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PROTOZOOLOGY - ORIGINAL PAPER

Haemosporidian parasites from captive Strigiformes in France

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Abstract

Haemosporidia infections may cause major damage to avian populations and represent a concern for veterinarians working in zoological parks or wildlife rescue centres. Following the fatal infection of 9 Great grey owls (*Strix nebulosa*) at Mulhouse zoological park, between summer 2013 and 2015, a prospective epidemiological investigation was performed in captive strigiform birds in France in 2016. The purpose was to evaluate the prevalence of haemosporidian parasites in captive Strigiformes and to estimate the infection dynamics around the nesting period. Blood samples were taken from 122 strigiform birds representing 14 species from 15 French zoological parks. Parasites were detected by direct examination of blood smears and by PCR targeting the mitochondrial cytochrome b gene. Haemosporidian parasites were detected in 59 birds from 11 zoos. Three distinct *Haemoproteus* mitochondrial cytochrome b sequences (haplotypes A and C for *H. syrnii* and haplotype B for *Haemoproteus* sp.) as well as two species of *Plasmodium* were detected. The overall prevalence of *Haemoproteus* infection was 12.8%. The percentage of birds infected by *Haemoproteus* varied according to the period of sampling. Nesting season seemed to be at greater risk with an average prevalence of 53.9% compared with winter season with an average prevalence of 14.8%, related to the abundance of the vectors. The prevalence of *Plasmodium* infection in Strigiformes did not exceed 8% throughout the year. This study confirmed how significant Haemosporidia infection could be in Strigiformes from zoological parks in France. The nesting season was identified as a period of higher risk of infection and consequently the appropriate period to apply prophylactic measures.

Keywords Haemosporidia · *Haemoproteus* · *Plasmodium* · Strigiformes · Prevalence · Zoological park

Introduction

Protozoan blood parasites of the order Haemosporidia are vector-borne parasites which have been reported in many bird species. Three genera can be distinguished: *Plasmodium* transmitted by mosquitoes (Culicidae), *Haemoproteus* transmitted by louse flies (Hippoboscidae) or biting midges (Ceratopogonidae) and *Leucocytozoon* transmitted by black flies (Simuliidae). Infections with multiple species and genera of Haemosporidia appear to be common (van Rooyen et al. 2013). These parasites may have negative fitness on the passerine host (Pearson 2001; Marzal et al. 2005) or may be pathogenic during energy-demanding or stressful phases such as breeding and the first year of life in juvenile snowy owls (*Nyctea scandiaca*) in the case of mixed infection with *H. noctuae* and *L. ziemanni* (Evans and Otter 1998). Cumulative effects of blood parasites on individuals may have serious consequences on columbiform host population (Earle et al. 1993). Following the fatal infection of 9 Great grey owls (*Strix nebulosa*) at Mulhouse zoological park, between summer 2013 and 2015, a prospective epidemiological investigation was performed in captive strigiform birds (both families Strigidae and Tytonidae) in 15 French zoological parks in 2016. The first objective of the study was to estimate the overall prevalence of Haemosporidia parasites and identify their genetic diversity in Strigiformes birds. The second objective was to describe the Haemosporidia infection dynamics during a year in strigiform birds throughout different periods of sampling.

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Methods

Study material and blood sampling

To estimate the prevalence of Haemosporidia in strigiform birds, a sample campaign was made between November 2015 and January 2016. All French zoological parks holding strigiform birds were solicited. Fifteen zoological parks finally participated to the present study, and a total of 122 birds (representing 14 different strigiform species) were collected.

To describe the infection dynamics, two additional campaigns were carried out with eight zoological parks among the fifteen zoos: one between April and June 2016 and another one between August and September 2016. A total number of 63 birds (representing 12 Strigidae species) were sampled during the second and the third periods.

The most frequently sampled species from family Strigidae were the snowy owl (*Bubo scandiacus*) (25 birds), the Great grey owl (*Strix nebulosa*) (23 birds) and the Eurasian eagle owl (*Bubo bubo*) (22 birds). The Barn owl (*Tyto alba*) (19 birds) was the only representative of the family Tytonidae.

