Blood parasites in noddies and boobies from Brazilian offshore islands – differences between species and influence of nesting habitat

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SUMMARY

Seabirds are often free from blood parasites, and a recent review suggested that phylogenetic, ecological and life-history parameters can determine the prevalence of blood parasites in seabirds. However, there is a lack of data available from many seabird groups, and a larger database is needed to understand prevalence patterns of blood parasites. We used a molecular screening approach to detect parasites of the genera *Plasmodium, Haemoproteus, Leucocytozoon* and *Babesia* in five species of two genera of seabirds that breed on Atlantic Ocean islands off Brazil. The observed patterns differed between the two bird genera. Like other Laridae, brown noddy, *Anous stolidus* adults were infected with *Haemoproteus* with low prevalence. Masked boobies, *Sula dactylatra* and brown boobies, *Sula leucogaster* were infected with *Babesia*. Of the latter, mainly juveniles were infected. In all species, intensity of infection (i.e. number of infected erythrocytes) was so low that parasites remained undetected in blood smears. This may explain the absence of major effects on the body condition of birds, although infected juvenile masked boobies were lighter than juveniles that were not infected with *Babesia*. Two tree-nesting species; black noddy, *Anous minutus* and red-footed booby, *Sula sula* did not have blood parasites, suggesting that tree-nesting may reduce the exposure to arthropod vectors compared with ground nesting in these species.

Key words: avian haematozoa, blood parasites, haemoparasites, innate immunity, seabirds.

INTRODUCTION

Birds are hosts to a number of intracellular blood parasites, including Haemosporidia of the genera *Plasmodium, Haemoproteus* and *Leucocytozoon*, Haemogregarinidae of the genus *Hepatozoon* and piroplasmids of the genus *Babesia*. These parasites may exert important ecological and evolutionary pressures on life-history traits of avian hosts (e.g. Merino *et al*. 2000; Hõrak *et al*. 2001; Marzal *et al*. 2005).

The prevalence of infection varies greatly among different bird taxa (e.g. Bennett *et al*. 1993; Valkiūnas 2005). For example, seabirds are often free from blood parasites (e.g. Peirce and Brooke, 1993; Merino *et al*. 1997*a*; Merino and Minguez, 1998; Engström *et al*. 2000). A recent review of blood parasite prevalence in birds suggested that multiple factors are responsible for patterns of association between parasitic infections and ecological and life history traits in seabirds (Quillfeldt *et al*. 2011). Indeed, life history and ecological parameters that increase the exposure time to arthropod vectors may be important. Furthermore, historical/phylogenetic factors may also influence susceptibility to different parasitic infections. For example, there is a relatively high prevalence of *Haemoproteus* in gulls and frigatebirds and *Plasmodium* in penguins, while *Hepatozoon* are apparently confined to albatross and storm-petrel species (Quillfeldt *et al*. 2011).

This study used genetic methods to detect parasites in five species belonging to two genera of seabirds that breed on tropical islands off Brazil. There have been no previous studies in this region that focus on protozoa in seabird blood (Quillfeldt *et al*. 2011). In particular, we were interested in inter- and intra-specific differences in prevalence patterns of blood parasites in these sympatrically breeding birds. Based on published data, we hypothesized that noddies would most likely be infected with *Haemoproteus* and boobies with *Babesia*.

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Table 1. Studies of blood parasite prevalence in noddies, including the present results. The results of PCR-based screening for *Haemoproteus* are given, and published studies of the same species are summarized (ad. = adults). In the present study, all individuals were negative in PCR-based screening for *Babesia* and *Leucocytozoon*

Species	Site	Date	Adults	Juveniles	*Haemoproteus*	Reference
Black noddy	São Pedro and São Paulo Archipelago (SPSPA)	August 2010	16	4	–	This study
Anous minutus	Atol das Rocas	August–September 2010	16	0	–	This study
	F. de Noronha	April 2011	0	4	–	This study
		July 2011	2	0	–	This study
	Total Black noddy	**(This study)**	**34**	**8**	–	This study
Brown noddy	Abrolhos	August 2011	15	16	–	This study
Anous stolidus	SPSPA	August 2010	23	9	7 ad. (30·4%)	This study
	Atol das Rocas	September 2010	35	15	1 ad. (2·9%)	This study
	F. de Noronha	March–April 2011	13	0	–	This study
		July 2011	1	14	–	This study
	Ilha da Trindade	February 2012	11	19	–	This study
	Total Brown noddy	**(This study)**	**98**	**73**	**8 ad. (8·2%)**	This study
	Aldabra Atoll, Indian Ocean		24	0	1 ad. (4·2%)	Lowery (1971)
	Oeno, Pitcairn Islands		1	0		Peirce and Brooke (1993)
	offshore islands of northern Mexico		2	0		Clark and Swinehart (1969)
	Bird I., Seychelles, W Indian Ocean		1	0		Peirce and Feare (1978)
	Tutuila, American Samoa, Pacific		1	0		Atkinson *et al.* (2006)
	Total Brown noddy	**(across studies)**	**126**	**73**	**9 ad. (7·1%)**	

