

***Plasmodium* (Apicomplexa) of the skylark (*Alauda arvensis*)**

**Jean-Marc CHAVATTE
Virginie GRÈS
Georges SNOUNOU
Alain CHABAUD
Irène LANDAU**

Muséum national d'Histoire naturelle,
Parasitologie comparée et modèles expérimentaux, USM 307,
case postale 52, 57 rue Cuvier, F-75231 Paris cedex 05 (France)
landau@mnhn.fr

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ABSTRACT

Plasmodium parasites were sought in 30 skylarks (*Alauda arvensis*) captured in the Département des Landes (France) during the post-mating migration. Parasite prevalence was low (6/30) while intra-specific diversity was high (14 species). Five species were morphologically indistinguishable from those found in the magpie (*Pica pica*) from the Paris region: *Plasmodium dorsti*, *P. bioccai*, *P. ghadiriani*, *P. dheriteae*, and *P. relictum quentini* n. subsp. Two species had been described in other birds: *P. rouxi* and *P. hexamerium*. The species *Plasmodium alaudae* (Celli & Sanfelice, 1891) is redefined in this article. Finally, five new species are described and named: *P. reniai* n. sp., *P. coluzzii* n. sp., *P. caloti* n. sp., *P. ginsburgi* n. sp. and *P. jeanriouxi* n. sp., and one, *Plasmodium* sp. is described but not named. The pattern of infection in the skylark, a migratory gregarious bird, is compared to that of the magpie, a solitary sedentary bird.

KEY WORDS

Apicomplexa,
Plasmodium,
Alauda arvensis,
migratory gregarious
bird,
Pica pica,
sedentary solitary bird,
new species.

RÉSUMÉ

Plasmodium (*Apicomplexa*) de l'alouette des champs (*Alauda arvensis*).

Trente alouettes (*Alauda arvensis*) dont certaines étaient porteuses de *Plasmodium* ont été capturées dans le département des Landes au cours de leur migration postnuptiale. La prévalence est faible (6/30) et la diversité intra-spécifique importante (14 espèces). Cinq espèces sont morphologiquement indistinguables de celles de la pie de la région parisienne : *Plasmodium dorsti*, *P. bioccai*, *P. ghadiriani*, *P. dhereteae*, *P. relictum quentini* n. subsp. ; deux sont connues d'autres oiseaux : *P. rouxi* et *P. hexamerium*. *Plasmodium alaudae* (Celli & Sanfelice, 1891), est redéfini ici. Enfin, cinq espèces sont nouvelles : *P. reniai* n. sp., *P. coluzzii* n. sp., *P. caloti* n. sp., *P. ginsburgi* n. sp., *P. jeanriouxi* n. sp. et une *Plasmodium* sp. est décrite non nommée. Le parasitisme de l'alouette, oiseau grégaire et migrateur, est comparé à celui de la pie, oiseau solitaire et sédentaire.

MOTS CLÉS

Apicomplexa,
Plasmodium,
Alauda arvensis,
oiseau grégaire
migrateur,
Pica pica,
oiseau solitaire
sédentaire,
espèces nouvelles.

INTRODUCTION

Bird *Plasmodium* form a highly varied group of parasites because of the large number of species, the extent of their host spectrum and their almost worldwide distribution. In a previous article (Chavatte *et al.* 2007) we showed that the number of species parasitizing a single bird species can be high (14 in magpies) and this fact, generally unknown, should be taken into account when analysing ecological or molecular data.

In this communication we present a morphological study of *Plasmodium* species from 30 skylarks (*Alauda arvensis* Linnaeus, 1758) caught in France during their post-mating migration. Six showed low-level infections. Three of these and one of the negative birds were maintained in captivity for five days. The negative skylarks remained negative during this period, while the parasitaemia of the other three birds increased. This study is based on the blood smears collected on the fifth day of captivity from these three skylarks, where *Plasmodium* were the only haematozoan species observed.

Six of the 14 species described or re-described in the magpie, *Pica pica* (Linnaeus, 1758) from the Paris region (Chavatte *et al.* 2007), were also observed in the skylarks: *P. hexamerium* Huff, 1935, parasite of *Sialis sialis sialis* (Linnaeus, 1758), *P. dorsti* Chavatte & Landau, 2007, *P. bioccai* Chavatte & Landau, 2007, *P. ghadiriani* Chavatte & Landau, 2007, *P. dhereteae* Chavatte & Landau, 2007 and *P. relictum quentini*

n. subsp. that was considered as *P. relictum* (Grassi & Felletti, 1891) when it was observed in the magpie. *Plasmodium rouxi* Sergent, Sergent & Catanei, 1928, parasite of the sparrow, *Passer hispaniolensis* (Temminck, 1820), was also observed, as was *P. alaudae* (Celli & Sanfelice, 1891) which is re-defined and re-described here. Five new species could be described: *Plasmodium coluzzii* n. sp., *P. caloti* n. sp., *P. jeanriouxi* n. sp., *P. ginsburgi* n. sp. and *P. reniai* n. sp. Finally, parasites observed in small numbers and whose gametocytes could not be identified, could not be attributed to a known species and have thus been referred to as *Plasmodium* sp. in this article.

The distribution of the *Plasmodium* species observed in the skylarks is presented in Table 1.

The traditional morphological identification of species as a prerequisite to molecular biology analysis is considered more and more by authors as indispensable to understand the relationships between species and their evolution (Valkiunas 2005). Data from sequencing parasite DNA amplified from skylark blood samples will be presented in a separate article and compared with those from the magpie (Chavatte *et al.* 2007).

MATERIALS AND METHODS

Blood smear examination was carried out for 30 skylarks (*Alauda arvensis*) captured on 20

October 1996 in the localities of Saint-Julien-en-Born (44°03'42"N, 1°13'33"W) and Messanges (43°48'56"N, 1°22'47"W) in the Département des Landes (France).

The material was collected, processed and stored as previously described (Chavatte *et al.* 2007). Aliquots of blood were stored in liquid nitrogen for molecular studies.

Since multiple parasite species are present, we have not adopted the designation of hapantotypes but rather selected for each of the species a holotype consisting of a characteristic schizont that was clearly delimited within a circle etched on the smear slide with a diamond, and that was photographed (Fig. 3D-J). These types are deposited in the MNHN, in the collection of Parasitologie comparée et Modèles expérimentaux, under the numbers 61-66, drawer PXII, 440 LV.

ABBREVIATIONS

RBC(s) red blood cell(s);
 MNHN Muséum national d'Histoire naturelle, Paris.