With a physical contention, approximately 30 μL of blood was collected from the brachial vein of each bird. One drop was used to make one or two blood smears. The smears were air-dried after their preparation. They were stored away from light before fixation and coloration. Within a 15-day deadline, all the smears were fixed by absolute methanol prior to Giemsa staining (10% in phosphate-buffered solution, pH = 7.4). The smears were then covered by a coverslip mounted with Eukitt® resin before examination under oil immersion. The remaining blood was stored with EDTA additive for molecular analysis. The samples were stored at 4 °C in the fridge of the respective zoological parks and then at − 20 °C in the laboratory (Muséum national d'Histoire naturelle).

Microscopic examination of blood smears

All the blood smears were examined with a microscope at low magnification (× 400), and they were studied at high magnification (× 1000) under immersion oil. When no parasites were detected after a 20-min examination, the sample was considered negative. The characteristics described by Valkiunas (2005) were used for parasite identification. The different development stages of gametocytes and meronts and the presence of volutin granules were precisely examined to determine the morphotype and the species.

Blood DNA extraction and PCR analysis

Total DNA was extracted, from positive blood samples by microscopy, using the QIAamp DNA Micro Kit (Qiagen) following the manufacturer's instruction for whole blood. For genetic analysis, a nested PCR (N-PCR) was performed. Amplification of the cytochrome b (cyt b) mitochondrial gene was obtained using specific primers and protocols from Duval et al. (2007) for molecular identification of *Haemoproteus, Plasmodium* and *Leucocytozoon.* All N-PCR amplifications were evaluated by running 10 μL of N-PCR products on a 2% agarose gel with one negative control and one positive control. The N-PCR products were sequenced using PLAS3 and PLAS4 primers by Eurofins Genomics (France). The partial cyt b gene sequence obtained included the cyt b gene region proposed as a standard for DNA bar-coding system for *Haemoproteus* species, according to Hellgren et al. (2004). All sequences were viewed, edited and aligned with CHROMAS software (Technelysium DNA) and MEGA7 (Kumar et al. 2016). The genetic analyser "Basic Local Alignment Search Tool" (https://www.ncbi.nlm.nih.gov/BLAST) was used to determine lineages of detected DNA sequences.

To determine phylogenetic relationships of the haemosporidian parasites detected in the present study with parasites from other Strigiformes and birds of prey, molecular phylogeny was performed with 432 bp of mitochondrial cyt b gene by using maximum likelihood methods with GTR model and nodal robustness evaluated by non-parametric bootstrapping (100 replicates). Haemosporidia cyt b sequences from birds of prey were retrieved from GenBank (http://www.ncbi.nlm.nih.gov) for phylogenetic reconstruction. The phylogenetic tree was rooted using avian *Leucocytozoon* parasites. Node values less than 70% were not displayed (Fig. 2) (Guindon et al. 2010). Cyt b mitochondrial gene is the most abundant marker in GenBank for a variety of haemosporidian parasites, and it is widely used in phylogenetic studies.

For the analysis of the results and their comparison according to different criteria, the Chi-square test with a Yates correction for the small values was used. The significance level was $p < 0.05$.

Results

Prevalence of Haemosporidia infection

Haemosporidia prevalence over the autumn/winter season was 16.4% (20 infected birds out of 122) (Table 1). Fourteen birds were infected by *Haemoproteus* sp., 5 birds were infected by *Plasmodium* sp., and one bird presented a mixed infection with *Haemoproteus* sp. and *Plasmodium* sp. None of the birds were infected by *Leucocytozoon* sp. (Table 1). Haemosporidian infection was reported in 5 Strigidae species (*Athene noctua*, *Bubo bubo*, *Bubo scandiacus*, *Strix nebulosa* and *Strix uralensis*). *Haemoproteus* parasites were found from *S. nebulosa*, *S. uralensis*, *B. bubo* and *B. scandiacus*; *Plasmodium* parasites from *A. noctua* and *B. scandiacus*; and the mixed infection from *B. scandiacus*.