METHODS

Study sites and species

Boobies and noddies are circumtropical species that breed yearly. They have mean body masses varying from 100–200 and 800–1500 g in noddies and boobies, respectively. These seabirds were sampled at five breeding colonies on offshore islands of the Atlantic coast of Brazil (for sample sizes, dates and locations see Tables 1 and 2):

1. Fernando de Noronha (3·854°S, 32·424°W): This site consists of one large island and 19 small adjacent islets, within a total area of 26 km^2. It contains the most diverse seabird community in Brazil, with 11 breeding seabird species and *c.* 25 000 breeding pairs (Antas, 1991; Schulz-Neto, 2004*a*). These islands provide different nesting habitats. For example, black noddies, *Anous minutus* and red-footed boobies, *Sula sula* nest in dense (vegetation cover 80%) forest of the Mulungu tree, *Erytrina velutina* (Schulz-Neto, 2004*a*), while brown noddies, *Anous stolidus* and brown boobies, *Sula leucogaster* nest on the rocky ground. Masked boobies, *Sula dactylatra* nest in medium (~ 0·5 m height) or tall grass (> 0·5 m height). Arthropods reported on these islands include ticks (Ixodidae – *Rhipicephalus microplus* and Astigmatina), flies and mosquitoes (Flechtmann, 1987). Black noddies on Fernando de Noronha also nest in small platforms on the ground of vertical, well-protected cliffs.
2. Abrolhos archipelago (17·926°S, 38·935°W) is a group of five small islands located ~65 km off the Brazilian coast. Six seabird species breed at this site, with *c.* 3000 breeding pairs (Alves *et al.* 2000). The percentage of vegetation cover varies between 0 and 40%. Rock, which is sparsely covered with short grass ~0·2 m high (*Alternanthera maritima, Cyperus imbricatus, Blutaparon portulacoides*; Hazin and Macedo, 2006) provides nesting habitat for brown noddies, while masked and brown boobies predominantly nest in sites containing tall grass (*C. imbricatus, Borreria verticilata*; IBAMA/FUNATURA, 1991) as well as short grass. Ticks, mosquitoes and flies (*Olfersia spinifera*; Graciolli and Carvalho, 2003) have been reported on these islands.
3. Ilha da Trindade (20·517°S, 29·300°W) is located at the far east of the Vitória-Trindade submarine ridge, 1160 km off Brazil. The island has five breeding species, including the brown noddy that nests in areas of large stones, pebbles, bare rock and medium grass (predominantly *Cyperus atlanticus* and *Bulbostylis nesiotis*, vegetation cover 40%).

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Table 2. Studies of blood parasites in boobies, including the present results. The results of PCR-based screening for *Babesia* are given, and published studies of the same species are summarized (ad. = adults, juv. = juveniles). In the present study, all individuals were negative in PCR-based screening for *Plasmodium*/*Haemoproteus* and *Leucocytozoon*

