

New species of haematozoa in Phalacrocoracidae and Stercorariidae in South Africa

Nola J Parsons^{1,2,3,4*}, Michael A Peirce^{5,6} and Venessa Strauss¹

¹ Southern African Foundation for the Conservation of Coastal Birds, PO Box 11116, Bloubergrant 7443, South Africa

² Animal Demography Unit, Department of Zoology, University of Cape Town, Rondebosch 7701, South Africa

³ Department of Environmental Affairs, Private Bag X2, Rogge Bay 8012, South Africa

⁴ Marine Research Institute, University of Cape Town, Rondebosch 7701, South Africa

⁵ MP International Consultancy, 6 Normandale House, Normandale, Bexhill-on-Sea, East Sussex, TN39 3NZ, UK

⁶ Corresponding Associate, International Reference Centre for Avian Haematozoa, Queensland Museum, PO Box 3300, South Brisbane, QLD 4101, Australia

* Corresponding author, e-mail: nolaparsons@yahoo.co.uk

New species of haematozoa, namely *Leucocytozoon ugwidi* sp. nov. from the Cape Cormorant *Phalacrocorax capensis* and *Haemoproteus skuae* sp. nov. from the Subantarctic Skua *Catharacta antarctica*, are described. These are the first species to be recorded from the families Phalacrocoracidae and Stercorariidae, respectively.

Introduction

Sick and injured marine and coastal birds are frequently recovered and sent to the Southern African Foundation for the Conservation of Coastal Birds (SANCCOB) for examination and rehabilitation when possible. Individual birds are subject to a range of veterinary investigations including screening for the presence of blood parasites (Parsons and Underhill 2005). This has presented a unique opportunity to examine for haematozoa in species of birds infrequently sampled and from which no host-specific species of parasites have hitherto been described (Peirce 2005).

Materials and methods

Birds were bled from the medial metatarsal vein as it lies over the tarsal bone with a 23G or 25G needle (depending on the size of the bird). Blood was collected directly from the hub of the needle into a heparinised capillary tube. A thin blood smear was made immediately, air-dried, fixed in methanol for 3 min and stained with a modified Wright-Giemsa stain (Kyro-Quick stain set, Kyron Laboratories (Pty) Ltd, Benrose, South Africa). All slides were initially screened at SANCCOB, each slide being examined for 10 min, using a 50× oil immersion lens. Slides found to contain parasites were retained, and those of particular interest were subsequently re-examined by MA Peirce. These slides were screened under a Zeiss binocular microscope at 160× and 800× magnification. All images were captured using a Nikon Coolpix 4500 digital camera attached to the Zeiss microscope. Morphometric measurements were obtained after the methods of Bennett and Campbell (1972) for haemoproteids, and Bennett et al. (1991) for leucocytozoids.

All birds were sampled on a weekly basis during their stay at SANCCOB, which allowed the course of the infection of

any parasitised birds to be followed and the parasitaemia monitored. Reference slides have been deposited in the International Reference Centre for Avian Haematozoa (IRCAH) Collection.

Results

Between 1 January 2001 and 31 March 2009, smears from 519 Cape Cormorants *Phalacrocorax capensis*, 52 Crowned Cormorants *P. coronatus*, 23 Bank Cormorants *P. neglectus* and 43 White-breasted Cormorants *P. carbo* were examined. Although a number of cormorants were found to be infected with a *Babesia* sp. (on-going study), only one *P. capensis* was found to harbour a hitherto undescribed species of *Leucocytozoon*. This bird was first examined on 22 January 2007 and found to have a very high parasitaemia with *Leucocytozoon* as well as a *Babesia* sp. It was resampled on 29 January and again on 5 February the same year. By the last sample point, the parasitaemia had dropped to just a few gametocytes per smear.

During the same period, seven Subantarctic Skuas *Catharacta antarctica* were examined for haematozoa. On 18 September 2006, a *C. antarctica* was found infected with a haemoproteid not previously recorded from any skua. Only a single slide was available from this bird and no subsequent samples were taken as the bird was released from the centre.

Taxonomic review

Family Phalacrocoracidae (cormorants)

Parasite: *Leucocytozoon ugwidi* sp. nov.

(Figure 1a–f)

Type host: *Phalacrocorax capensis* (Sparrman).

Type locality: SANCCOB, Bloubergrant, South Africa.