REVIEW OF THE LITERATURE

Two species were mainly reported from the skylark (Garnham 1966; Bennett *et al.* 1982; Valkiūnas 2005): *P. alaudae* (Celli & Sanfelice, 1891) and *P. subpraecox* (Grassi & Feletti, 1892). It is necessary to clarify the taxonomic status of these species. *Plasmodium subpraecox*, has been mentioned in the skylark and described in the little owl, *Athene noctua* (Scopoli, 1769) (Grassi & Feletti 1892: fig. 8), and in *Passer hispaniolensis* (Grassi & Feletti 1892: fig. 7). The first accurate re-description was made by Giovannola (1939), his description and drawings made from the little owl were quite concordant with those of Grassi & Feletti (1892). Thus, *Athene noctua*, and not the skylark, is the type host of *P. subpraecox*. A closely related but not identical species (*P. caloti* n. sp.) was noted in the skylark and is described below. In 1891, Celli & Sanfelice described a *Plasmodium* in the skylark with some forms that develop rapidly while others more slowly. When examining their drawings, it is clear that multiple species were represented. Here we re-describe this species from the birds we col-

TABLE 1. — *Plasmodium* Marchiafava & Celli, 1885 distribution in the Skylarks (*Alauda arvensis* Linnaeus, 1758).

Bird number	<i>Plasmodium</i> species
1 (738U)	<i>P. bioccai</i> Chavatte & Landau, 2007
	<i>P. coluzzii</i> n. sp.
	<i>P. dorsti</i> Chavatte & Landau, 2007
2 (740U)	<i>P. ginsburgi</i> n. sp.
	<i>P. alaudae</i> (Celli & Sanfelice, 1891)
	<i>P. coluzzii</i> n. sp.
	<i>P. dorsti</i>
	<i>P. jeanriouxi</i> n. sp.
	<i>P. relictum quentini</i> n. subsp.
3 (741U)	<i>P. rouxi</i> Sergent, Sergent & Catanei, 1928
	<i>Plasmodium</i> sp.
	<i>P. alaudae</i>
	<i>P. bioccai</i>
	<i>P. caloti</i> n. sp.
	<i>P. coluzzii</i> n. sp.
	<i>P. dheriteae</i> Chavatte & Landau, 2007
	<i>P. dorsti</i>
	<i>P. ghadiriani</i> Chavatte & Landau, 2007
	<i>P. hexamerium</i> Huff, 1935
	<i>P. jeanriouxi</i> n. sp.
<i>P. relictum quentini</i> n. subsp.	
<i>P. reniai</i> n. sp.	
<i>P. rouxi</i>	
<i>Plasmodium</i> sp.	

lected, and we designate as lectotype of *P. alaudae* the specimen depicted by Celli & Sanfelice (1891: pl. 3 fig. 16).

SYSTEMATICS

Family PLASMODIIDAE Mesnil, 1903

Genus *Plasmodium* Marchiafava & Celli, 1885

REMARKS

All the *Plasmodium* parasites were found within erythrocytes, and except for a few rings none were seen in reticulocytes. The infected RBC were at times deformed, rounded or discoloured by certain parasites, but the deformability observed was not more pronounced than that observed in other birds such as the magpie (Chavatte *et al.* 2007) or the sparrow (Landau *et al.* 2003). On the other hand, the red blood cell nucleus was often slightly tilted even by parasites that did not truly displace it.

For the known *Plasmodium* species, only the main characters are briefly described.

Molecular biology studies (Martinsen *et al.* 2007) evidenced that some of the avian *Plasmodium* sub-genera created by Corradetti *et al.* (1963) are polyphyletic and until the situation is clarified we prefer not to use them.

Plasmodium bioccai Chavatte & Landau, 2007
(Fig. 1A)

MATERIAL EXAMINED. — France. Landes, Saint-Julien-en-Born, 44°03'42"N, 1°13'33"W, blood smears of *A. arvensis* number 738U, 25.X.1996 (MNHN P2-XXV, 1-23); 741U, 25.X.1996 (MNHN P2-XXV, 44-64).

DISTRIBUTION. — Seine-Saint-Denis, France (Chavatte *et al.* 2007); new record for the Landes, France.

HOSTS. — *Pica pica* (type host); new record for *Alauda arvensis*.

REMARKS

This species is comparable to that described in the magpie. The parasites lie in a rounded, prematurely hypertrophied and non-discoloured RBC with a condensed ellipsoid nucleus displaced to one of the edges. The schizonts are regular in shape, rounded or approximately oval with smooth contours. The chromatin, initially as granular masses of variable sizes, progressively fragments into distinct nuclei. The pigment is fine, loosely assembled in irregular rows, though agglomerated in some areas. Two mature schizonts were observed with 26 nuclei dispersed in the cytoplasm. The gametocytes lead to the same RBC modification as do the schizonts.

Plasmodium coluzzii Chavatte & Landau, n. sp.
(Fig. 1B)

TYPE MATERIAL. — Holotype: France. Landes, Saint-Julien-en-Born, 44°03'42"N, 1°13'33"W, blood smear of *A. arvensis* number 741U, 25.X.1996 (MNHN 440LV PXIII, 61) (Fig. 3I).

Paratypes: same data as holotype, blood smears of *A. arvensis* number 738U, 25.X.1996 (MNHN P2-XXV, 1-23); 740U, 25.X.1996 (MNHN P2-XXV, 24-43); 741U, 25.X.1996 (MNHN P2-XXV, 44-64).

ETYMOLOGY. — This species is dedicated to the memory of Alberto Coluzzi.

DISTRIBUTION. — Known only from the type locality, Saint-Julien-en-Born, Landes, France.

HOST. — *Alauda arvensis* (type host).

DESCRIPTION

The young schizonts cover $\frac{1}{5}$ to $\frac{1}{3}$ of the slightly enlarged RBC, where the median or sub-median nucleus is tilted sideways. The RBC rounds up as the schizonts mature, pushing the host nucleus to the edge of the cell, and often the parasites are found in RBCs without nucleus. Throughout schizont maturation, chromatin occurs as a peripheral crown in which one distinguishes granules and little nuclei around a clear vacuolated centre. The pigment in the more mature stages forms rough aggregates of agglomerated granules. The most characteristic stage is round in a rounded enucleate RBC, with the parasite nuclei tightly packed around a clear sinuous centre. Fully mature stages were not seen, and the number of nuclei is estimated to exceed 30.

Gametocytes are large and round and are often found in RBCs devoid of nuclei.

DIFFERENTIAL DIAGNOSIS

Plasmodium coluzzii n. sp. should be compared to *P. bioccai*, a parasite described in the magpie and found in the skylark. The two species differ by the smaller size of the schizonts of *P. coluzzii* n. sp. where the immature forms have dispersed and diffuse chromatin, features that contrast with the well-defined nuclei that can be observed even in the young schizonts of *P. bioccai*. The RBC nuclei, present in *P. bioccai*, are often absent in *P. coluzzii* n. sp.

Some schizonts similar to those of *P. coluzzii* n. sp. had been observed in the magpie studied by Chavatte *et al.* (2007), but the paucity in the numbers observed had not allowed to distinguish the species.