Species	Site	Date	Adults	Juveniles	*Babesia*	Reference
Red-footed booby	Atol das Rocas	September 2010	9	16	–	This study
Sula sula	F. de Noronha	July 2011	1	0	–	This study
	Total Red-footed booby	**(This study)**	**10**	**16**	–	This study
	Oahu, Hawaii		35	34	–	Work (1996)
	Aldabra Atoll		28		–	Lowery (1971)
	Genovesa, Galápagos[a]		23		–	Padilla *et al*. (2006)
	Oeno, Pitcairn Islands			15	–	Peirce and Brooke (1993)
	Christmas Island, Indian Ocean[b]		12		–	Quillfeldt *et al*. (2010)
	Total Red-footed booby	**(Across studies)**	**108**	**65**	–	
Masked booby	Abrolhos	February 2011	30	0	2 ad. (6·7%)	This study
Sula dactylatra		August 2011	5	17	3 juv. (17·6%)	This study
	Atol das Rocas	September 2010	31	19	9 juv. (47·4%)	This study
	F. de Noronha	March 2011	31	0	–	This study
		July 2011	17	18	2 ad. (11·8%) 6 juv. (33·3%)	This study
	Total Masked booby	**(This study)**	**114**	**54**	**4 ad. (3·5%)** **18 juv. (33·3%)**	This study
	Desnoeufs, Amirantes, Indian Ocean			9	2 juv. (22·2%)	Peirce and Feare (1978)
	Total Masked booby	**(Across studies)**	**114**	**63**	**4 ad. (3·5%)** **20 juv. (31·7%)**	
Brown booby	Abrolhos	February 2011	30	0	–	This study
Sula leucogaster		August 2011	10	13	1 juv. (7·7%)	This study
	SPSPA	August 2010	31	14	1 juv. (7·1%)	This study
	Atol das Rocas	September 2010	15	15	10 juv. (66·7%)	This study
	F. de Noronha	March 2011	35	7	–	This study
		July 2011	0	2	–	This study
	Total Brown booby	**(This study)**	**121**	**51**	**12 juv. (23·5%)**	This study
	Johnston Atoll		70	35	54% in chicks, 13% in adults	Work and Rameyer (1997)
	Christmas Island, Indian Ocean[b]		12		–	Quillfeldt *et al*. (2010)
	Offshore islands of northern Mexico		1		–	Clark and Swinehart (1969)
	Total Brown booby	**(Across studies)**				

[a] 9% of the Red-footed boobies on Genovesa were infected with *Haemoproteus*.

[b] Samples analysed by Quillfeldt *et al*. (2010) for *Plasmodium*/*Haemoproteus* and *Leucocytozoon* were subsequently also screened for *Babesia* using the methods outlined in the present paper (Javier Martínez, unpubl. data).

Table 3. PCR settings and primer pairs used to perform the molecular screening in the present study (PaluF/PaluR, Leunew1F/LDRd, and Bab600F/IsospR). All amplicons achieved were sequenced. The primers Palu F/R can detect both *Plasmodium* and *Haemoproteus*. In order to achieve a larger amplicon to perform a reliable phylogenetic inference, one *Babesia* and one *Haemoproteus* isolate were amplified by using the sets of primers NBA1Bab/Hep1615R and L14902/H15725

Primers	Sequence 5′ → 3′	Size bp	Annealing	Extension	Parasites (gene)
Palu-F(Martinez *et al.* 2009)	GGGTCAAATGAGTTTCTGG	390	54 °C/30 s	72 °C/30 s	*Plasmodium/Haemoproteus* (cytB)
Palu-R(Martinez *et al.* 2009)	DGGAACAATATGTARAGGAGT				
Leunew1F[a]	GGWCAAATGAGTTTCTGGG	340	56 °C/30 sec	72 °C/30 s	*Leucocytozoon* (cytB)
LDRd(Merino *et al.* 2008)	CTGGATGWGATAATGGWGCA				
Bab600F[a]	TCGTAGTTGAACTTCTGCTG	797	58 °C/30 s	72 °C/60 s	*Babesia* (18S rDNA)
IospR[a]	ATTGCCTCAAACTTCCTTGC				
NBA1Bab[a]	GGATAACCGTGCTAATTGT	1484	58 °C/30 s	72 °C/90 s	*Babesia* (18S rDNA)
Hep1615R(Merino *et al.* 2006)	AAAGGGCAGGGACGTAATC				
L14902 (Ricklefs and Fallon, 2002)	TTATTAGCCACTTGTTATACTCC	869	48 °C/30 s	72 °C/60 s	*Haemoproteus* (cytB)
H15725(Ricklefs and Fallon, 2002)	CATCCAATCCATAATAAAGCAT				

[a] Primers designed in this study.

Flies, *O. spinifera* have been recorded (Graciolli and Carvalho, 2003), however there are no records of ticks or haematophagous mosquitoes.