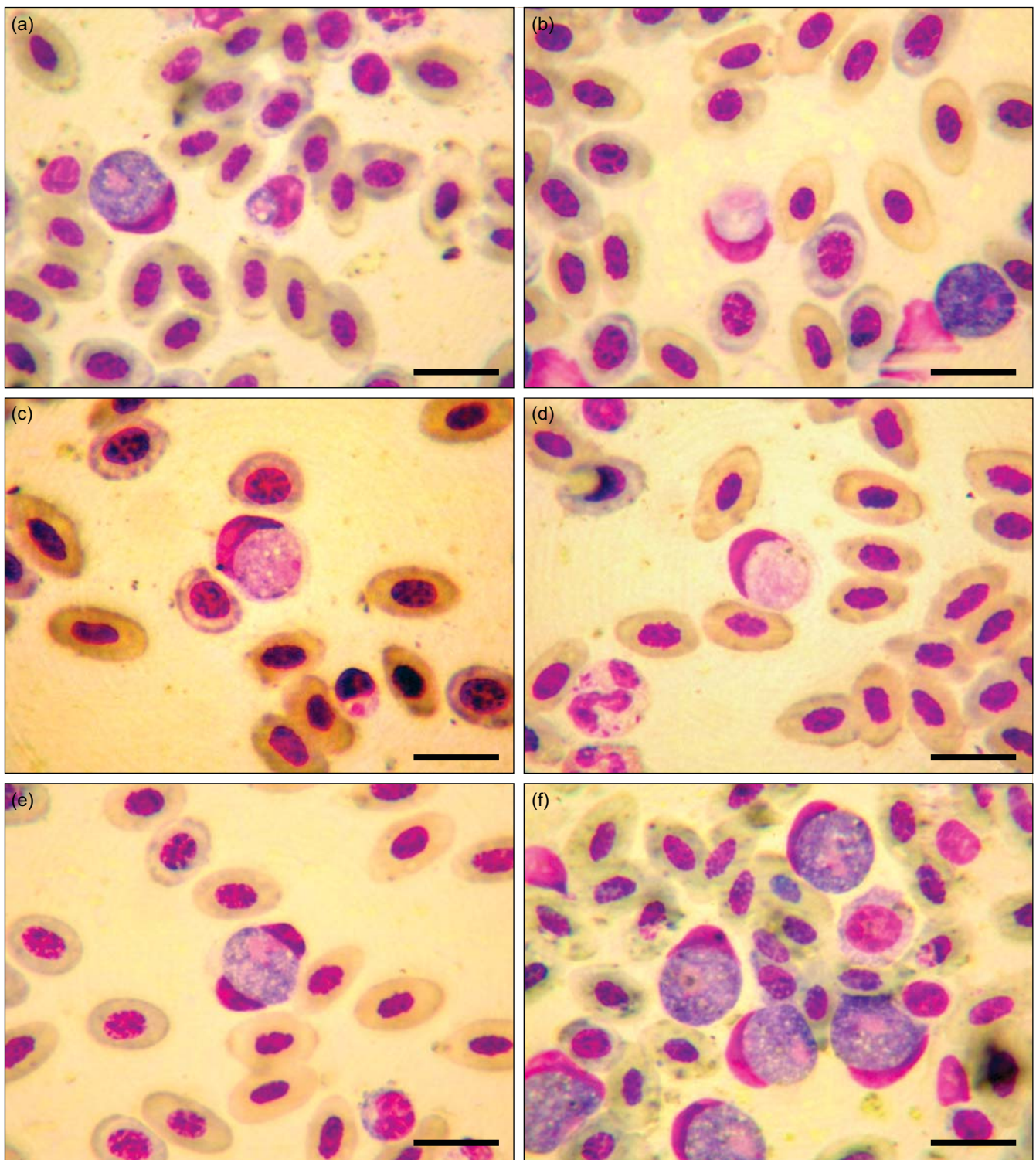


Figure 1: *Leucocytozoon ugwidi* sp. nov. from *Phalacrocorax capensis*. (a) Immature gametocyte (centre) and mature macrogametocyte to the left. (b) Immature microgametocyte centre and macrogametocyte free of host cell nucleus on the right. (c) Mature macrogametocyte showing distinct karyosome within the nucleus. (d) Mature microgametocyte. (e) Mature macrogametocyte in a host cell in which the host cell nucleus has been cleaved into two equal halves. (f) Group of mature macrogametocytes. Scale bars = 10 µm

