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LEUCOCYTOZOON (APICOMPLEXA: LEUCOCYTOZOIDAE) FROM WEST AFRICAN BIRDS, WITH DESCRIPTIONS OF TWO SPECIES

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ABSTRACT: Five species of *Leucocytozoon* were recovered from 35/828 birds of 95 species examined from 6 sites in West Africa between May 1995 and June 2001. *Leucocytozoon pogoniuli* n. sp. is described from the tinker barbets *Pogoniulus subsulphureus* and *Pogoniulus atroflavus*. *Leucocytozoon trachyphoni* n. sp. is described from the barbet *Trachyphonus purpureus*. No leucocytozoids have been reported previously in species of *Pogoniulus*. *Leucocytozoon nectariniae* was identified from the sunbird *Nectarinia olivacea*, and *Leucocytozoon brimonti* was recovered from 4 species of Pycnonotidae (bulbuls), all of which are new host records. We also report the first *Leucocytozoon* to be recovered from the phylogenetically isolated bird, *Picathartes* sp. (Picathartidae). This parasite is similar in appearance to *Leucocytozoon sakharoffi*, and probably represents a previously undescribed species. In view of the intraspecific variability and, frequently, relatively minor interspecific differences within Leucocytozoidae, we suggest that the development and application of molecular techniques would greatly advance understanding of speciation and relationships within this family.

Species of *Leucocytozoon* are widespread, intracellular blood parasites of birds. First seen by Danilewsky (1885), the Leucocytozoidae continue to present many problems of taxonomy and host-specificity. Species that occur as a round form are often morphologically similar, and the appearance of the deformed nucleus of the host cell is used as an important character in descriptions. Initially thought to be host-species specific, experiments by Fallis et al. (1974) failed to transmit infections to hosts from other bird families. The concept of host-family/host-subfamily specificity for *Leucocytozoon* has hitherto been considered the most prudent (Desser and Bennett, 1993), though this may need to be reevaluated in the light of future research and the application of nonmorphological techniques. A further difficulty with this concept is the uncertainty surrounding the taxonomic status of many birds, especially in Africa because the application of DNA-DNA hybridization techniques has caused the systematic position of many bird groups to be reassessed, especially in the passeriformes. This article reports the species of *Leucocytozoon* identified from 828 birds sampled over a 7-yr period from sites in Côte d'Ivoire, Cameroon, and Equatorial Guinea (Sehgal et al., in press).

MATERIALS AND METHODS

The samples used in this study were collected opportunistically as part of an on-going study of avian evolution in Central and West Africa (Smith et al., 1997, 2000; Sehgal et al., 2001). Eight-hundred and twenty-eight blood smears were collected from 95 species of bird at sites in a variety of habitats in Cameroon, Côte d'Ivoire, and Equatorial Guinea between 1995 and 2001; the geographical coordinates and location names from each site where *Leucocytozoon* spp.-infected individuals occurred are given in the text. Birds were collected in mist nets and released after a small amount of blood had been taken from the brachial vein. A band was affixed to the leg of each bird caught so that it could be identified if recaught. Blood smears were air dried, fixed in 100% methanol for 2 min, and subsequently stained with 3% Giemsa stain for 20 min. Slides were examined for a minimum of 20 min at 200×, 400×, and 1,000× magnification. In examining and describing the species of *Leucocytozoon* in this article, we follow the protocols established by Bennett et al. (1991). Drawings were made with the aid of a drawing tube, and measurements were made on parasites not in contact with neighboring cells to avoid the effects of distortion. All specimens have been deposited at the International Reference Centre for Avian Haematozoa (IRCAH) in Brisbane, Australia.

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RESULTS

Five species of *Leucocytozoon* were identified in 35 passerine birds of 9 species from 4 families, i.e., Nectariniidae (sunbirds), Capitonidae (=Lybiidae) (barbets), Picathartidae (jungle fowl or rock crows), and Pycnonotidae (bulbuls). This represents an overall prevalence of 3.6% and a prevalence of 10.2% at the 6 collection sites at which *Leucocytozoon* spp. were recovered. *Leucocytozoon* spp. were present at low intensity (<0.025% of red cells) in all infected birds. One of 4 *Pogoniulus subsulphureus* and 1/1 *Pogoniulus atroflavus* were infected with a new species of *Leucocytozoon*, and 1/1 *Trachyphonus purpuratus* was infected with a second species of *Leucocytozoon*, originally assigned to *Pogoniulus fringillinarum*. These are described below.

DESCRIPTION

Leucocytozoon pogoniuli n. sp.