Plasmodium dorsti Chavatte & Landau, 2007
(Fig. 1C)

MATERIAL EXAMINED. — France. Landes, Saint-Julien-en-Born, 44°03'42"N, 1°13'33"W, blood smears of *A. arvensis* number 738U, 25.X.1996 (MNHN P2-XXV,

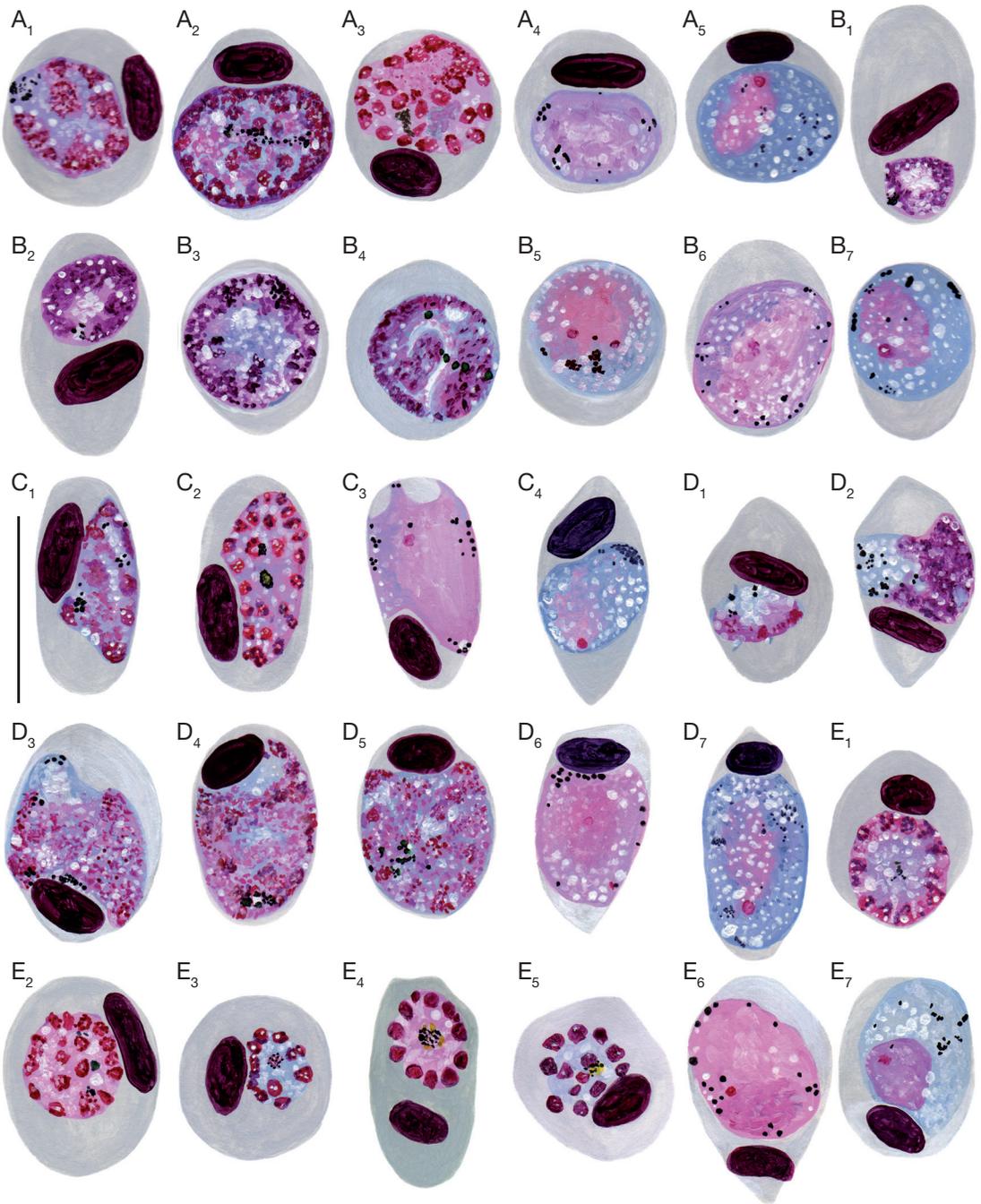


FIG. 1. — **A**, *Plasmodium bioccai* Chavatte & Landau, 2007; **A**₁–**A**₃, schizonts; **A**₄, microgametocyte; **A**₅, macrogametocyte; **B**, *P. coluzzii* Chavatte & Landau, n. sp.; **B**₁–**B**₄, schizonts; **B**₅, **B**₇, macrogametocyte; **B**₆, microgametocyte; **C**, *P. dorsti* Chavatte & Landau, 2007; **C**₁, **C**₂, schizonts; **C**₃, microgametocyte; **C**₄, macrogametocyte; **D**, *P. ginsburgi* Chavatte & Landau, n. sp.; **D**₁–**D**₅, schizonts; **D**₆, microgametocyte; **D**₇, macrogametocyte; **E**, *P. relictum quentini* Chavatte & Landau, n. subsp.; **E**₁–**E**₅, schizonts; **E**₆, microgametocyte; **E**₇, macrogametocyte. Scale bar: 10 µm.

1-23); 740U, 25.X.1996 (MNHN P2-XXV, 24-43); 741U, 25.X.1996 (MNHN P2-XXV, 44-64).

DISTRIBUTION. — Seine-Saint-Denis, France (Chavatte *et al.* 2007); new record for the Landes, France.

HOSTS. — *Pica pica* (type host); new record for *Alauda arvensis*.

REMARKS

The examined specimens are closely similar to *P. dorsti* from the magpie. This large species lies in a little or non-enlarged RBC whose nucleus is pushed to one of the extremities. The schizonts that fill three-quarters of the RBC, are elongated with irregular shapes and contours. The oldest schizonts observed had 26 nuclei. In contrast to what is observed in the magpie, RBCs in the skylark, are neither deformed nor discoloured.

Plasmodium ginsburgi Chavatte & Landau, n. sp.
(Fig. 1D)

TYPE MATERIAL. — Holotype: **France**, Landes, Saint-Julien-en-Born, 44°03'42"N, 1°13'33"W, blood smear of *A. arvensis* number 738U, 25.X.1996 (MNHN 440LV PXIII, 65) (Fig. 3H).

Paratypes: same data as holotype, blood smears of *A. arvensis* number 738U, 25.X.1996 (MNHN P2-XXV, 1-23).

ETYMOLOGY. — This species is dedicated to Hagai Ginsburg.

DISTRIBUTION. — Known only from the type locality, Saint-Julien-en-Born, Landes, France.

HOST. — *Alauda arvensis* (type host).

DESCRIPTION

This large species lies in an enlarged ellipsoid RBC where extremities are often narrower and sometimes discoloured. The RBC nucleus is dense and sub-globular and is pushed towards one of the extremities. The young and sub-mature schizonts are irregular in form and are disposed transversally in the middle part of the RBC whose nucleus is tilted. They comprise a blue cytoplasmic area scattered with vacuoles, a rather rough pigment, and a highly granular and abundant diffuse chromatin

mass in which nuclei are yet to be differentiated. The older schizonts occupy nearly the whole of the RBC, whose nucleus is consequently found in a notch. The parasite chromatin is still highly granular and covers the whole of the schizont, except for one or two central vacuolated areas, and progressively condenses to form nuclei. The rough pigment is assembled and is often associated with a white vacuole. Fully mature schizonts could not be observed, but we evaluate the number of nuclei to exceed 30.