Table 1: Morphometric parameters of gametocytes of *Leucocytozoon ugwidi* sp. nov. from *Phalacrocorax capensis*. *n* = sample size

<i>Leucocytozoon ugwidi</i>	Morphometric parameter	Macrogametocytes (<i>n</i> = 40)				Microgametocytes (<i>n</i> = 25)			
		Mean	SD	Min.	Max.	Mean	SD	Min.	Max.
Parasite	Maximum diameter (μm)	10.6	0.6	9.8	11.8	10.9	0.9	9.3	12.5
	Minimum diameter (μm)	10.1	0.4	9.2	10.8	9.3	0.9	7.6	10.8
	Periphery (μm)	32.4	1.2	30.7	34.8	31.6	2.2	27.1	36.1
	Area (μm ²)	83.8	6.4	71.1	96.7	80.2	11.5	58.6	104.1
Parasite nucleus	Maximum diameter (μm)	3.8	0.5	2.9	4.9				
	Minimum diameter (μm)	2.4	0.3	2.0	3.3				
	Nucleus area (μm ²)	7.3	1.8	6.1	12.9				
Host–parasite complex	Maximum length (μm)	12.9	1.0	10.3	14.6	12.4	1.0	10.9	14.5
	Minimum length/diameter (μm)	10.4	0.9	9.1	11.8	9.4	0.9	7.6	10.8
	Complex area (μm ²)	107.3	16.0	75.2	140.6	93.8	15.5	70.3	123.8

Vector: Unknown, but assumed to be a species of simuliid.

Etymology: Named after the Xhosa name for cormorant.

Immature gametocyte: Merozoites usually invade leucocytes, most commonly lymphocytes. Developing gametocytes (Figures 1a–b) cause indentation of the host cell nucleus, which gradually becomes stretched around the periphery of the parasite as it matures.

Macrogametocyte (Figure 1a, c, e and f; Table 1): Mature gametocytes exhibit the usual sexual differentiating characteristics with the macrogametocyte cytoplasm staining a deep blue; the nucleus is round to ovoid staining pale red with a distinct darker red karyosome and occupies about 8% of the area of the parasite; cytoplasm is granular and in some mature parasites fine purple-staining volutin granules may be present. The parasite is small, almost totally round, occupying about 77% of the host cell–parasite complex; nucleus of host cell–parasite complex extends as a band around parasite; occasionally a slightly raised central point may be seen and ends of band are usually thinner, covering 40–45% of the periphery of the parasite and occupying 23% of the area of the host cell–parasite complex. Very occasionally, a parasite is seen in which the host cell nucleus is split into two relatively equal segments (Figure 1e).

Microgametocyte (Figure 1d; Table 1): Morphologically similar to macrogametocyte but averaging about 4% smaller in size. Cytoplasm pale blue with dispersed nucleus; karyosome occasionally seen. Nucleus of host cell–parasite complex extends as a band around the parasite periphery occupying a similar area as in macrogametocytes. Ratio of macrogametocytes to microgametocytes is 90:1.

Hapantotype: IRCAH: G465376 from *P. capensis* coll. Parsons, 22 January 2007, SANCCOB, Bloubergrant, South Africa; deposited in IRCAH Collection, Queensland Museum, Brisbane, Australia.

Parahapantotypes: IRCAH: G465377 and IRCAH: G465378 from *P. capensis* coll. Parsons, 29 January 2007 and 5 February 2007, respectively, SANCCOB, Bloubergrant, South Africa; deposited in IRCAH Collection, Queensland Museum, Brisbane, Australia.

All slides are from the same bird and also contain intra-erythrocytic parasites of a *Babesia* sp. Other hosts: None known at present time but assumed to occur throughout the geographic range of the Phalacrocoracidae.

Comments: *Leucocytozoon ugwidi* sp. nov. is a small round morph currently known only from southern Africa. The high parasitaemia observed in the smear from the first sample point clearly shows this to be a parasite well established in the host species even though prevalence appears to be low. The other distinguishing characteristic of *L. ugwidi* sp. nov. is the very low ratio of microgametocytes to macrogametocytes.

Family Stercorariidae (skuas)

Parasite: *Haemoproteus skuae* sp. nov.

(Figure 2a–f)

Type host: *Catharacta antarctica* (Lesson).

Type locality: SANCCOB, Bloubergrant, South Africa.