4. Atol das Rocas (3·856°S, 33·817°W) is located 145 km west of Fernando de Noronha (Kikuchi and Leão, 1997) and has the largest seabird colony with *c.* 150 000 individuals (Schulz-Neto, 1998), including five seabird species which breed on both islands (Schulz-Neto, 2004*b*). However, although black noddies and red-footed boobies breed on Fernando de Noronha they also rest and forage on Atol das Rocas (Schulz-Neto, 2004*b*). These islands are mostly sandy with bushes. Brown noddies, masked and brown boobies nest on the ground, in areas with 80–90% of short grass vegetation cover, *Portulaca oleracea*, as well as medium grass, *Cyperus ligularis* (Schulz-Neto, 2004*b*). Black noddies breed in coconut palm trees (*Cocos nucifera*), while red-footed boobies rest in coconut palm trees.
5. The São Pedro and São Paulo Archipelago (SPSPA) (0·917°N, 29·335°W) is a remote group of 10 small rocky islands located ∼1000 km from the Brazilian coast, and 610 km from Fernando de Noronha. It contains the smallest colony of seabirds, with three breeding species of approximately 1000 individuals (Both and Freitas, 2004). There is no vegetation on SPSPA, so birds are crowded in a small area of rocky outcrops.

Sample collection and PCR screening

Chicks and adults were caught either by hand or by using dip nets. Each bird was weighed to the nearest 5 or 10 g using a Pesola® spring balance. Standard structural measurements were recorded as follows: culmen and tarsus lengths to the nearest 0·1 mm using callipers, wing and tail lengths to the nearest mm using stopped wing and feather rulers, respectively.

All adults were sampled during the breeding season, while on their nest incubating eggs or attending young, except for black noddies and red-footed boobies, which were sampled at Atol das Rocas, even though they breed on Fernando de Noronha (Schulz-Neto, 2004*a*, *b*). Each bird was individually marked with a numbered metal ring to avoid sampling the same bird multiple times. For each bird, a drop of blood was obtained from the tarsal or brachial vein and stored on FTA classic cards (Whatman International Ltd., UK). Small pieces of FTA card were cut with sterilized scissors. Genomic DNA was extracted from FTA cards as indicated by Martínez *et al.* (2009). Polymerase chain reactions (PCR) were used to detect haemoparasites. Information about primers can be found in Table 3.

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PCR were performed in a 10 μL reaction volume, containing 20–100 ng template DNA, 50 mM KCl, 10 mM Tris–HCl, 1·5 $MgCl_2$, 0·2 mM dNTPs, 0·25 μM primer, and 1·25 U of AmpliTaq Gold 360 (Applied Biosystems, Foster City, CA, USA). PCR using the Veriti thermal cycler (Applied Biosystems, Foster City, CA, USA) conditions are as follows: 95 °C for 10 min (polymerase activation), 40 cycles at 95 °C for 30 s, annealing temperature (see Table 2), extension temperature (see Table 3), and a final extension at 72 °C for 10 min. PCR assays were checked using agarose gel electrophoresis. Amplicons were recovered from agarose gels (UltraClean GelSpin DNA Purification kit, *MO BIO*) and subjected to direct sequencing using an ABI 3130 (Applied Biosystems) automated sequencer. Positive and negative controls were routinely used.

Phylogenetic analysis

DNA sequences for *Haemoproteus* (cytochrome B) obtained from noddies were aligned with 77 other sequences belonging to *Haemoproteus* or *Parahaemoproteus* species that can be found on GenBank. The alignment was performed using the CLUSTALW algorithm implemented in BIOEDIT (Hall, 1999). The final alignment contained 558 positions and 80 sequences, including two lineages of *Leucocytozoon* as the outgroup. The alignments were analysed using Bayesian inference, implemented in MrBayes 3.2 (Ronquist and Huelsenbeck, 2003), setting the substitution model GTR + G. The model was previously selected using corrected AIC (Akaike Information Criterion) implemented in JMODELTEST 0.0.1 (Posada, 2008). This analysis consisted of 2 runs of 4 chains each, with 3 000 000 generations per run and a burn-in of 300 000 generations (54 000 trees for consensus tree). The final standard deviation of the split frequencies was lower than 0·01. Convergence was checked using TRACER v1.5 software (a program for analysing the trace files generated by Bayesian MCMC runs; Rambaut and Drummond, 2007). All model parameters were higher than 100 indicating convergence.