Gametocytes are ellipsoid and contain numerous little vacuoles.

DIFFERENTIAL DIAGNOSIS

Plasmodium ginsburgi n. sp. can be distinguished from *P. bioccai* by its shape and that of the RBC which is ellipsoid and elongated in the former and rounded in the latter. These species also differ by their pigment, which is gathered and rough in the former and finer and more dispersed in the latter. Schizont maturation and nucleus formation differ between the two species: the chromatin remains diffuse until the late stages in *P. ginsburgi* n. sp. in contrast to the early formation of individual nuclei in *P. bioccai*. Finally, the number of nuclei exceeds 30 in *P. ginsburgi* n. sp. but is around 24 to 30 in *P. bioccai*.

The ellipsoid elongate *P. ginsburgi* n. sp. with a high number of nuclei is comparable to *Plasmodium maior* Raffaele, 1930, a parasite of *Passer hispaniolensis* and *P. domesticus* (Linnaeus, 1758). The schizonts illustrated by Raffaele (1930) are comparable to those observed here: the ellipsoid schizonts are large even in young forms, they fill the RBC whose globular nucleus is pushed to one of the extremities. However, the two species differ by the chromatin that remain diffuse throughout a large portion of *P. ginsburgi* n. sp. maturation whereas it is gathered up in a few large masses in *P. maior*, and by the rough pigment amassed around a vacuole that contrasts to the pigment distributed in several masses in *P. bioccai*. Raffaele had not described any mature forms, but in the re-description (Landau *et al.* 2003), it was noted that 20 to 24 merozoites are formed, a number inferior to that estimated for *P. ginsburgi* n. sp.

Plasmodium relictum quentini

Chavatte & Landau, n. subsp.
(Fig. 1E)

Plasmodium relictum – Chavatte & Landau 2007 (parasite of *Pica pica*).

TYPE MATERIAL. — Holotype: **France**. Landes, Saint-Julien-en-Born, 44°03'42"N, 1°13'33"W, blood smear of *A. arvensis* number 740U, 25.X.1996 (MNHN 440LV PXIII, 62) (Fig. 3E).

Paratypes: same data as holotype, blood smears of *A. arvensis* number 740U, 25.X.1996 (MNHN P2-XXV, 24-43); 741U, 25.X.1996 (MNHN P2-XXV, 44-64).

ETYMOLOGY. — This subspecies is dedicated to Jean-Claude Quentin.

DISTRIBUTION. — Seine-Saint-Denis, France (Chavatte *et al.* 2007) and type locality, Saint-Julien-en-Born, Landes, France.

HOSTS. — *Alauda arvensis* (type host); *Pica pica*.

DESCRIPTION

The schizonts lie in an enlarged and often rounded and sometimes discoloured RBC, whose condensed and sub-globular nuclei is pushed to the periphery. The schizont's 12 to 18 nuclei are dense, rounded and well delimited. Initially peripheral and slightly protruding towards the outside, the nuclei are then distributed to the interior of the schizont. The cytoplasm is clear with a few white sharp-edged vacuoles. The pigment is most often central with the fine grains gathered up. The gametocytes are rounded and induce the same alterations in the RBC as the schizonts.

TAXONOMIC STATUS

The specimens studied are identical to the species described from the magpie (Chavatte *et al.* 2007) where it was identified to *P. relictum*. However, this parasite presented some minor differences with respect to the *P. relictum* re-described (Landau *et al.* 2003), in *Passer domesticus*, namely, a more pronounced RBC deformation, and crown of nuclei that are more regular and protuberant than those in the sparrow. The authors opted then to designate these parasites as *P. relictum*, as these differences are rather minor. However, since both the parasites of the lark and the magpie display

these very same differential characters, we consider that in these two hosts they represent a vicariant form of the *P. relictum* described in the sparrow. We designate it as *P. relictum quentini* n. subsp. (= *P. relictum* sensu Chavatte & Landau 2007, parasite of *Pica pica*).

Plasmodium reniai Chavatte & Landau, n. sp.
(Fig. 2A)

TYPE MATERIAL. — Holotype: **France**. Landes, Saint-Julien-en-Born, 44°03'42"N, 1°13'33"W, blood smear of *A. arvensis* number 741U, 25.X.1996 (MNHN 440LV PXIII, 64) (Fig. 3G).

Paratypes: same data as holotype. Blood smears of *A. arvensis* number 741U, 25.X.1996 (MNHN P2-XXV, 44-64).

ETYMOLOGY. — This species is dedicated to Laurent Rénia.

DISTRIBUTION. — Known only from the type locality, Saint-Julien-en-Born, Landes, France.

HOST. — *Alauda arvensis* (type host).

DESCRIPTION

The parasite lies in a non-deformed, non-hypertrophied RBC whose nucleus is slightly displaced by the larger parasite forms. The young schizonts have 2 to 6 nuclei, and are crescent-shaped and found at the apex of the RBC, generally attached to its edge. Their cytoplasm is abundant, vacuolated and the nuclei though of irregular shape are clearly distinguished individually. As they develop, the schizonts extend along one of the RBC borders, keeping contact with RBC membrane and separated from its nucleus by a band of erythrocytic cytoplasm. Throughout its development, the schizont retains a curved shape, larger on the top of the RBC and narrower along the host nucleus. The number of nuclei is estimated to be around 30, though no fully mature schizonts could be observed.

Gametocytes are elongated and have roughly the same shape and position as the schizonts.

DIFFERENTIAL DIAGNOSIS

The closest species is *P. golvani* Chavatte & Landau, 2007, parasite of the magpie.

This parasite is distinguished from the one in the magpie by its general shape which is thinner and more elongated. Moreover, and in contrast to *P. reniai* n. sp., *P. golvani* shows a long thin protrusion along the RBC nucleus, its young forms have few nuclei and these are arranged in two rows in the mature forms.

Plasmodium caloti Chavatte & Landau, n. sp.
(Fig. 2B)

TYPE MATERIAL. — **France.** Landes, Saint-Julien-en-Born, 44°03'42"N, 1°13'33"W, blood smear of *A. arvensis* number 741U, 25.X.1996 (MNHN 440LV PXIII, 63) (Fig. 3D).

Paratypes: same data as holotype, blood smears of *A. arvensis* number 741U, 25.X.1996 (MNHN P2-XXV, 44-64).

ETYMOLOGY. — This species is dedicated to the memory of Jacques Calot.

DISTRIBUTION. — Known only from the type locality, Saint-Julien-en-Born, Landes, France.

HOST. — *Alauda arvensis* (type host).

DESCRIPTION

The average sized parasite is found in a rounded highly hypertrophied RBC that is sometimes discoloured and whose nucleus is off-centre though not in close contact with the cell edge. Among the small or medium sized (<10 nuclei) *Plasmodium* species of the skylark, *P. caloti* n. sp. is the only one where RBCs are modified to this extent.

