**Haemoproteus ilanpapernai** n. sp. (Apicomplexa, Haemoproteidae) in *Strix seloputo* from Singapore: morphological description and reassignment of molecular data

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Received 31 October 2013, Accepted 26 March 2014, Published online 24 April 2014

**Abstract** – *Haemoproteus ilanpapernai* Karadjian and Landau n. sp. from the Spotted Wood Owl, *Strix seloputo*, in Singapore is described from material from Ilan Paperna’s collection of slides. The species was previously identified as *Haemoproteus syrnii* (Mayer, 1910). However, comparisons between the material from *Strix seloputo* and our own material from *Strix aluco*, the type host of *H. syrnii*, revealed morphological and molecular differences. *H. ilanpapernai* n. sp. differs morphologically from *H. syrnii* by the much smaller size of the gametocytes, the different position of the mature gametocytes in the erythrocyte (apical, subapical, or lateral in *H. ilanpapernai* vs. always lateral in *H. syrnii*), the effect on the erythrocyte nucleus (frequently tilted in *H. ilanpapernai* but not displaced laterally vs. straight and displaced laterally in *H. syrnii*) and characters of the pigment (aggregated in the gametocytes of *H. ilanpapernai* vs. dispersed in *H. syrnii*). A molecular analysis showed that the two species differ by 2.9% at the cyt b and 3.1% at the COI genes.

**Key words:** *Haemoproteus ilanpapernai* n. sp., *Strix seloputo*, Singapore, cyt b, COI.

### Introduction

Several *Haemoproteus* have been reported in Strigidae from different localities in South-East Asia but only two were described morphologically by Ilan Paperna [1]: *H. noctuae* Celli and San Felice, 1891 [2] in the Brown Hawk-Owl *Ninox scutulata* (Raffles, 1822), and *H. cf. noctuae* in *Glaucidium brodiei* (Burton, 1836). A third *Haemoproteus* species,
identified as *H. syrni* (Mayer, 1910) [3], was also found in one *N. scutulata* and in a *Strix seloputo* Horsfield, 1821 [1].

Using a blood sample collected by Paperna from a *S. seloputo* in Singapore, Martinsen et al. [4] published the first gene sequences from this parasite. The corresponding material from Ilan Paperna’s collection was later deposited in the Muséum National d’Histoire Naturelle, Paris. After study of the corresponding blood samples we were able to describe the present species and differentiate it from *H. syrni*.

We found that the morphology of the parasites of *Strix* from Singapore corresponded neither to the original description by Mayer (1910) of *H. syrni* in *Strix aluco* (Linnaeus, 1758) from Germany and Austria [3] nor to the morphology of *H. syrni* in *S. aluco* from different regions in France [5]. Furthermore, the mitochondrial sequences obtained by Martinsen et al. [4] from the cyt b and COI genes of the parasites from *S. seloputo* differ significantly from those we obtained from the parasites of *S. aluco* in France [5]. We were therefore dealing with two different species.

**Material and methods**

**Biological material**

According to Paperna et al. [1], the birds were collected with mist nets in Singapore, in two forests in the central water catchment area (Nee Soon and MacRitchie 1° 22′ N, 103° 48′ E [6]).

Two raptor species were found infected with the parasite identified at the time as *H. syrni*: *N. scutulata*, Owl 1, June 2001, and *S. seloputo*, Owl 3, 2003. Owl 1 (*Ninox scutulata*) was also infected by *Plasmodium ninoxii* [1].

The material of the present description is based on slides from *S. seloputo* (Owl 3) sampled on the same day and harboring a pure infection. It comprises blood smears and a blood spot from this bird which were sent to Martinsen for molecular analysis [4]. There is no indication of the number of birds examined in Singapore. Morphological comparisons with *H. syrni* were made with blood smears of seven adult *S. aluco* from the Cévennes, Hérault (France), and molecular characterization was performed on two blood samples (one EDTA tube and one blood spot) which harbored single infections with *H. syrni*.

**Methods**

All blood smears were fixed using absolute methanol prior to Giemsa staining (10% in phosphate-buffered solution, pH = 7.4) for 1 h. They were then covered by a cover slip mounted with Eukitt® resin before examination under oil immersion, as previously described [5].

The DNA extractions and PCR protocols have previously been described [4, 5]. A p-distance analysis was performed on the common gene portions (360 bp for cyt b and 945 bp for COI).

**Photographs and measurements**

The blood smears were examined with an Olympus BX63 microscope and the microphotographs performed with an Olympus DP72 camera. Measurements were performed on the microphotographs using the cellSens Dimension 1.9 software.

**Statistical analysis**

Kolmogorov-Smirnov [7] and Shapiro-Milk [8] normality tests were performed at first. The values of the parasites’ sizes do not follow a normal distribution and Mann and Whitney [9] tests were performed to analyze the differences between the two parasite species’ length and width. The values of the red blood cells’ sizes follow a normal distribution and one-way ANOVA tests were performed to measure the length and the width of non-parasitized red cells and cells parasitized by male and female gametocytes. Data analyses were performed with the GraphPad Prism 5 software.

**Haemoproteus ilanpapernai** Karadjian and Landau n. sp.

 urn:lsid:zoobank.org:act:17FC0A4D-DE7E-47E7-9A3E-0F A1DF49FBA6

Type host: *Strix seloputo* Horsfield, 1821.