(Table I, Figs. 1–4)

Macrogametocytes: Parasites round or slightly elongated. Cytoplasm dark-blue staining, with between 12 and 25 small clear vacuoles scattered throughout. Parasite nucleus conspicuous, usually elliptical, pale. Microgametocytes of similar size, round or slightly elongated but too pale for internal structure to be defined, less numerous than macrogametocytes. Host-cell nucleus present as an uneven band around most of periphery of parasite, often with 2 swellings in contact with 47–80% (mean, 62%) of the parasite periphery. In almost half the parasites measured, this nucleus was stretched so thinly around the parasite that it appeared as 2 separate caps on opposite sides of gametocyte (Figs. 2, 4).

Taxonomic summary

Type host: Pogoniulus subsulphureus (Fraser); (Capitonidae).

Other hosts: Pogoniulus atroflavus, collected at same location and date as Hapantotype.

TABLE I. Measurements (μm) of macrogametocytes of *Leucocytozoon pogoniuli* n. sp. and *Leucocytozoon trachyphoni* n. sp. (standard deviations in parentheses).

	Leucocytozoon pogoniuli (n = 20)	*Leucocytozoon trachyphoni* (n = 20)
Parasite		
Maximum diameter	11.3 (0.8)	12.8 (07)
Minimum diameter	10.1 (0.8)	10.8 (1.1)
Periphery	31.5 (4.1)	31.6 (2.1)
Area (μm)	85.0 (6.4)	103.1 (11.0)
Parasite nucleus		
Maximum diameter	3.0 (0.6)	2.4 (0.3)
Minimum diameter	2.3 (0.2)	1.6 (0.2)
Area (μm)	5.0 (1.2)	4.1 (0.7)
Host–parasite complex		
Maximum diameter	14.4 (1.3)	13.7 (1.1)
Minimum diameter	11.0 (1.0)	12.2 (1.2)
Area (μm)	111.6 (10.1)	125.8 (11.2)
Host cell nucleus		
Area (μm)	27.0 (9.1)	22.0 (4.5)
Parasite periphery covered (%)	62.4% (9.8)	43.2% (7.1)

Site and locality: Mt. Alen, Equatorial Guinea, 1°39.04′N, 10°18.09′E, May 1998.

Specimens deposited: Hapantotype blood film no. G464641, parahapantotype blood film no. G464642.

Remarks

One species of *Leucocytozoon* has been described hitherto from the Capitonidae, i.e., *Leucocytozoon capitonis*, from the Asian barbets *Megalaima viridis* and *Megalaima franklini* in south India and in Thailand (Bennett et al., 1993). Several surveys of African birds have reported '*Leucocytozoon* sp.' (Peirce and Backhurst, 1970; Ashford et al., 1976; Bennett and Herman, 1976; Peirce, 1976), but no descriptions were given. Bennett et al. (1993) assigned all these records from Capitonidae to *L. capitonis*, although stating that the available material from these African studies in the International Reference Centre for Avian Haematozoa were not suitable for taxonomic purposes.

Leucocytozoon capitonis is significantly larger in all morphometric parameters than *L. pogoniuli*. In addition, it differs from *L. pogoniuli* in the crescentic shape of the host-cell nucleus, which, in *L. pogoniuli*, often appears as 2 separate caps at opposite poles of the parasite and in the scarcity of vacuoles in the parasite cytoplasm. *Leucocytozoon pogoniuli* differs principally from *Leucocytozoon fringillinarum* in its larger size, in the greater extent to which the host-cell nucleus surrounds the periphery of the parasite and by its frequent apparent split into 2 masses at opposite poles of the parasite. Other leucocytozoids with bulbous terminal portions include *Leucocytozoon nectariniae* and *Leucocytozoon dubreuili* in the thrushes (Turdinae; redescribed by Khan and Fallis, 1970). *Leucocytozoon pogoniuli* differs from *L. nectariniae* examined in the present study in being larger, the presence of vacuoles more consistent, and in higher numbers, a more differentiated nucleus, and a higher proportion having the host-cell nucleus divided into 2 portions. Four other species in the African *Pogoniulus*, the tinker birds or tinker barbets, have been examined previously (N = 27; Bennett et al., 1992a) and no *Leucocytozoon* were seen.

DESCRIPTION

Leucocytozoon trachyphoni n. sp.

