitize carnivores, and their ability to form cysts in intermediate/paratenic hosts that is observed in other sister taxa has been lost secondarily during host switching.

The morphological features of disporocystic and tetrasporozoic oocysts without Stieda bodies and the phylogenetic characteristics reported here and by others, indicates that Hyaloklossia belongs to the Sarcocystidae; however, Hyaloklossia can easily be distinguished from the other four subfamilies with a heteroxenous or facultatively heteroxenous life cycles. Thus, we suggest that Hyaloklossia belongs to a lineage independent from other members of the Sarcocystidae and propose a new subfamily to accommodate them.

Declaration of competing interest

None.

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