Table 1 Detection of Haemosporidia parasites by direct examination of blood smears and PCR in 122 Strigiformes birds from French zoological parks

Strigiformes species		Number of birds	Number of infected birds		
			Parasites		Co-infections
			Haemoproteus	*Plasmodium*	*Haemop.* + *Plasmodium*
Little owl	*Athene noctua*	3	0	1	0
Eurasian eagle owl	*Bubo bubo*	22	4	0	0
African eagle owl	*Bubo africanus*	3	0	0	0
Siberian eagle owl	*Bubo bubo sibiricus*	3	0	0	0
Mackinder's eagle owl	*Bubo mackinderi*	1	0	0	0
Snowy owl	*Bubo scandiacus*	25	2	4	1
Great horned owl	*Bubo virginianus*	4	0	0	0
Spectacled owl	*Pulsatrix perspicillata*	2	0	0	0
Tawny owl	*Strix aluco*	2	0	0	0
Brown wood owl	*Strix leptogrammica*	3	0	0	0
Great grey owl	*Strix nebulosa*	23	7	0	0
Ural owl	*Strix uralensis*	11	1	0	0
Northern hawk-owl	*Surnia ulula*	1	0	0	0
Barn owl	*Tyto alba*	19	0	0	0
Total			14	5	1

The exact age of the sampled animals was not known in all the parks. Animals have been classified as either adults or juveniles (born in 2015). There was a significant difference between the percentage of young birds infected by *Haemoproteus* sp. 67% (51%; 82%) and the percentage of infected adult birds 18% (13%; 22%) ($p < 0.05$).

The prevalence of *Haemoproteus* infection was 13% (11.7%; 14.26%) in males and 10% (8.5%; 11.5%) in females, a percentage close to the global *Haemoproteus* prevalence of the study of 12.8% (11.7%; 13.9%).

The prevalence of haemosporidian infection in the eight zoological parks, which participated to the whole study (3 sampling periods), was variable: 16.4% in autumn/winter, up to 53.9% in spring and down to 34.9% in late summer. The prevalence variation was associated with *Haemoproteus* spp. which were parasites mostly observed in birds sampled during the survey.

Morphological and molecular characteristics of *Haemoproteus* parasites

Two well-distinct *Haemoproteus* morphotypes, *H. syrnii* and *Haemoproteus* sp., and three distinct *Haemoproteus* mitochondrial cytochrome b sequences were detected (haplotypes A, B and C).

Haplotype A was found in 24.5% of *Haemoproteus* infections. It was found in two species of *Strix* genus (*S. nebulosa* and *S. uralensis*) and *Bubo bubo.* Haplotype A was identical to *H. syrnii* mitochondrial cytochrome b sequence identified by Karadjian et al. (2013). Morphological characteristics of *Haemoproteus* associated with haplotype A were identified as *H. syrnii* according to the redescription of the morphology of the parasite on *Strix aluco* (Karadjian et al. 2013) (Fig. 1). The gametocyte grows along the core of the erythrocyte asymmetrically since one side is less rounded. The gametocyte continues to evolve along the erythrocyte nucleus and surround its ends. As it matures, the gametocyte ends up surrounding the erythrocyte nucleus without compressing it. Gametocyte is loaded with volutin granules that progressively occupy the extremities of the parasite.

Haplotype C was the most prevalent haplotype (60.4%) in strigiform birds sampled in this study. It was isolated from species from the genus *Bubo* and to a lesser extent from the genus *Strix*. Haplotype C sequence was very close to *H. syrnii* haplotype A with a molecular divergence of 0.5%. Morphological characteristics associated with haplotype C were similar to those of *H. syrnii*.

Haplotype B was the less prevalent one (15.1% of infection cases). It has been isolated in 2 species, *Strix nebulosa* and *Bubo bubo*, in simple infection or in mixed infection with *H. syrnii* haplotype A. Haplotype B sequence diverged molecularly by 2.9% from *H. syrnii* haplotypes A and C.

Morphologically, *Haemoproteus* sp. haplotype B presents some characteristics which distinguish it from *H. syrnii.* During its development, the gametocyte grows along the core of the erythrocyte in a symmetric way. Also, gametocytes do not present volutin granules compared with *H. syrnii* (Fig. 1).