The *Babesia* DNA sequence (18S rDNA) obtained from boobies was aligned together with 20 other sequences belonging to *Babesia* species that were listed in GenBank. The alignment was performed using PROBCONS (http://toolkit.tuebingen.mpg.de/probcons). Poorly aligned positions and divergent regions of the alignment were suppressed using GBlocks (Talavera and Castresana, 2007) selecting the following options: 'Minimum Number of Sequences for a Conserved Position' to 11, 'Minimum Number of Sequences for a Flank Position' to 17, 'Maximum Number of Contiguous Nonconserved Positions' to 3, 'Minimum Length of a Block' to 10, and 'Allowed Gap Positions' to 'With Half'. The final alignment contained 1404 positions. In this case, the substitution model GTR + I + G was selected to perform the Bayesian analysis. Only 1 000 000 generations were necessary to obtain convergence. The tree was rooted on the midpoint.

In addition, the maximum-likelihood inference was also performed using PhyML (Guindon *et al*. 2010). This analysis was performed with the two alignments. The substitution models were those indicated above, the subtree pruning and regrafting and the nearest-neighbour interchange tree-rearrangements were selected, and the approximate likelihood-ratio test was used to obtain the clade support.

Microscopic analyses

Studies using blood smears may not detect parasites if the intensity of infection is low (e.g. Valkiūnas, 2005). Genetic methods using PCR can detect infections missed by blood smears (e.g. Feldman and Freed 1995; Bensch *et al*. 2000; Perkins and Schall, 2002; Ricklefs *et al*. 2005; Parker *et al*. 2006; Merino *et al*. 2008). PCR has also been shown to be more sensitive than microscopic-based diagnosis of *Babesia* spp. (Almeria *et al*. 2001; Ano *et al*. 2001). However, for identification of the parasite species, molecular methods should be combined with light microscopy (Valkiūnas *et al*. 2008).

A drop of blood from each bird was immediately smeared and air-dried, fixed with methanol and later stained with Giemsa stain (1/10 v/v) for 30 min. Blood smears from birds that were positive for blood parasite infections by PCR were scanned using an optic microscope following methods described by Merino *et al*. (1997*b*). In brief, one-half of every blood smear was scanned at ×200 to look for extracellular parasites. Intracellular stages of haematozoa were sought at ×400 in the other half of the sample. The oil immersion objective was used when a possible parasite was sought at ×400. All samples were scanned using a microscope Olympus B061.

Statistical data analysis

Statistical analyses were carried out in SigmaStat 3.5. and SPSS 11.0. Prevalence was given with 95% confidence intervals (95% CI). In order to compare the body condition of birds infected or free from parasites, and among birds of archipelagos with different rates of infection, we calculated mass residuals, i.e. the difference between observed mass and predicted mass. Predicted masses were calculated using a linear regression of body mass on four measures of structural size (e.g. Dehnhard *et al*. 2011). To account for temporal changes in body mass, only birds sampled in August–September were

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included in these analyses. Predicted masses were calculated for brown noddy adults according to the regression equation: $M_{mean} = -330{\cdot}9 + 1{\cdot}4$*Culmen + 3·2*Tarsus + 1·2*Wing + 0·3*Tail ($R = 0{\cdot}597$, $F = 9{\cdot}3$, $P < 0{\cdot}001$). The calculated mass residuals were compared among birds of the different archipelagos using analysis of variance (ANOVA). Predicted masses for juvenile masked boobies were calculated according to the regression equation: $M_{mean} = -2278{\cdot}2 + 17{\cdot}1$* Culmen + 27·8*Tarsus − 0·9*Wing + 5·3*Tail ($R = 0{\cdot}612$, $F = 7{\cdot}3$, $P < 0{\cdot}001$). The calculated mass residuals were compared among individuals with and without parasites using *t*-tests.

RESULTS

Noddies

We did not detect any parasites in black noddies ($N = 34$ adults and 8 juveniles, Table 1). In brown noddies, 8 of 98 adult birds (8·2 ± 5·4%) were infected with *Haemoproteus* (GenBank accession number KC754967), while none of the juveniles ($N = 73$) were infected. *Haemoproteus* DNA was detected in adult brown noddies from two of five sampling sites (Table 1), namely a high prevalence on SPSPA (30·4 ± 18·8%, N = 23) and a single bird at Atol das Rocas (2·9 ± 5·5%, $N = 35$). The proportions of infected adult brown noddies varied among colonies (Chi-square test: $\chi^2 = 20{\cdot}1$, D.F. = 4, $P < 0{\cdot}001$). The mass residuals of adult brown noddies did not differ among archipelagos with different occurrence of *Haemoproteus* (ANOVA: $F_{2,69} = 1{\cdot}4$, $P = 0{\cdot}248$). We did not detect parasites from blood smears, indicating a low intensity of infection.