In general the shape of the immature and nearly mature schizonts is rounded, their contours regular and the nuclei, usually 8 in number, are dense and are predominantly found at the periphery. As they near maturity, these nuclei can bulge slightly to the outside. The abundant and homogeneous cytoplasm is pink coloured and contains a small number of vacuoles. The pigment is fine and agglomerated.

Gametocytes are rounded like the schizonts and lie in rounded RBCs.

DIFFERENTIAL DIAGNOSIS

Plasmodium caloti n. sp. should be compared to *P. subpraecox*, a parasite described briefly and

illustrated by Grassi & Feletti in *Athene noctua* (Grassi & Feletti 1892: fig. 8). These authors have also recorded it in *Alauda arvensis* and in *Passer hispaniolensis*.

According to these authors the number of merozoites is 5 to 12. In a re-description of this species, Giovannola (1939), enumerated 10 to 12 merozoites in the mature schizont, and from his illustrations one can note a few similarities with *P. caloti* n. sp.: defined outlines, cytoplasmic abundance, and a rather peripheral location of the nuclei in mature schizonts. On the other hand, the parasite described here produces an average of 8 merozoites and leads to a much more pronounced hypertrophy of the RBC.

Plasmodium caloti n. sp. and *P. alaudae* differ by the following features: the larger size of *P. caloti* n. sp., its more or less central position in a considerably enlarged RBC with a displaced nucleus, contrasts with the small size of the apically located *P. alaudae* where infected RBCs are not modified nor their nuclei displaced.

Plasmodium alaudae (Celli & Sanfelice, 1891)
(Fig. 2C)

MATERIAL EXAMINED. — **France.** Landes, Saint-Julien-en-Born, 44°03'42"N, 1°13'33"W, blood smears of *A. arvensis* number 740U, 25.X.1996 (MNHN P2-XXV, 24-43); 741U, 25.X.1996 (MNHN P2-XXV, 44-64).

DISTRIBUTION. — Italy (Celli & Sanfelice 1891); new record for the Landes, France.

HOSTS. — *Pica pica* (type host); new record for *Alauda arvensis*.

REDESCRIPTION

The parasites are small in size, and are found located at the apex of non-modified RBCs where nuclei are not displaced or only a little. The schizonts are compact and rounded, and the nuclei, eight in number, are dense roughly round with a clear centre. The cytoplasm is relatively abundant with a few dispersed vacuoles and two granules of black pigment. The gametocytes have not been identified but are probably elongated.

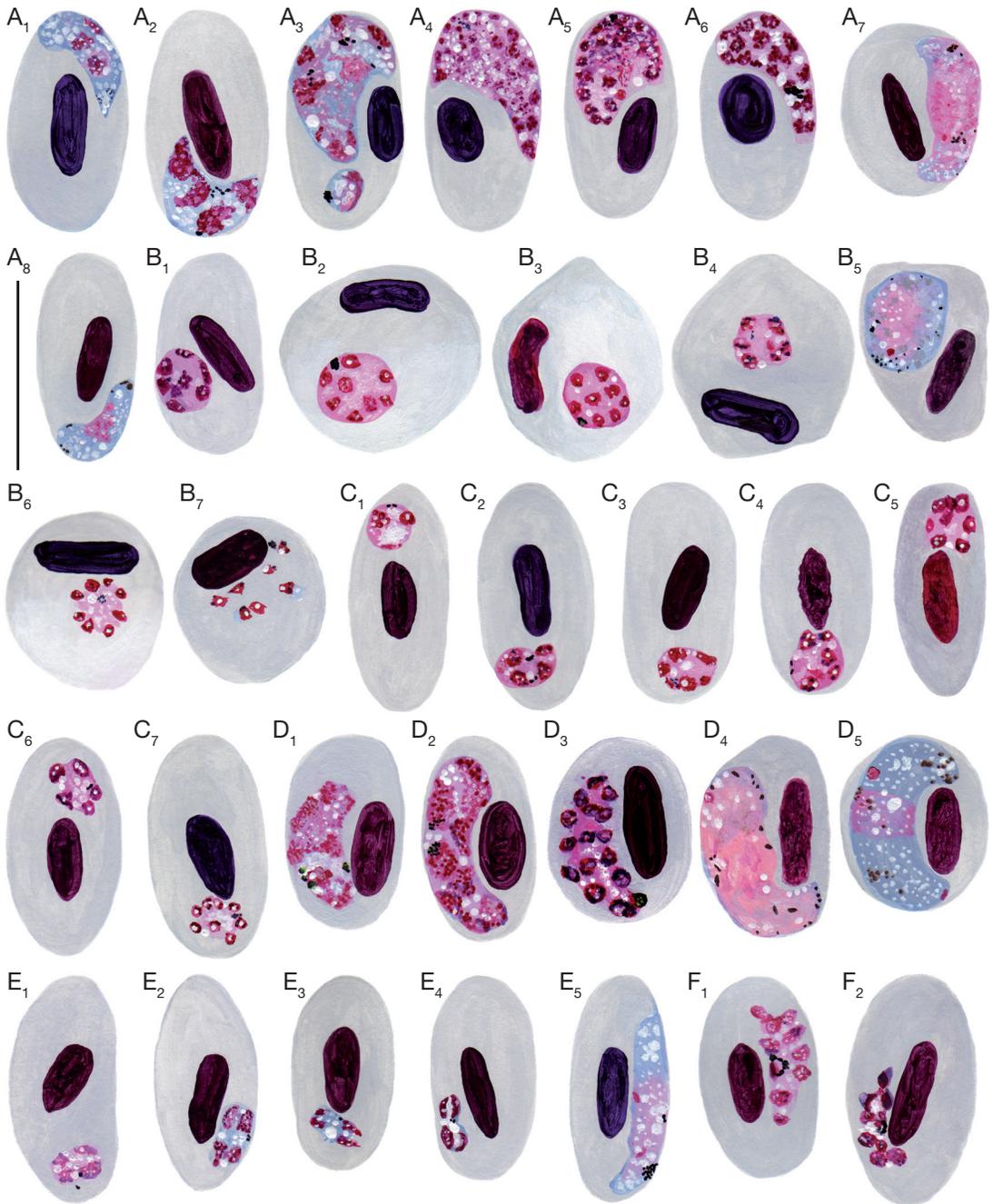


FIG. 2. — **A**, *Plasmodium reniai* Chavatte & Landau, n. sp.; **A**₁-**A**₆, schizonts; **A**₇, microgametocyte; **A**₈, macrogametocyte; **B**, *P. caloti* Chavatte & Landau, n. sp.; **B**₁-**B**₄, **B**₆, **B**₇, schizonts; **B**₅, macrogametocyte; **C**₁-**C**₇, *P. alaudae* (Celli & Sanfelice, 1891), schizonts; **D**, *P. ghadiriani* Chavatte & Landau, 2007; **D**₁-**D**₃, schizonts; **D**₄, microgametocyte; **D**₅, macrogametocyte; **E**, *P. rouxi* Sergent, Sergent & Catanei, 1928; **E**₁-**E**₄, schizonts; **E**₅, macrogametocyte; **F**₁, **F**₂, *P. dhertheae* Chavatte & Landau, 2007, schizonts. Scale bar: 10 µm.