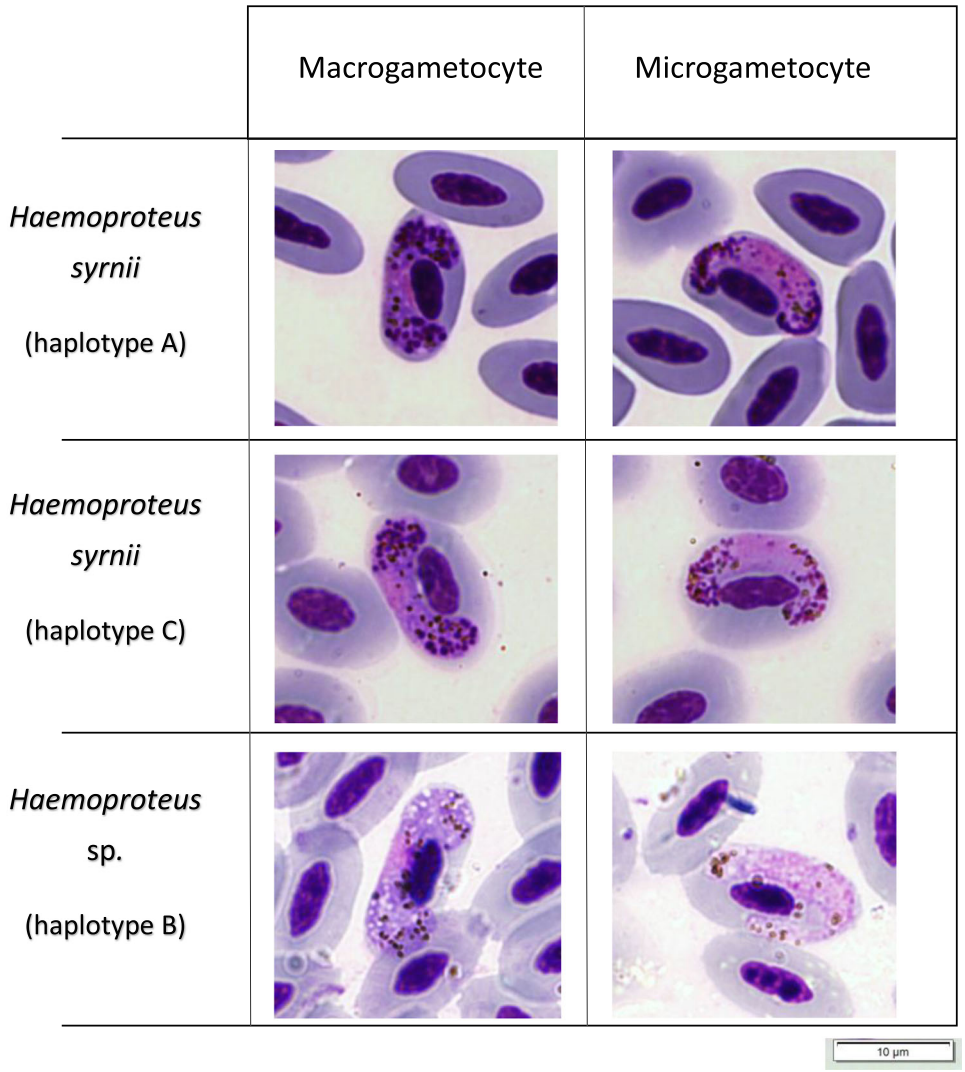


Fig. 1 Microscopic aspect of the gametocytes of *Haemoproteus syrnii* (haplotypes A and C) and *Haemoproteus* sp. (haplotype B)

Haplotype B isolated from *Strix nebulosa* formed a well-supported group with two *Haemoproteus* lineages, *Haemoproteus* STRURA01 (LC230127) from *Strix uralensis* and STAL hCULKIB01 (KP794611) from *Strix aluco* assigned to *H. syrnii* species (Fig. 2). The genetic distance between *H. syrnii* STAL 154ZI (KF279523) and *H. syrnii* STAL hCULKIB01 (KP794611) was 3.3% both *Haemoproteus* from *Strix aluco*.