Vector: Unknown, but either hippoboscoid or ceratopogonid.

Etymology: Named after the host.

Immature gametocyte (Figure 2a–b): Early forms usually occupy a lateral position within the erythrocyte and this is maintained throughout development. Parasite outline is amoeboid in most developing gametocytes and vacuoles may be present.

Macrogametocyte (Figure 2c–f; Table 2): Macrogametocytes exhibit the usual sexual differentiating characteristics. Parasite halteridial with irregular and amoeboid outlines although some parasites may be smooth and almost entire; occupying about 61% of the host cell cytoplasm. Cytoplasm is granular and partially vacuolated. Occasionally, a mature gametocyte may be almost circumnuclear. Parasite nucleus is compact, round to ovoid and generally located centrally; pigment granules, small, golden brown, scattered evenly throughout the cytoplasm; Mature parasites cause little displacement of the host cell nucleus. There is little hypertrophy of infected erythrocytes, in fact the opposite is observed with about 4% atrophy. Occasionally, double infections may be observed when all host cell cytoplasm is occupied (Figure 2f).

Microgametocyte (Figures 2b and e; Table 2): Similar in shape to the macrogametocyte and presenting the usual staining characteristics. Nucleus is stranded in appearance and less diffuse than in many species; occupying a central position and staining pale red. Parasite cytoplasm very pale and hyaline; pigment granules fewer than in macrogametocytes and usually located in clusters terminally either side of the nucleus. Infected erythrocytes