TAXONOMIC STATUS

Celli & Sanfelice (1891) described *Haemoproteus alaudae* (later transferred to *Plasmodium*) in the blood of the skylark. At that time the authors elected to reserve *Plasmodium* to the parasites of humans. This species was considered to be composed of three types in the blood that were distinguished by the time required for development. When the figures published by these authors are carefully examined (Celli & Sanfelice 1891: pl. III), it became clear that they were in fact dealing with multiple species, not an unexpected occurrence in skylarks: a large species with schizonts of 22-30 nuclei where the RBC nuclei are displaced or even expelled (Celli & Sanfelice 1891: figs 19-21), a smaller apical species with 8 or 9 nuclei that does not displace the RBC nucleus or only little (same, figs 16, 17), a latero-apical species with 18 nuclei where the RBC nuclei is unaffected (same, fig. 18), and finally a very small species with 8 nuclei where the RBC is slightly rounded and its nucleus displaced (same, fig. 15). For the most part these species could not be confidently ascribed to a known species. However, the taxon *H. alaudae* could be retained for the form depicted in Celli & Sanfelice's plate III, figs 16, 17, for the species described above in view of the following similarities: small size, apical position, number of nuclei = 8, and little or no displacement of the nuclei in RBCs that are neither rounded nor hypertrophied.

Gametocytes could not be linked with confidence with the gametocytes observed for this species.

DIFFERENTIAL DIAGNOSIS

Plasmodium alaudae, by virtue of the number of nuclei (8) in the schizont, could be compared to *Plasmodium vaughani merulae* Corradetti & Scanga, 1972, a parasite of *Turdus merula* Linnaeus, 1758, where 8 merozoites can be observed per schizont albeit rarely. However, the absence of a bluish refractile globule in *P. alaudae* clearly distinguishes it from *P. vaughani*.

Plasmodium alaudae is close to the rounded apical forms of *P. ashfordi* that also produce 7 or 8 nuclei on average. It differs with respect to the absence of the fan-shaped schizonts that characterise *P. ashfordi*, and by its denser and clearly delimited nature.

Plasmodium alaudae must also be distinguished from *P. caloti* n. sp. (see below).

Plasmodium ghadiriani Chavatte & Landau, 2007
(Fig. 2D)

MATERIAL EXAMINED. — France. Landes, Saint-Julien-en-Born, 44°03'42"N, 1°13'33"W, blood smears of *A. arvensis* number 741U, 25.X.1996 (MNHN P2-XXV, 44-64).

DISTRIBUTION. — Seine-Saint-Denis, France (Chavatte *et al.* 2007); new record for the Landes, France.

HOSTS. — *Pica pica* (type host); new record for *Alauda arvensis*.

REMARKS

This parasite is identical to that described in the magpie. The schizonts are large and elongate and lie next to the laterally displaced nucleus of the RBC that is often enlarged. Young schizonts characteristically contain a few large chromatin masses that will fragment into smaller rounded nuclei. The older schizonts observed contained 17 nuclei, consistent with the 18 to 24 nuclei enumerated in the magpie parasite.

Plasmodium rouxi

Sergent, Sergent & Catanei, 1928
(Fig. 2E)

MATERIAL EXAMINED. — France. Landes, Saint-Julien-en-Born, 44°03'42"N, 1°13'33"W, blood smears of *A. arvensis* number 740U, 25.X.1996 (MNHN P2-XXV, 24-43); 741U, 25.X.1996 (MNHN P2-XXV, 44-64).

DISTRIBUTION. — Algeria (Sergent *et al.* 1928); new record for the Landes, France.

HOSTS. — *Culex pipiens* (Sergent *et al.* 1928) (type host); *Serinus canaria* (Sergent *et al.* 1928); *Passer hispaniolensis* (Sergent *et al.* 1929); new record for *Alauda arvensis*.

REMARKS

The characters that allow to identify this species to *P. rouxi* are: the small size of the schizont, the rectangular form, the presence of four nuclei, the two unequal pigment grains, and the absence of

the refractile globule characteristic of *P. vaughani* subspecies.

Plasmodium dheriteae Chavatte & Landau, 2007
(Fig. 2F)

MATERIAL EXAMINED. — France. Landes, Saint-Julien-en-Born, 44°03'42"N, 1°13'33"W, blood smears of *A. arvensis* number 741U, 25.X.1996 (MNHN P2-XXV, 44-64).

DISTRIBUTION. — Seine-Saint-Denis, France (Chavatte *et al.* 2007); new record for the Landes, France.

HOSTS. — *Pica pica* (type host); new record for *Alauda arvensis*.

DESCRIPTION

This species is quite comparable to the one we have previously described in the magpie. The red blood cell is unaltered; schizonts are elongated and often extending beyond the RBC nucleus. Schizonts have six to 10 nuclei distributed irregularly, with one terminal nucleus at one extremity. The pigment is lateral and agglomerated.

REMARKS

This species should be compared to *Plasmodium ashfordi* Valkiūnas, Zehtindjiev, Hellgren, Ilieva, Iezhova & Bensch, 2007, a recently described parasite of the great reed-warbler *Acrocephalus arundinaceus* (Linnaeus, 1758). Some of *P. ashfordi* elongated 7 or 8 nuclei schizonts resemble those of *P. dheriteae*, but the fan-shaped schizonts considered to be "characteristic" of *P. ashfordi* were not observed in any of the skylarks we sampled.

Plasmodium jeanriouxi Chavatte & Landau, n. sp.
(Fig. 3A)

TYPE MATERIAL. — Holotype: France. Landes, Saint-Julien-en-Born, 44°03'42"N, 1°13'33"W, blood smear of *A. arvensis* number 741U, 25.X.1996 (MNHN 440LV PXIII, 66) (Fig. 3J).

Paratypes: same data as holotype, blood smears of *A. arvensis* number 740U, 25.X.1996 (MNHN P2-XXV, 24-43); 741U, 25.X.1996 (MNHN P2-XXV, 44-64).

ETYMOLOGY. — This species is dedicated to Jean Rioux.

DISTRIBUTION. — Known only from the type locality, Saint-Julien-en-Born, Landes, France.

HOST. — *Alauda arvensis* (type host).

DESCRIPTION

This is a small parasite that is found in unaltered RBCs, most often in apico-lateral position. Throughout its development, it is closely apposed to the RBC nucleus that is sometimes slightly tilted. The trophozoites and young schizonts are rounded, though flattened at the level where they are in contact with the host nucleus. The chromatin is found as two elongated and granular peripheral masses lying on either sides of a relatively abundant cytoplasm. The arrangement of the chromatin in the young forms is highly characteristic. The cytoplasm is scattered with vacuoles of variable sizes and contains 2 to 3 pigment grains. As it matures the schizont adopts a roughly trapezoid shape, 6 or 7 nuclei become distinguishable and are disposed in a crown and then protrude at the periphery of the parasite. The vacuoles are less numerous than in the less mature forms but of larger size. The pigment grains are agglomerated in a black mass.