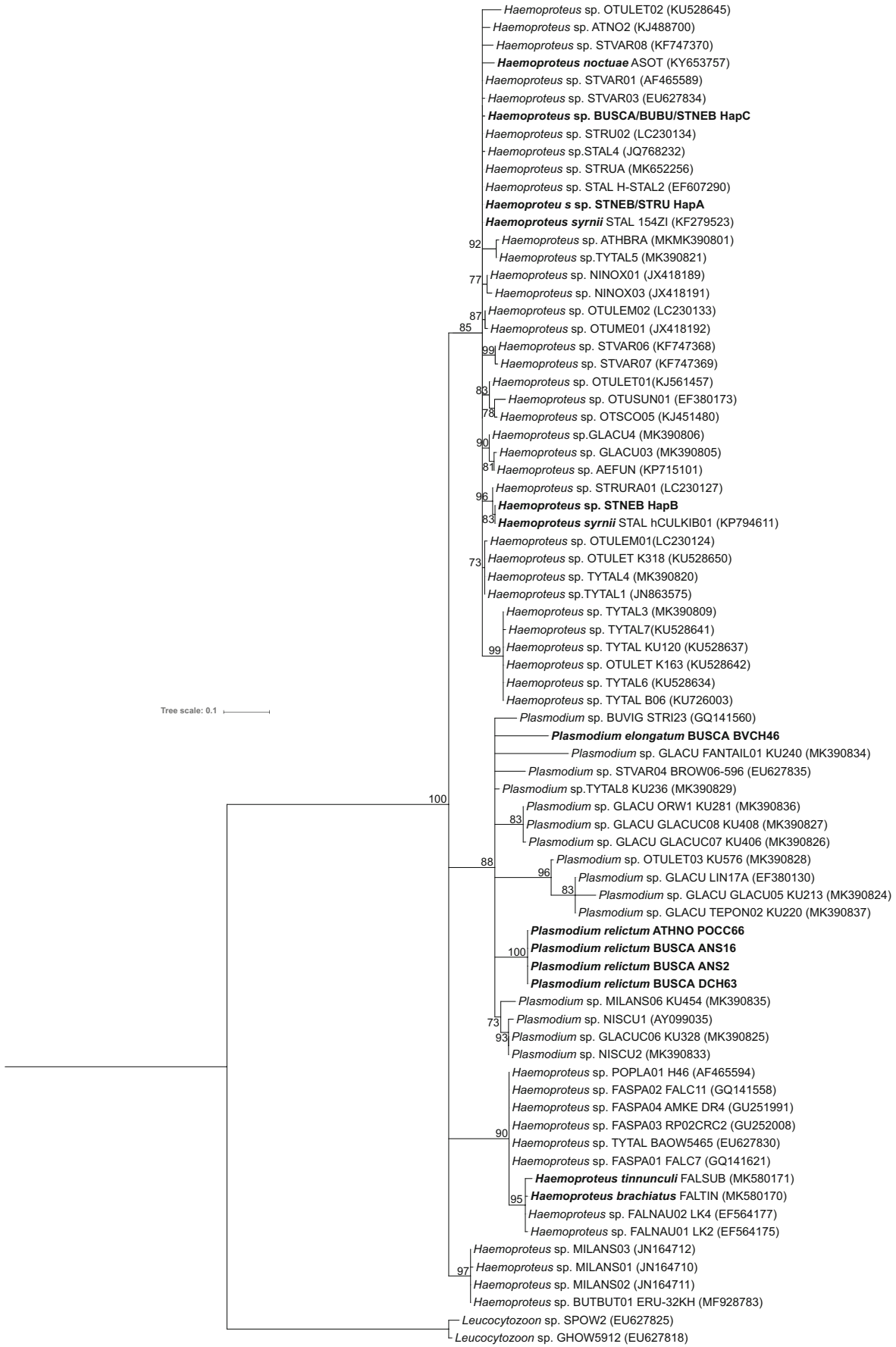
H. syrnii (haplotypes A and C) formed a group with *H. noctuae* and other *Haemoproteus* spp. infecting a variety of Strigidae species.

All *Haemoproteus* parasites from Strigidae family clustered together. Interestingly, *Haemoproteus* sp. from Tytonidae (second bird family included in Strigiformes) were included in the Strigidae *Haemoproteus* clade but also grouped with another clade composed with *Haemoproteus* from Falconidae family.