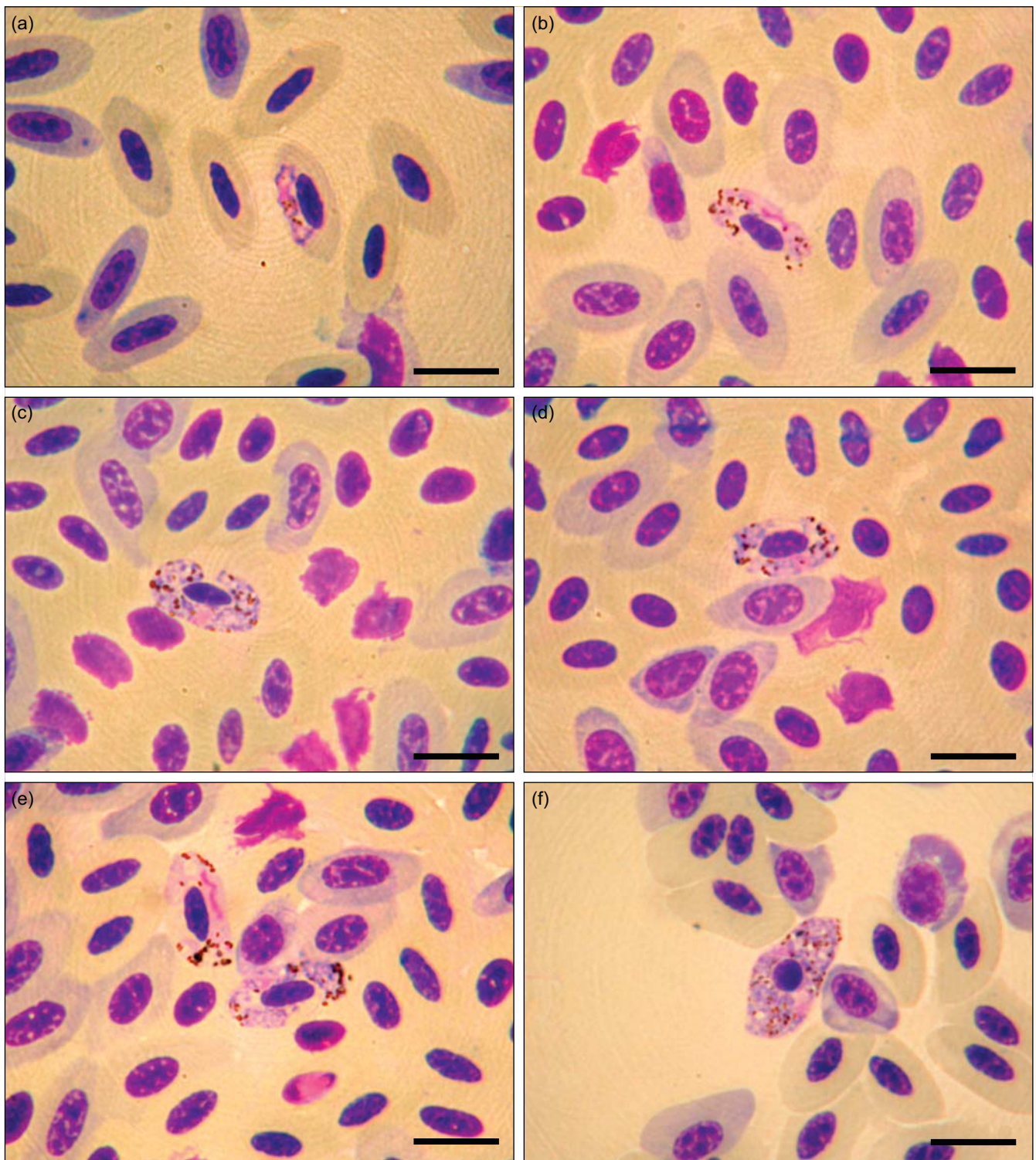


Figure 2: *Haemoproteus skuae* sp. nov. from *Catharacta antarctica*. (a) Immature macrogametocyte. (b) Immature microgametocyte. (c) Macrogametocyte with outline almost entire. (d) Macrogametocyte with amoeboid outline. (e) Macrogametocyte (lower) and microgametocyte. (f) Two macrogametocytes in the same host erythrocyte. Scale bars = 10 μ m

Table 2: Morphometric parameters of gametocytes of *Haemoproteus skuae* sp. nov. from *Catharacta antarctica* (n = 25)

<i>Haemoproteus skuae</i>	Morphometric parameter	Mean	SD	Range
Macrogametocyte				
Uninfected erythrocyte	Length (µm)	13.4	0.7	12.2–14.8
	Width (µm)	7.4	0.4	6.6–8.4
	Area (µm ²)	78.5	6.9	66.6–93.4
Uninfected erythrocyte nucleus	Length (µm)	6.6	0.5	5.1–7.4
	Width (µm)	3.1	0.3	2.5–3.7
	Area (µm ²)	16.3	2.1	12.1–20.9
Infected erythrocyte macrogametocyte	Length (µm)	13.7	0.8	12.5–15.6
	Width (µm)	7.0	0.4	6.2–7.8
	Area (µm ²)	75.5	7.4	61.4–95.9
Infected erythrocyte nucleus	Length (µm)	5.6	0.8	3.5–7.0
	Width (µm)	2.9	0.4	2.4–3.7
	Area (µm ²)	12.9	2.4	9.2–18.4
Macrogametocyte	Length (µm)	21.1	3.5	15.6–27.5
	Width (µm)	2.1	0.3	1.7–2.8
	Area (µm ²)	46.4	8.5	35.6–66.2
Macrogametocyte nucleus	Length (µm)	2.4	0.3	1.8–3.0
	Width (µm)	1.8	0.3	1.3–2.7
	Area (µm ²)	3.4	0.7	2.7–6.4
Pigment granules		27.9	4.1	20–39
Nuclear displacement ratio		0.8	0.1	0.6–1.0
Microgametocyte				
Infected erythrocyte microgametocyte	Length (µm)	13.1	1.0	11.2–15.6
	Width (µm)	6.9	0.6	6.0–8.1
	Area (µm ²)	71.6	8.8	57.5–90.0
Infected erythrocyte nucleus	Length (µm)	5.7	0.3	5.1–6.4
	Width (µm)	2.9	0.4	2.4–4.3
	Area (µm ²)	13.3	2.2	11.0–19.3
Microgametocyte	Length (µm)	14.1	2.3	11.8–22.4
	Width (µm)	2.3	0.3	1.8–3.0
	Area (µm ²)	32.4	6.2	25.2–45.1
Microgametocyte nucleus	Length (µm)	5.1	0.7	4.1–6.6
	Width (µm)	2.0	0.4	1.5–3.0
	Area (µm ²)	10.8	3.0	5.5–18.9
Pigment granules		19.7	3.4	15–29
Nuclear displacement ratio		0.8	0.1	0.4–1.0

show about 9% atrophy. Ratio of macrogametocytes to microgametocytes is 60:40. Multiple invasion of host cells with microgametocytes not observed.

Hapantotype: IRCAH: G465379 from *C. antarctica* coll. Parsons, 18 September 2006, SANCCOB, Bloubergrant, South Africa; deposited in IRCAH Collection, Queensland Museum, Brisbane, Australia.