(Table I, Figs. 5–8)

Macrogametocytes: Parasites round or slightly elongated. Cytoplasm dark-blue staining, without vacuoles. Parasite nucleus small, well defined, round to elliptical. Microgametocytes pale, present in similar numbers to macrogametocyte, all measurements similar to macrogametocyte. Host-cell nucleus forming a uniform thin crescent around less than half periphery of parasite (range 33–64%, mean 43%)

Taxonomic summary

Type host: Trachyphonus purpuratus Verreaux & Verreaux (Capitonidae).

Site and locality: Tai Forest, Côte d'Ivoire, 5°49.98′N, 7°20.56′W, June 2001.

Specimens deposited: Hapantotype, blood film no. G464643.

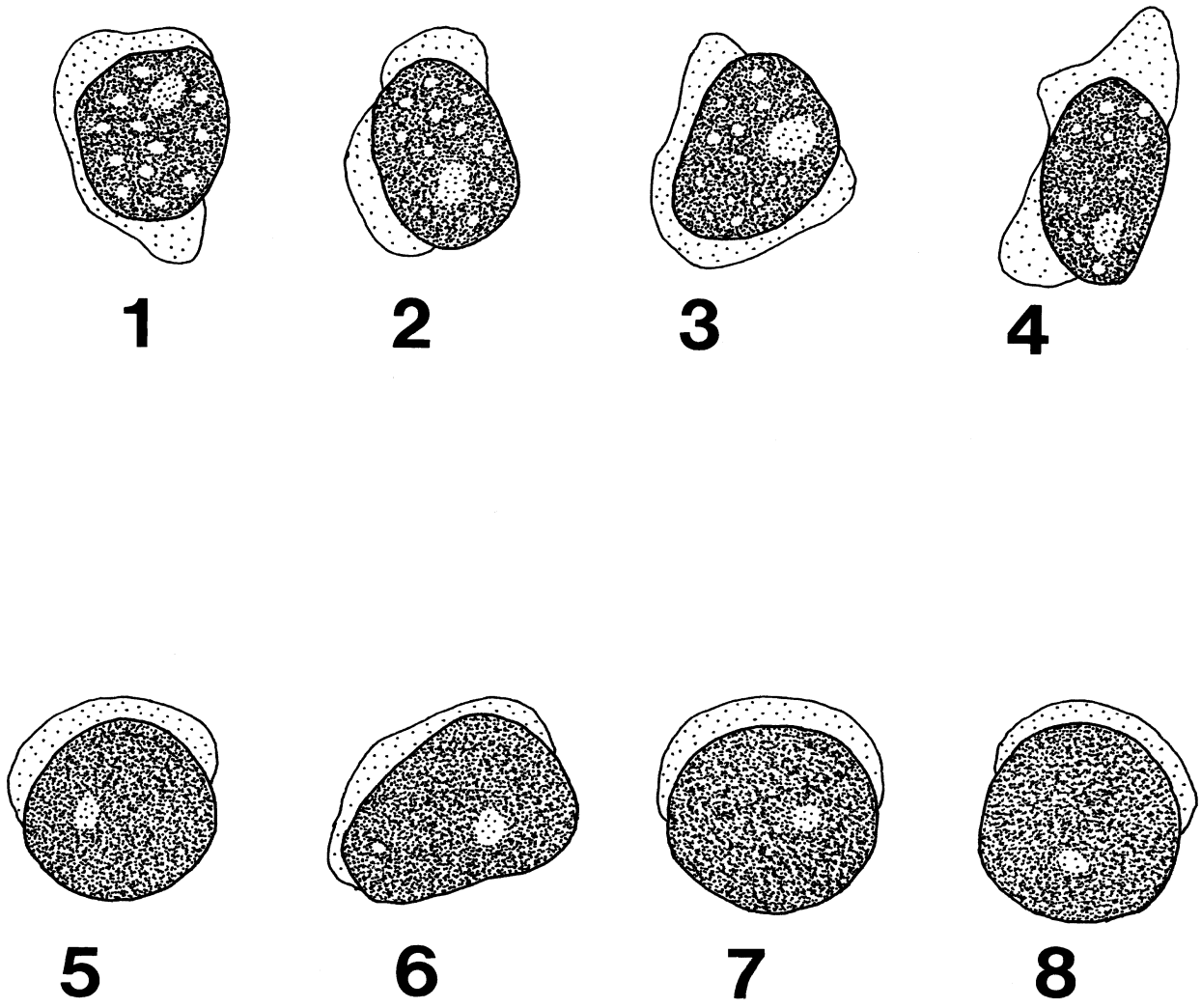
Remarks

Leucocytozoon trachyphoni resembles *L. capitonis* in having a crescentic host-cell nucleus and few or no cytoplasmic vacuoles. It differs principally in being significantly smaller than *L. capitonis* in all morphometric measures. The 2 parasites from Capitonidae described in this article differ from one another in the larger size of the parasite nucleus, smaller area of the parasite, and the presence of numerous conspicuous vacuoles in *L. pogoniuli*. Furthermore, in this species, the host-cell nucleus covers a larger extent of the parasite periphery (about two thirds) than in *L. trachyphoni* (less than half) and is usually bipolar in shape, compared with the crescentic appearance of this structure in *L. trachyphoni*. *Leucocytozoon trachyphoni* is similar to *L. fringillinarum* in appearance and in all measurements, differing only in the scarcity of vacuoles in the cytoplasm (Bennett et al., 1992b). *Leucocytozoon* sp. infections have been reported in 5/31 *Trachyphonus* of 4 species examined previously (Bennett et al., 1992a). *Leucocytozoon fringillinarum* was identified in 1 *Trachyphonus darnaudii* (Peirce et al., 1977), but this was subsequently stated to be in error (Bennett et al., 1993), and in 1 *Trachyphonus erythrocephalus* (Peirce and Backhurst, 1970) in northern Kenya. Parasites recovered from 1 *T. purpuratus* (Peirce, 1984) and 2 *Trachyphonus vaillanti* (IRCAH collection) were not identified to species.

Other species identified

Leucocytozoon nectariniae was identified in 8/123 olive sunbirds, *Nectarinia olivacea*, examined. In addition, another 3 *N. olivacea* were infected with *Leucocytozoon*, which cannot yet be assigned to species; these showed a range of measurements and forms of host nuclear size and shape, which do not conform to the descriptions and measurements of *L. nectariniae*.

Leucocytozoon brimonti Mathis and Leger, 1910, occurred in 13/101 yellow-whiskered greenbuls, *Andropadus latrirostris*, 8/101 little greenbuls, *Andropadus virens*, 1/2 Xavier's green-