The phylogenetic analysis showed that the haplotype 288 isolated from noddies clustered with three *Haemoproteus* haplotypes isolated from other seabirds (Fig. 1), of which *Haemoproteus jenniae* isolated from swallow-tailed gulls (*Creagrus furcatus*) was the closest species (99·5%). The genetic distance between haplotype 288 and *Haemoproteus iwa* isolated from magnificent frigatebird *Fregata magnificens* was 1·3%.

Boobies

We did not detect any parasites in red-footed boobies ($N = 10$ adults and 16 juveniles, Fig. 2, Table 2). Brown boobies and masked boobies were infected with *Babesia* (GenBank accession number KC754965).

In brown boobies, only juveniles were infected (23·5 ± 11·6%, $N = 51$), while all adults ($N = 121$) were free from blood parasites. *Babesia* DNA was detected in brown booby juveniles from three of four breeding sites (Table 2), with low prevalence on Abrolhos (7·7 ± 14·5%, $N = 13$) and SPSPA (7·1 ± 13·5%. $N = 14$), but high prevalence in the largest colony, on Atol das Rocas (66·7 ± 23·9%, $N = 15$). The proportions of infected brown booby juveniles varied among colonies ($\chi^2 = 22{\cdot}2$, D.F. = 3, $P < 0{\cdot}001$). However, brown booby juveniles from archipelagos with different occurrence of *Babesia* did not differ in their mass residuals (ANOVA: $F_{2,36} = 0{\cdot}2$, $P = 0{\cdot}830$). Likewise, the mass residuals were similar between brown booby juveniles whether infected or free of *Babesia* (*t*-test: $t = 0{\cdot}2$, D.F. = 37, $P = 0{\cdot}824$).

In masked boobies, the prevalence was nearly ten times higher in juveniles (33·3 ± 12·6%, $N = 54$) than in adults (3·5 ± 3·4%, $N = 114$). The difference in prevalence between adults and juveniles was statistically significant ($\chi^2 = 26{\cdot}1$, D.F. = 1, $P < 0{\cdot}001$). Adults were infected in two of three colonies, with low prevalences of 5·7 ± 7·7% at Abrolhos ($N = 35$) and 4·2 ± 5·7% at Fernando de Noronha ($N = 48$). Juveniles were infected at all three breeding sites, with intermediate to high prevalence of 17·6 ± 18·1% (Abrolhos, $N = 17$), 33·3 ± 21·8% (Fernando de Noronha, $N = 18$) and 47·4 ± 22·5% (Atol das Rocas, $N = 19$). The proportions of infected masked boobies did not vary significantly among colonies for either adults ($\chi^2 = 1{\cdot}7$, D.F. = 2, $P = 0{\cdot}429$, power 0·18) or juveniles ($\chi^2 = 3{\cdot}6$, D.F. = 2, $P = 0{\cdot}168$, power 0·36).

We did not detect parasites from blood smears, indicating the low intensity of infection. However, masked booby juveniles infected with *Babesia* were on average 74·1 ± 44·6 g lighter than the population mean, while uninfected masked booby juveniles were on average 37·0 ± 28·6 g heavier than the population mean. This difference of *c.* 7% of body mass was statistically significant (*t*-test: $t = 2{\cdot}2$, D.F. = 52, $P = 0{\cdot}034$).

Phylogenetic analysis showed that the haplotype 211 isolated from boobies clustered with two *Babesia* haplotypes isolates from seabirds, *Babesia poelea* from brown booby and *Babesia* sp. from common murre *Uria aalge* (Fig. 3). The genetic distance between these *Babesia* species and the haplotype 211 was 0·3 and 0·8%, respectively. However, other *Babesia* species (*Babesia kiwiensis* and *Babesia bennetti*) isolated from birds were phylogenetically distant from this clade.