Young gametocytes are elongated, with pointed extremities and, akin to the schizonts, are partially apposed to the host nucleus. As they mature they associate more intimately with this nucleus that is sometimes thereby altered. They contain grains of black pigment, most often rounded, and an accessory chromatin granule.

DIFFERENTIAL DIAGNOSIS

The close apposition to the RBC nucleus and the low number of nuclei (6 or 7) make this species comparable to *Plasmodium snounoui* Chavatte & Landau, 2007, a parasite of *Pica pica*, and to *P. nucleophilum* Manwell, 1935, a parasite of *Dumetella carolinensis* (Linnaeus, 1766). *P. jeanriouxi* n. sp. can be differentiated from *P. snounoui* by its gathered-up dense shape, by the distribution of chromatin in two elongated lateral masses in the young forms and in a crown in the older forms. By contrast, *P. snounoui* is elongated in shape, its cytoplasm is clear, and the nuclei are dispersed in the young schizont and disposed in a fan-shape in the mature forms.

Plasmodium jeanriouxi n. sp. also differs from *P. nucleophilum* by its shape and size: the first has a compact rounded then roughly trapezoid shape and is apposed to the RBC nucleus over a limited surface, whereas for the second by contrast the schizonts are elongated and closely associated with the nucleus over their whole length, a feature specifically depicted by Manwell (1935: pl. I, figs 15, 16), and considered by this author as “especially characteristic”.

Plasmodium sp.
(Fig. 3B, F)

TYPE MATERIAL. — Holotype: **France**. Landes, Saint-Julien-en-Born, 44°03'42"N, 1°13'33"W, blood smear of *A. arvensis* number 740U, 25.X.1996 (MNHN P2-XXV, 24-43); 741U, 25.X.1996 (MNHN P2-XXV, 44-64).

DISTRIBUTION. — Known only from the type locality, Saint-Julien-en-Born, Landes, France.

HOST. — *Alauda arvensis* (type host).

REMARKS

This parasite of the skylark is close to *P. rouxi*. A detailed description will be published in another work on the tetranucleate *Plasmodium*. This species differs from *P. rouxi* principally by its rounded shape that contrasts with the rectangular *P. rouxi*, and by the disposition of the protruding nuclei in a semi-circle rather than as two opposed bunches as in *P. rouxi*.

Plasmodium hexamerium Huff, 1935
(Fig. 3C)

MATERIAL EXAMINED. — **France**. Landes, Saint-Julien-en-Born, 44°03'42"N, 1°13'33"W, blood smears of *A. arvensis* number 741U, 25.X.1996 (MNHN P2-XXV, 44-64).

DISTRIBUTION. — Illinois, USA (Huff 1935); Seine-Saint-Denis, France (Chavatte *et al.* 2007); new record for the Landes, France.

HOSTS. — *Sialis sialis sialis*, Huff (1935) (type host); new record for *Alauda arvensis*.

REMARKS

As for the magpie, the small apical or sub-apical schizonts with 6 nuclei are indistinguishable from those of *P. hexamerium*.

DISCUSSION

PREVALENCE

The salient difference between the infections observed in the skylark in this study and those observed in the magpies collected in the Paris region (Chavatte *et al.* 2007) is the low prevalence of infected skylarks (6 out of 30 or 20%) as compared to the high prevalence in the magpie where 28 out of 33 (85%) were found infected. An explanation for this observation might be found in the contrasting biology of the two bird species.

The skylarks considered in the present study were captured in a migratory corridor. They would have nested in the previous summer in the cultivated plains of northern countries where the abundance and diversity of the Culicidae vectors are low. The birds were on their way to the Mediterranean region at the time of capture. It would thus seem likely that the skylarks would have acquired the infections before their post-mating migration in the Mediterranean regions where they would have wintered and assembled in the previous season. In these areas *Aedes* bite very early in the season.

The magpies on the other hand are sedentary during the nesting period, and those studied live in a biotope where the vectors are numerous and diverse.

SPECIES MULTIPLICITY

The intra-generic diversity was high with 14 *Plasmodium* species found. For both bird species, the high diversity could be due to phenomena of speciation due to isolation, either through the vectors (richness of *Aedes* for the magpie) or geographical isolation (skylarks are exposed to infection in a variety of distinct locations). The large number of parasite species observed might at first appear surprising, however, a multiplicity of species of the same genus in a given host is a phenomenon that has been frequently observed in numerous parasite groups (Chabaud & Durette-Desset 1978). In birds, this is often overlooked, because of the dominance of one of the species, or because morphological variation is invoked to conclude that fewer species are present. Yet morphological studies have demonstrated that under normal conditions as observed in the natural