FIGURES 1–4. *Leucocytozoon pogoniuli* macrogametocytes.

FIGURES 5–8. *Leucocytozoon trachyphoni* macrogametocytes (scale bar = 10 μm).

bul, *Phyllastrephus xaveri*, and 1/7 western nicator, *Nicator chloris*.

Three macrogametocytes of *Leucocytozoon* sp. were found in the 1 blood slide obtained from the Rockfowl *Picathartes oreas*; in 2 of these parasites, the host-cell nucleus had hemolyzed and only 1 specimen was suitable for detailed examination. Its characteristics and deformation of the host-cell nucleus resembled *Leucocytozoon sakharoffi* Sambon, 1908.

Leucocytozoon nectariniae occurred in *Nectarinia olivacea* from Mt. Alen (Equatorial Guinea, 1°39.04′N, 10°18.09′E), Ncoho (Equatorial Guinea, 1°14.2′N, 9°57.11′E), Sakbayeme (Cameroon, 4°02.29′N, 10°34.45′E), and Sangmbengue (Cameroon, 4°04.10′N, 10°33.68′E), *L. brimonti* in 4 species of Pycnonotidae from Mt. Alen, Sakbayeme, and Sangmbengue, and *L. pogoniuli* n. sp., and *Leucocytozoon* sp. from *Picathartes oreas* only from Mt. Alen; the single infection of *T. purpuratus* with *L. trachyphoni* n. sp. was the only *Leucocytozoon* recovered from 111 birds examined in Tai Forest (Côte d'Ivoire).

DISCUSSION

The frequent attribution of small leucocytozoids with a cap-like host-cell nucleus to *L. fringillinarum*, regardless of the bird host family, has engendered considerable confusion. Bennett et al. (1992b), in discussing this species, state that records of this parasite from nonfringillid hosts are in error and that these leucocytozoids should be referred to the appropriate bird family. It is on the basis of this concept of host-family specificity that the leucocytozoid from *T. purpureus* is described as a new species, despite its close morphological similarity to *L. fringillinarum*. This underlines the need for nonmorphological techniques to clarify the status of this parasite.

A number of Nectariniidae (sunbirds) have been recorded as being infected with species of *Leucocytozoon* (Bennett et al., 1982; Bishop and Bennett, 1992). As noted above, the belief that *L. fringillinarum* Woodcock, 1910, and *Leucocytozoon majoris* Laveran, 1902, had a wide host specificity, resulted in

leucocytozoids from *Nectarinia* species, including *N. olivacea*, being assigned to these hosts. Bennett et al. (1992b), in reviewing leucocytoids from sunbirds, assigned all previous records of *Leucocytozoon* sp. in sunbirds to *L. nectariniae*, which they assert is the only species of *Leucocytozoon* occurring in this bird family. In the present study, none of the specimens from 3 *N. olivacea*, which differed from the description of *L. nectariniae*, were present in sufficiently high numbers or, in 1 case, sufficiently well preserved and stained to enable their status to be confirmed.