DISCUSSION

In accordance with other seabird studies (reviewed by Quillfeldt *et al.* 2011), parasite prevalences were low in five species of tropical Brazilian seabirds from Atlantic Ocean islands. Two species were free from parasites, and the other three species had low blood parasite prevalences with parasites detected being the typically found parasite taxa for each genus, with no multiple infections. The absence of infections in black noddies and red-footed boobies might be explained by their different nesting

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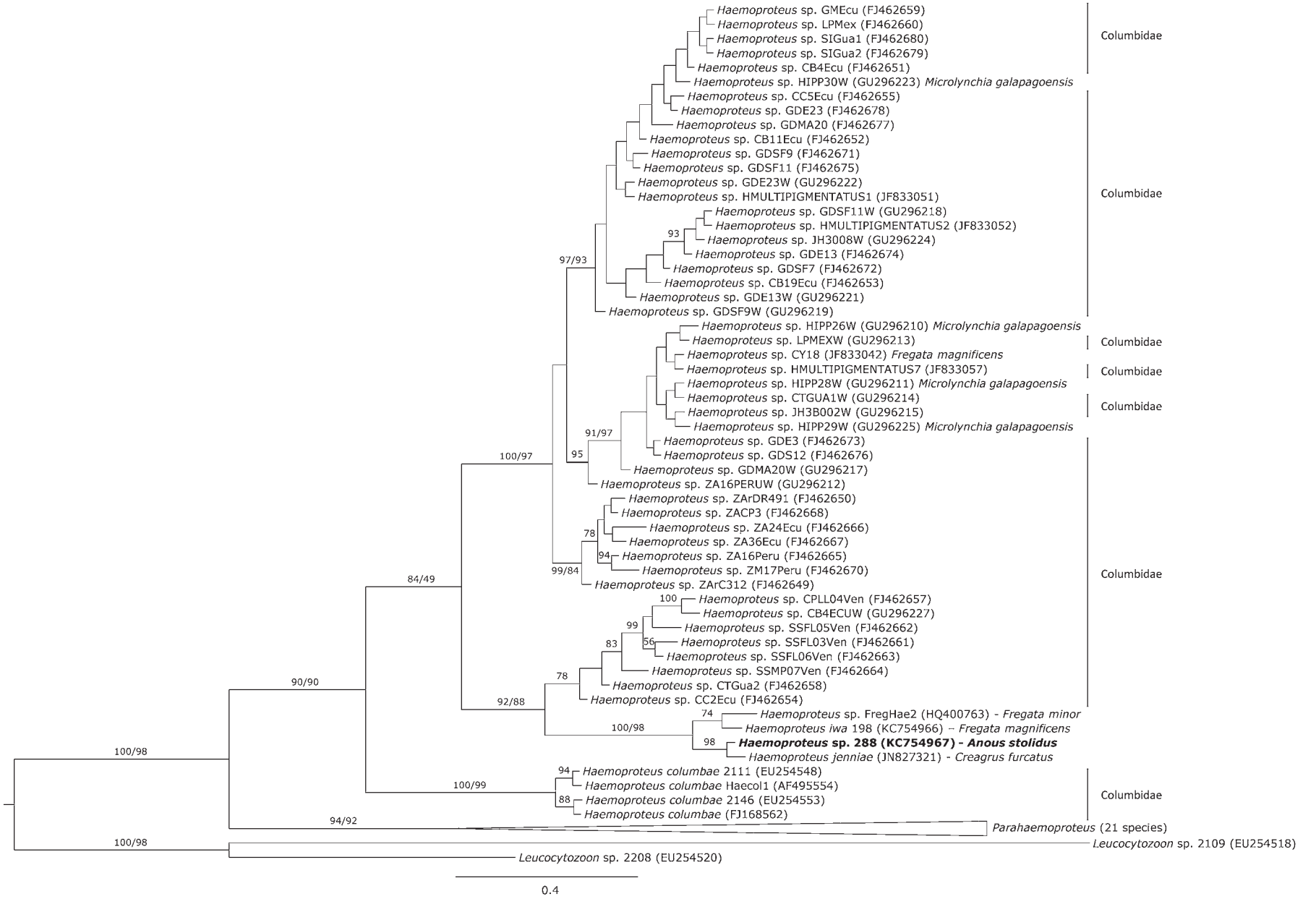

Fig. 1. Phylogenetic inference of the *Haemoproteus* haplotype found in brown noddies *Anous stolidus*. Phylogenetic tree was obtained with the program MrBayes v3.2 using the substitution model GTR + G. When clades present two support values, the first one corresponds with the Bayesian support and the second one with that achieved by maximum likelihood inference. The *Haemoproteus* haplotype isolated in the present study is marked in bold.