Morphological and molecular characteristics of *Plasmodium* parasites

Two species of the genus *Plasmodium* were observed morphologically and identified molecularly as *P. relictum* and *P. elongatum*. *Plasmodium* sp. BUSCA BVCH46 isolated from *Bubo scandiacus* was identical molecularly to *P. elongatum* found in other non-bird of prey families. Four *Plasmodium* lineages, ANS2, ANS16, DCH63 and POCC66, found in *Bubo scandiacus* and *Athene noctua*, were

Fig. 2 Phylogeny of Haemosporidia parasites derived by maximum ▶ likelihood using partial sequences of *cytochrome b* gene of the mitochondrial DNA from Strigiformes birds in this study and previously reported avian parasites obtained from GenBank. Sequences from the present study and sequences of namely *Haemoproteus* species are in bold. Numbers above branches indicate bootstrap values (1000 bootstrap replicates)


Haemoproteus sp. OTULET02 (KU528645)
Haemoproteus sp. ATNO2 (KJ488700)
Haemoproteus sp. STVAR08 (KF747370)
Haemoproteus noctuae ASOT (KY653757)
Haemoproteus sp. STVAR01 (AF465589)
Haemoproteus sp. STVAR03 (EU627834)
Haemoproteus sp. BUSCA/BUBU/STNEB HapC
Haemoproteus sp. STRU02 (LC230134)
Haemoproteus sp.STAL4 (JQ768232)
Haemoproteus sp. STRUA (MK652256)
Haemoproteus sp. STAL H-STAL2 (EF607290)
Haemoproteu s sp. STNEB/STRU HapA
Haemoproteus syrnii STAL 154ZI (KF279523)
92
Haemoproteus sp. ATHBRA (MKMK390801)
Haemoproteus sp.TYTAL5 (MK390821)
77
Haemoproteus sp. NINOX01 (JX418189)
Haemoproteus sp. NINOX03 (JX418191)
87
Haemoproteus sp. OTULEM02 (LC230133)
85
Haemoproteus sp. OTUME01 (JX418192)
99
Haemoproteus sp. STVAR06 (KF747368)
Haemoproteus sp. STVAR07 (KF747369)
83
Haemoproteus sp. OTULET01(KJ561457)
Haemoproteus sp. OTUSUN01 (EF380173)
78
Haemoproteus sp. OTSCO05 (KJ451480)
90
Haemoproteus sp.GLACU4 (MK390806)
Haemoproteus sp. GLACU03 (MK390805)
81
Haemoproteus sp. AEFUN (KP715101)
96
Haemoproteus sp. STRURA01 (LC230127)
Haemoproteus sp. STNEB HapB
83
Haemoproteus syrnii STAL hCULKIB01 (KP794611)
Haemoproteus sp. OTULEM01(LC230124)
73
Haemoproteus sp. OTULET K318 (KU528650)
Haemoproteus sp. TYTAL4 (MK390820)
Haemoproteus sp.TYTAL1 (JN863575)
Haemoproteus sp. TYTAL3 (MK390809)
Haemoproteus sp. TYTAL7(KU528641)
99
Haemoproteus sp. TYTAL KU120 (KU528637)
Haemoproteus sp. OTULET K163 (KU528642)
Haemoproteus sp. TYTAL6 (KU528634)
Haemoproteus sp. TYTAL B06 (KU726003)
Tree scale: 0.1
Plasmodium sp. BUVIG STRI23 (GQ141560)
Plasmodium elongatum BUSCA BVCH46
Plasmodium sp. GLACU FANTAIL01 KU240 (MK390834)
Plasmodium sp. STVAR04 BROW06-596 (EU627835)
Plasmodium sp.TYTAL8 KU236 (MK390829)
100
Plasmodium sp. GLACU ORW1 KU281 (MK390836)
83
Plasmodium sp. GLACU GLACUC08 KU408 (MK390827)
Plasmodium sp. GLACU GLACUC07 KU406 (MK390826)
88
Plasmodium sp. OTULET03 KU576 (MK390828)
96
Plasmodium sp. GLACU LIN17A (EF380130)
83
Plasmodium sp. GLACU GLACU05 KU213 (MK390824)
Plasmodium sp. GLACU TEPON02 KU220 (MK390837)
Plasmodium relictum ATHNO POCC66
100
Plasmodium relictum BUSCA ANS16
Plasmodium relictum BUSCA ANS2
Plasmodium relictum BUSCA DCH63
Plasmodium sp. MILANS06 KU454 (MK390835)
73
Plasmodium sp. NISCU1 (AY099035)
93
Plasmodium sp. GLACUC06 KU328 (MK390825)
Plasmodium sp. NISCU2 (MK390833)
Haemoproteus sp. POPLA01 H46 (AF465594)
Haemoproteus sp. FASPA02 FALC11 (GQ141558)
Haemoproteus sp. FASPA04 AMKE DR4 (GU251991)
Haemoproteus sp. FASPA03 RP02CRC2 (GU252008)
90
Haemoproteus sp. TYTAL BAOW5465 (EU627830)
Haemoproteus sp. FASPA01 FALC7 (GQ141621)
Haemoproteus tinnunculi FALSUB (MK580171)
95
Haemoproteus brachiatus FALTIN (MK580170)
Haemoproteus sp. FALNAU02 LK4 (EF564177)
Haemoproteus sp. FALNAU01 LK2 (EF564175)
Haemoproteus sp. MILANS03 (JN164712)
97
Haemoproteus sp. MILANS01 (JN164710)
Haemoproteus sp. MILANS02 (JN164711)
Haemoproteus sp. BUTBUT01 ERU-32KH (MF928783)
Leucocytozoon sp. SPOW2 (EU627825)
Leucocytozoon sp. GHOW5912 (EU627818)