Andropadus latrirostris, *A. virens*, *Phyllastrephus xaveri*, and *Nicator chloris* are new host records for *L. brimonti*. This parasite has been identified in 1 other species of *Andropadus* (*Andropadus importunus*; Peirce et al., 1977) and 1 species of *Phyllastrephus* (*Phyllastrephus terrestris*). Five other *Phyllastrephus* spp. and 1 species of *Nicator* have been examined and no leucocytozoids were seen (see Bennett et al., 1992a; Bishop and Bennett, 1992).

Both *L. brimonti* and *Leucocytozoon pycnonoti* have been identified from many species of birds in the Pynonotidae (Bulbuls), and the 2 species can occur concurrently (Bennett et al., 1992b). No *L. pycnonoti* were seen in the present study. All *L. brimonti* seen in this study conformed to the redescription by Bennett et al. (1992b), although in some specimens from both *Andropadus latrirostris* and *Andropadus virens*, the host nucleus extended round more than 50% of the periphery of the parasite. None, however, approached the extent of host nucleus deformation seen in *L. pycnonoti* (Bennett et al., 1992b).

Thus, in some hosts, both *L. nectariniae* and *L. brimonti*, though conforming closely in most respects to their descriptions or redescriptions, differed from them significantly, principally in the extent and deformity of the adhered host-cell nucleus. These are the first records of *L. brimonti* for these 4 avian species, and it is possible that this fact and the geographical distance from the areas in central, southern, and eastern Africa from where these species of *Leucocytozoon* were described may account for these differences. The paucity of material precluded a more detailed analysis of the degree by which these specimens departed from published descriptions.

This is the first examination of a member of the Picathartidae for blood parasites. The family comprises 2 species and is confined to the tropical forests of west Africa. Its systematic position remains uncertain. It was reviewed by Delacour and Amadon (1951), who considered and rejected evidence that the *Picathartes* sp. be placed with the starlings (Sturnidae) or crows (Corvidae), and, on the basis of behavioral and anatomical traits, considered that they had more in common with babblers (Timaliinae). They have also been placed in the subfamily Picathartini of the Muscicapidae (Howard and Moore, 1984), in the tribe Picathartini of the muscapid subfamily Timaliinae, the babblers (Landsborough Thompson, 1964), and, most recently, on the basis of DNA-DNA hybridization, as '*Incertae Sedis*', but probably within the parvorder Corvida (Sibley and Monroe, 1990). The suggestion by Olson (1979) that it may be a rainforest relic with Asian affinities emphasizes its distance from contemporary bird groupings. The prevailing view being that *Leucocytozoon* spp. are host-family specific, the macrogametocytes seen in the single *Picathartes* sp. examined are likely to belong to a hitherto undescribed species, though its status cannot be firmly ascertained at this stage. In appearance, it is similar to *L. sakharoffi*, which has a wide host range within the Corvidae. It is, however, of interest that no *Leucocytozoon* were seen in 9 crows of 3 species examined in sub-Saharan Africa (Bennett et al., 1992a).

The findings in this study reveal marked variability within what appear to be single species, in a family of protozoa that can display little morphological difference between species and in which cross-infection experiments are required to determine with certainty a species' validity. Recently, a portion of the mitrochondrial DNA of *Leucocytozoon* sp. was sequenced (Perkins and Schall, 2002). We feel that the study of the Leucocytozoidae may benefit considerably from the further development and application of molecular methodologies involving DNA (Tautz et al., 2003). Recently, molecular methods have been developed to detect *Leucocytozoon* from avian blood samples (Hellgren et al., 2004). These studies show that many lineages of *Leucocytozoon* exist and that, in a survey of bluethroats (*Luscinia svecica*; Muscicapidae), 5 mitochondrial DNA lineages were distinguished from 86 individual birds tested. In addition, a study of blood-feeding blackflies (*Simulium* spp), which commonly transmit *Leucocytozoon*, has revealed that the flies exhibit a high degree of feeding specificity, with certain species of blackfly feeding preferentially on birds and others feeding on mammals (Malmqvist et al., 2004). These types of studies, once linked with morphological data, will clarify relationships between *Leucocytozoon* species and their insect and avian hosts.

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