Other hosts: None known at present time but assumed to occur throughout the geographic range of the Stercorariidae.

Comments: A haemoproteid occurring in a family of birds from which parasites have not hitherto been recorded. The morphology of some gametocytes resembles that of *H. lauae* from the Laridae and this is discussed below.

Discussion

Most species of *Leucocytozoon* and *Haemoproteus* are host-family and sometimes subfamily specific (Peirce 2005), although there are some exceptions, most notably amongst a few closely related groups of small passerines. As there are only two morphological forms of *Leucocytozoon* recognised, round and elongate, many of the species described appear

similar. The advent of molecular techniques in recent years has been used as an adjunct to traditional morphological taxonomy, although sequencing of species has primarily concentrated on *Plasmodium*, with few sequences available for species of *Leucocytozoon* and *Haemoproteus*. Recent studies have suggested that rather than fewer species there may be complexes of more than one morphologically similar species present in a specific bird family that can only be separated molecularly (Sehgal et al. 2006). Thus, the justification for describing *L. ugwidii* as a new species is based on morphology and host-family specificity only. Also, the morphology is quite different from the only other species of *Leucocytozoon* seen in coastal birds at SANCCOB, namely *L. tawaki* in the African Penguin *Spheniscus demersus* (Earlé et al. 1992).

The low prevalence of *L. ugwidii* observed in the SANCCOB birds was not surprising, as the level of infected birds was similar to that seen with *L. tawaki* in African Penguins (NP and VS unpubl. data). Also, it could reasonably be assumed that some birds would have had latent or subclinical infections when first admitted and were blood-screened before any infection relapsed as most do, particularly when birds are

under stress (Peirce 2008). However, subsequent weekly monitoring of most birds did not highlight any such infections.

There are few records of haematozoa from species of Phalacrocoracidae (Bennett et al. 1982, Bishop and Bennett 1992). There is only one previous record for *Leucocytozoon* in *P. melanoleucos* in Australia, from which Mackerras and Mackerras (1960) reported the presence of *L. vandenbrandeni*, a parasite originally described from the Anhingidae (the authors misidentified the type host as a cormorant instead of a darter), but requiring review (Peirce 2005) and having a morphology very distinct from *L. ugwidii*. The parasite illustrated from *P. melanoleucos* (Mackerras and Mackerras 1960) is also morphologically distinct from *L. ugwidii*.

There are no records for any haematozoa having been found in members of the Stercorariidae (Bennett et al. 1982; Bishop and Bennett 1992). In the past, some ornithologists included skuas in the family Laridae, but the advent of DNA–DNA hybridization studies in recent years has clearly placed them in the family Stercorariidae (Furness 1996). However, birds in both families are closely related and occur in the suborder Lari. It may therefore be expected that any parasite in skuas would be a different species to that found in gulls for the reasons stated above. The only haemoproteid described from Laridae is *H. laeae*. This parasite does share some similarities with *H. skuae*. *Haemoproteus laeae* was redescribed by Peirce (1981) from the Lesser Black-backed Gull *Larus fuscus* in the United Kingdom, but the range of morphometric measurements provided were far fewer than the range subsequently established and required for species descriptions. No taxonomic status was ascribed to the material as both the host and geographic area were not compatible with the original description by Yakunin (1972). Whereas Peirce (1981) noted that a few gametocytes showed some degree of being amoeboid during development, this was not illustrated by Valkiunas (1997) who based his redescription on infection from the type host species, the Black-headed Gull *L. ribidundus*. Therefore, while there are some similarities in morphometric measurements between *H. laeae* (Valkiunas 1997) and those presented here for *H. skuae*, the former is classified as a circumnuclear haemoproteid with outline entire, whereas *H. skuae* is halteridial and amoeboid.

As noted in the description above, some gametocytes of *H. skuae* do have the outline entire, but a greater number are amoeboid. On this basis and on other criteria noted above, there is clear justification for establishing *H. skuae* as a distinct species. However, if and when more material from skuas and gulls is available, it would be interesting to compare molecular sequences of *H. skuae* and *H. laeae* to determine if they are closely related as the morphological evidence suggests.

Jones et al. (2002) surveyed 125 South Polar Skuas *C. maccormicki* in the Antarctic but did not find any parasites. The absence of haemoproteids in particular was not surprising as the vectors, particularly ceratopogonids, would almost certainly be absent. Hippoboscids were not found on any of the handled birds when examined. Therefore at the present time the vector for *H. skuae* remains unknown.