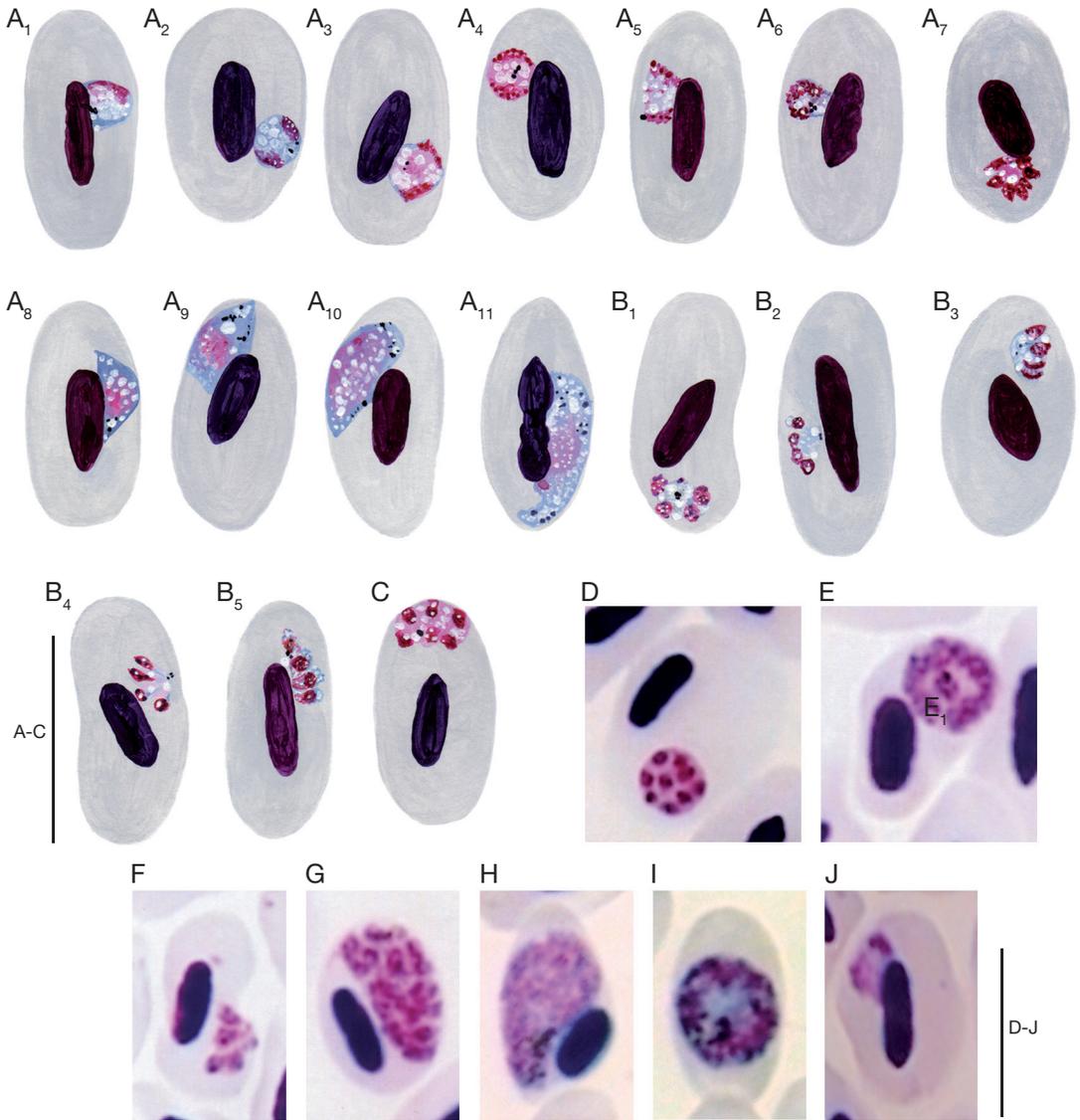


FIG. 3. — **A**, *Plasmodium jeanriouxi* Chavatte & Landau, n. sp.; **A**₁, trophozoite; **A**₂-**A**₇, schizonts; **A**₈, **A**₁₀, microgametocytes; **A**₉, **A**₁₁, macrogametocytes; **B**₁-**B**₅, *Plasmodium* sp., schizonts; **C**, *Plasmodium hexamerium* Huff, 1935, schizont; **D**-**J**, photomicrograph of holotypes of the newly described species of *Plasmodium* from *Alauda arvensis* Linnaeus, 1758; **D**, *P. caloti* n. sp.; **E**, *P. relictum* *quentini* n. subsp.; **F**, *Plasmodium* sp.; **G**, *P. reniai* n. sp.; **H**, *P. ginsburgi* n. sp.; **I**, *P. coluzzii* n. sp.; **J**, *P. jeanriouxi* n. sp. Scale bars: 10 µm.

infection, the morphological variations are relatively minor for a given species. On the other hand, these variations can be substantially more pronounced when parasitaemias reach high levels, as is the case in some experimental infections where many parasites display an atypical course of infection.

In recent years, approaches based on gene sequences have led to propose that avian *Plasmodium* parasites are highly diverse, but that many of the species are “cryptic” (see e.g., Bensch *et al.* 2004.) The concordance between morphology and molecular markers was only investigated in a minority

of studies (Martinsen *et al.* 2006; Valkiūnas *et al.* 2007) but it is conceivable that correspondences might be established between these “cryptic” species and the morphology.

Preliminary data (not shown) obtained by sequencing the magpies *Plasmodium* revealed that at least eight clearly different “types” from the small subunit ribosomal 18S gene could be isolated from these birds. Although we cannot at present link the sequences to a particular species, these observations corroborate nevertheless the species multiplicity of *Plasmodium* in these birds.

In general, it is pointless to draw conclusions

from the molecular analysis of material poorly or no identified.

In the case of our parasites, because of the multiplicity of species, because some species may be undetectable, and the sequencing results are preliminary it is unwise to interpret results. However, by PCR with primers specific to each type of *Plasmodium* from magpies, we amplify two similar sequences from the skylark. The sequences were shared by the skylark and the magpie only when one or several species of the trio *Plasmodium dorsti*, *P. bioccai*, *P. relictum* were present. These are precisely the three species belonging to the subgenus *Haemamoeba*.

IDENTIFICATION KEY FOR THE *PLASMODIUM* MARCHIAFAVA & CELLI, 1885 SPECIES
FOUND IN *ALAUDA ARVENSIS* LINNAEUS, 1758

1. Rounded or oval gametocytes; RBC nucleus displaced to one of the extremities 2
— Elongated gametocytes, RBC nucleus laterally displaced if displaced 7
2. Schizonts with more than 18 nuclei, contour clearly defined by a smooth membrane; nuclei not protruding at the periphery of the schizont. RBC enlarged to a degree related to the size and shape of the schizont 3
— Schizonts with less than 18 nuclei, nuclei bulging at the periphery of the immature forms; RBC very hypertrophied but those infected by parasites distinctly of smaller size 6
3. Rounded schizonts 4
— Ellipsoid or elongate schizonts 5
4. Clearly defined individual nuclei, distributed in the schizont throughout development, 28-30 nuclei, loosely gathered pigment *Plasmodium bioccai*
— Individual nuclei poorly defined in sub-mature forms, with granular indistinct chromatin that predominates at the periphery for most of the development period, more than 30 nuclei *Plasmodium coluzzi* n. sp.
5. Ellipsoid, elongate or oval schizonts of regular shape and contour, nearly completely filling the RBC, poorly defined nuclei prior to maturation, granular indistinct chromatin, more than 30 nuclei *Plasmodium ginsburgi* n. sp.
— Schizonts variable in shape, irregular but rather elongated, occupying $\frac{3}{4}$ of the RBC, well-defined individual nuclei, 24 to 30 nuclei *Plasmodium dorsti*
6. RBC rarely discoloured, schizonts occupying at most half of the RBC, fine pigment loosely assembled in a rounded area, 14 nuclei *Plasmodium relictum quentini* n. subsp.
— Discoloured RBC, schizonts occupying at most a third of the RBC, paucity of pigment assembled in a little black mass, 8 nuclei *Plasmodium caloti* n. sp.
7. Schizonts lying along the RBC nucleus 8
— Schizonts not lying along the RBC nucleus 9
8. Large schizonts that displace the RBC nucleus laterally, 18-24 nuclei
..... *Plasmodium ghadiriani*
— Small schizonts that do not displace the RBC nucleus, 8 nuclei *Plasmodium dherteeae*

9. Large schizonts, partially apposed to the membrane at the apex of the RBC, more than 30 nuclei *Plasmodium reniai* n. sp.
 — Small schizonts, not touching the RBC membrane, less than 10 nuclei 10
10. Schizonts with 6 or 8 nuclei 11
 — Schizonts with 4 nuclei 13
11. Schizonts with 6 nuclei *Plasmodium hexamerium*
 — Schizonts with 8 nuclei 12
12. Parasites partially apposed to the RBC nucleus *Plasmodium jeanriouxi* n. sp.
 — Parasites not in contact with the RBC nucleus *Plasmodium alaudae*
13. Schizonts rectangular in shape *Plasmodium rouxi*
 — Rounded schizonts displaying a fan-shape *Plasmodium* sp.

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