Type locality: Singapore.

Collector and date: Ilan Paperna, 2001–2003.

Etymology: named after the late Ilan Paperna.

Other host: *Ninox scutulata* (Raffles, 1822).

Type material: 8 blood films from a *Strix seloputo* deposited in the collections of the Muséum National d’Histoire Naturelle, Paris (MNHN 176BF, PXIV58–63).

Authority: The authors of the new taxon are different from the authors of this paper; Article 50.1 and Recommendation 50A of the International Code of Zoological Nomenclature [10].

**Description (Figs. 1–16, Table 1)**

Young gametocytes (Figs. 1–5) at first round or oval with the nucleus at one end and a large intra-cyttoplasmic vacuole (Figs. 3–5); then elongated along the RBC nucleus, parasite nucleus median, and both extremities containing large white vacuoles (Figs. 6–9). Small dark brown granules and fine rods of dark brown pigment scattered in the cytoplasm. Gametocytes along the erythrocyte nucleus, sometimes at its end (Fig. 6). Volutin granules at the periphery, round and well limited. Disappearance of the large vacuoles with few aggregations of chromatin. Macrogametocyte nucleus adpressed to it (Figs. 10–16). Microgametocyte, compact, ellipsoid, or rounded, and located near the erythrocyte’s nucleus, touch the nucleus without being closely adpressed to it (Figs. 10–16). Microgametocyte nucleus, diffuse with few aggregations of chromat. Macrogametocyte nucleus rounded and well limited. Disappearance of the large vacuoles of the immature stages; numerous small volutin grains scattered in the cytoplasm, particularly at the periphery (Figs. 10–16). Dark brown pigment of the microgametocytes aggregated, forming a dense mass (Figs. 10, 11, 16), pigment of the macrogametocytes more dispersed (Figs. 12, 14, 15). Mature
Figures 1–16. Microphotographs of gametocytes of *Haemoproteus ilanpapernai* Karadjian & Landau n. sp. in the blood of *Strix seloputo*. 1–5: Young gametocytes; 6 and 7: immature gametocytes; 8 and 9: nearly mature gametocytes; 10 and 16: microgametocytes with agglomerated pigment (arrows); 11 and 13: microgametocytes with the erythrocyte nucleus tilted; 12: macrogametocyte with the erythrocyte nucleus tilted; 14 and 15: macrogametocytes with aggregation of pigment (arrows). Giemsa staining. Scale bar = 5 μm.
Microgametocytes (micro), macrogametocytes (macro), uninfected red blood cells (uninf RBC), red blood cells harboring microgametocytes (micro RBC), macrogametocytes (macro RBC). Results are expressed as mean length and width ± SEM, n = 30. The sizes of the microgametocytes and the macrogametocytes were analyzed by the Mann-Whitney test; mature microgametocytes are significantly larger than macrogametocytes (length, \( p \) value < 0.0001; width, \( p \) value < 0.001). The sizes of RBC were tested by a one-way analysis of variance and showed no significant difference (length, \( p \) value = 0.11; width, \( p \) value = 0.79).

Discussion

Paperna et al. [1], noticing the small size of the gametocytes, thought that only immature parasites were present in the blood smears of the owl. In fact, the majority of gametocytes are fully differentiated into mature micro- and macrogametocytes. Since, at that time, no sequence of identified parasites from Strix was available in GenBank, the cyt b and COI sequences from S. seloputo were therefore assigned to H. syrnii. Two other non-identified cyt b sequences from Haemoproteus parasites of Strix varia (Barton, 1799) from Austria [11, 12] can be retrieved from GenBank and show 0.5% differences with H. syrnii. They are probably another haplotype of H. syrnii.

In view of the important morphological differences between H. ilanpapernai n. sp. and H. syrnii, we consider that these two parasites should be considered as two different species. The cyt b and COI sequences of H. ilanpapernai n. sp. show differences of, respectively, 2.9% and 3.1% with H. syrnii, which confirms the morphological analysis.

The sequences previously deposited in GenBank and assigned to H. syrnii [4] should be reassigned to H. ilanpapernai n. sp. and the geographical origin of the samples stated mistakenly as Israel should be changed to Singapore.

The number of sequences of bird Haemoproteus deposited in databases is increasing and their specific identification is very often a problem, as pointed out by Valkiunas et al. [13] and Karadjian et al. [5]. This problem arises mainly from the diversity of parasite species present in a single host. In the case of H. ilanpapernai, we are as confident as possible that the owl harbored a single species of Haemoproteus.

Acknowledgements. The slides from Ilan Paperna’s collection were deposited in the collections of The Museum National d’Histoire Naturelle de Paris, through the courtesy of Prof. Jaap van Rijn, Director of the Department of Animal Sciences, The Robert H. Smith Faculty of Agriculture, Food and Environment, Rehovot, Israel. LD was supported by a postdoctoral fellowship from the Labex BCDiv (Biological and Cultural Diversities), Muséum National d’Histoire Naturelle, Paris.

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Cite this article as: Karadjian G, Martinsen E, Duval L, Chavatte J-M & Landau I: Haemoproteus ilanpapernai n. sp. (Apicomplexa, Haemoproteidae) in Strix seloputo from Singapore: morphological description and reassignment of molecular data. Parasite, 2014, 21, 17.