molecularly very close (genetic divergence ranging from 0.13 to 0.26%) and identified as *P. relictum*. All these four *P. relictum* grouped together (Fig. 2). *Plasmodium relictum* lineages ANS16 and DCH63 were similar to *P. relictum* lineages GRW11 and Peng14-121Br2AF, respectively. *Plasmodium* lineages ANS2 and POCC66 were very close to *P. relictum* lineages pSGS1 and pGRW11, respectively.

Discussion

To diagnose Haemosporidia infection, all specimens were first analysed by examining blood smears. All the slides were read carefully at least 4 times. Only samples from microscopically positive birds were analysed by molecular biology. According to the study from Krone et al. (2008), both methods (blood smear examination and PCR) are valid for the detection of Haemosporidia. Ideally, PCR should have been made on all the samples, but limited time and budget made it necessary to reduce the number of PCR analyses. Moreover, at present, a positive PCR analysis without the morphological examination can lead to misidentifications as much as a positive morphological examination without PCR analysis (Karadjian et al. 2013).

During the present study, infection with *Haemoproteus* parasites was found in *Bubo scandiacus*, *B. bubo*, *Strix nebulosa* and *S. uralensis*. In contrast, no infection could be detected in *Strix aluco*, *Pulsatrix perspicillata* or *S. leptogrammica*. *Haemoproteus* infection has already been reported in *S. nebulosa* and *S. aluco* by *H. syrnii*; *Bubo scandiacus, Athene noctua and Asio otus* by *H. noctuae; Strix seloputo* and *Ninox scutulata* by *H. ilanpapernai*; and *Strix varia* and *Bubo virginianus* by *Haemoproteus* sp. (Evans and Otter 1998; Mutlow and Forbes 2000; Ricklefs and Fallon 2002; Valkiunas 2005; Karadjian et al. 2014; Bukauskaitė et al. 2015; Pacheco et al. 2018). The differences between our study and previous investigations could be explained by the small number of samples for some species like *Strix aluco*.

In the present study, the prevalence of Haemosporidia was significantly different between juvenile and adult birds ($p < 0.05$). This result was not in accordance with an observation in non-captive Tawny owls (*Strix aluco*) which demonstrated a low prevalence in young animals (Karadjian et al. 2013).

May/June seemed to be at greater risk for *Haemoproteus* sp. infection in captive Strigiformes in France. This might be related to the fact that during this period, most of the strigiform birds are in breeding season (Marks et al. 1999). The increase in *Haemoproteus* infection prevalence was mainly related to infection of the breeding birds and the increase of vector populations. During the breeding season, the birds limit their movement. Moreover, *Haemoproteus* parasites use Ceratopogonidae and Hippoboscidae as vectors, and the survival of these insects is favoured in warm and shaded areas such as nests.