It is unlikely that either *L. ugwidii* or *H. skuae* have any clinical significance in their hosts under normal conditions,

as neither genus is usually pathogenic in non-domestic birds, although as concomitant infections with other disease agents they can affect the morbidity of the host (Peirce 2008).

Acknowledgements — We would like to thank the staff and volunteers of SANCCOB, especially N Fleming for looking at blood smears. SANCCOB is supported by a wide range of donors, particularly the International Fund for Animal Welfare (IFAW). NJP acknowledges support from the Norway South Africa Fisheries Agreement (NORSA), the National Research Foundation (SEACHANGE Programme), Earthwatch Institute and the University of Cape Town Research Committee.

References

- Bennett GF, Campbell AG. 1972. Avian Haemoproteidae. 1. Description of *Haemoproteus fallisi* n.sp. and a review of the haemoproteids of the family Turdidae. *Canadian Journal of Zoology* 50: 1269–1275.
- Bennett GF, Earlé RA, Peirce MA, Huchzermeyer FW, Squires-Parsons D. 1991. Avian Leucocytozoidae: the leucocytozoids of the Phasianidae *sensu lato*. *Journal of Natural History* 25: 1407–1428.
- Bennett GF, Whiteway M, Wodworth-Lynas C. 1982. A host-parasite catalogue of the avian haematozoa. *Memorial University of Newfoundland Occasional Papers in Biology* 5: 1–243.
- Bishop MA, Bennett GF. 1992. Host parasite catalogue of the avian haematozoa, supplement 1 and bibliography of the avian blood-inhabiting haematozoa, supplement 2. *Memorial University of Newfoundland Occasional Papers in Biology* 15: 1–244.
- Earlé RA, Bennett GF, Brossy JJ. 1992. First African record of *Leucocytozoon tawaki* (Apicomplexa: Leucocytozoidae) from the jackass penguin *Spheniscus demersus*. *South African Journal of Zoology* 27: 89–90.
- Furness RW. 1996. Family Stercorariidae (skuas). In: del Hoyo J, Elliott A, Sargatal J (eds), *Handbook of the birds of the world*, vol. 3. Barcelona: Lynx Edicions. pp 556–571.
- Jones HI, Gallagher JM, Miller GD. 2002. Survey of South Polar skuas (*Catharacta maccormicki*) for blood parasites in the Vestfold Hills region of Antarctica. *Journal of Wildlife Diseases* 38: 213–215.
- Mackerras MJ, Mackerras IM. 1960. The haematozoa of Australian birds. *Australian Journal of Zoology* 8: 226–260.
- Parsons NJ, Underhill LG. 2005. Oiled and injured African penguins *Spheniscus demersus* and other seabirds admitted for rehabilitation in the Western Cape, South Africa, 2001 and 2002. *African Journal of Marine Science* 27: 289–296.
- Peirce MA. 1981. Haematozoa of British birds. VI. Redescription of *Haemoproteus laeae* Yakunin from the lesser black-backed gull *Larus fuscus*. *Journal of Natural History* 15: 459–462.
- Peirce MA. 2005. A checklist of the valid avian species of *Babesia* (Apicomplexa: Piroplasmorida), *Haemoproteus*, *Leucocytozoon* (Apicomplexa: Haemosporida), and *Hepatozoon* (Apicomplexa: Haemogregarinidae). *Journal of Natural History* 39: 3621–3632.
- Peirce MA. 2008. Hemoparasites. In: Samour J (ed.), *Avian medicine* (2nd edn). London: Elsevier. pp 337–346.
- Sehgal RNM, Hull AC, Anderson NL, Valkiunas G, Markovets MJ, Kawamura S, Tell LA. 2006. Evidence for cryptic speciation of *Leucocytozoon* spp. (Haemosporida, Leucocytozoidae) in diurnal raptors. *Journal of Parasitology* 92: 375–379.
- Valkiunas G. 1997. Bird Haemosporida. *Acta Zoologica Lituanica* 3–5: 1–607 (in Russian).
- Yakunin MP. 1972. Blood parasites of wild birds of South-East Kazakhstan. *Trudy Instituta Zoologii Akademiiy Nauk Kazakhskoi SSR* 33: 69–79 (in Russian).