No *Leucocytozoon* parasites were detected during the present study. This genus of Haemosporidia was described in the European non-captive avifauna (Krone et al. 2008), and *L. ziemanni* has been isolated in juvenile snowy owls in England (Evans and Otter 1998). In the USA, *Leucocytozoon* parasites have been described in three occasions in snowy owls (Baker et al. 2018). *Leucocytozoon* parasites are mainly transmitted by black flies (Forrester and Greiner 2008) whose larvae need waterfall or water in movement for their development. This kind of environment is usually not found inside or in close contact to avian enclosures in zoological parks.

The present study identified two different *Haemoproteus* species. Karadjian et al. (2013) found *H. syrnii* (haplotype A) more frequently in *Strix aluco* in wild non-captive French avifauna. *Haemoproteus syrnii* (haplotype C) was the most prevalent *Haemoproteus* in the French zoological parks. According to Valkiunas (2005), only two species of Haemosporidia were described in Strigiformes: *H. syrnii* in *Strix aluco* and *H. noctuae* in *Athene noctua*. In captive strigiform population, *H. noctuae* and *H. syrnii* have been isolated from snowy owls (Evans and Otter 1998; Baker et al. 2018). Phylogenetic relationships within *Haemoproteus* parasites infecting Strigiformes are not clearly defined yet. In the present study, *Haemoproteus* sp. haplotype B formed a group with *H. syrnii*. *Haemoproteus* sp. haplotype B parasites were morphologically and molecularly divergent from *H. syrnii* (haplotypes A and C). Further morphological description and genomic data are needed to fully describe this potential new species.

Plasmodium elongatum and *P. relictum*, both generalist and widespread *Plasmodium* species in avian hosts, were found in Strigiform birds. *Plasmodium relictum* lineages found in captive Strigidae birds were similar to *P. relictum* lineages GRW11 and Peng14-121Br2AF and very close to *P. relictum* lineages pSGS1 and pGRW11. *Plasmodium relictum* pSGS1 has been shown experimentally virulent and cause malaria in birds (Valkiunas et al. 2018).

The origin of the present study was the report of several fatal cases in Great grey owls infected with *Haemoproteus* parasites in a French zoological park. Birds of prey are often considered not susceptible to clinical infection with Haemosporidia. Necropsy revealed a low body condition score, marked anaemia and hepatomegaly in all individuals. Histological analysis showed marked degeneration and multifocal hepatocyte necrosis. The clinical signs and lesions were similar to those described by Evans and Otter (1998) in Little owls infected with *H. noctuae* and *L. ziemanni*. Necrotic lesions of the hepatic parenchyma were also observed in a 6-year-old flamingo (*Phoeniconaias minor*) infected by *Haemoproteus* sp. (Ferrell et al. 2007). Olsen and Gaunt (1985) found that birds of prey infected with Haemosporidia had a lower attenuation and a higher mortality rate. Haemosporidia infections may not be the cause of death but a key factor. The death of individuals is likely an association of different factors: parasitaemia, opportunistic infection and

vectors. In a case, heat stress is mentioned as a factor contributing to the mortality of a snowy owl infected by *Haemoproteus* spp. and West Nile Virus (Harasym 2008). The role that vectors can play on hosts could be important. Lloyd (2002) reported several cases of anaemia in young birds infected by Hippoboscidae flies. It has been suggested that Hippoboscidae flies could be potential vectors for *H. syrnii* (Karadjian et al. 2013).

Plasmodium infections with *P. relictum* and *P. elongatum* have been identified in captive strigiform birds throughout the year. *Plasmodium relictum* is recognized as highly pathogenic in some bird groups especially Sphenisciformes (Grilo et al. 2016). Recently, co-infection with *Haemoproteus* sp. or *Leucocytozoon* sp. appear to have a significant impact in some species of Strigiformes such has snowy owls (Baker et al. 2018).

Conclusion

This study indicated that several species of *Haemoproteus* are able to infect captive Strigiformes, particularly the Great grey owl, the snowy owl and the Eurasian eagle owl. The nesting period (May/June) appears to be the period at higher risk of infection. The acute nature of the infections limits successful medical intervention, making the prevention by exclusion during the period at risk, the principal means to control infection.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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