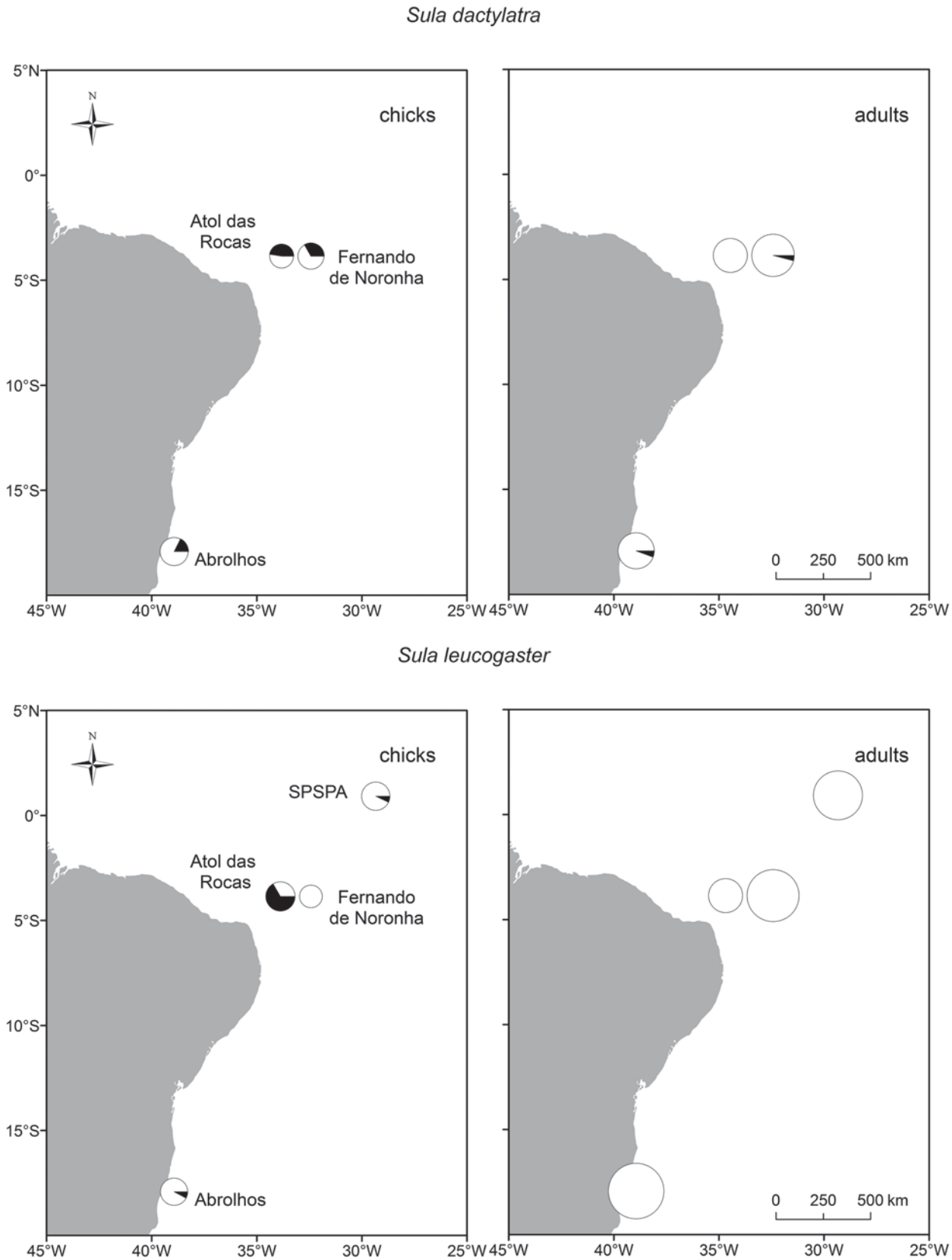

Fig. 2. Infection of brown and masked boobies with *Babesia poelea* on Brazilian Atlantic islands. The size of the symbols represents the sample size, and the portion of black in the circle represents the percentage of infected animals.

habitats. In contrast to ground-nesting species, these two species nest on trees or bushes, where louse flies (Hippoboscidae), common vectors for *Haemoproteus*, and ticks (Ixodidae), common vectors for *Babesia* (e.g. Smith, 1996), may be less likely to occur.

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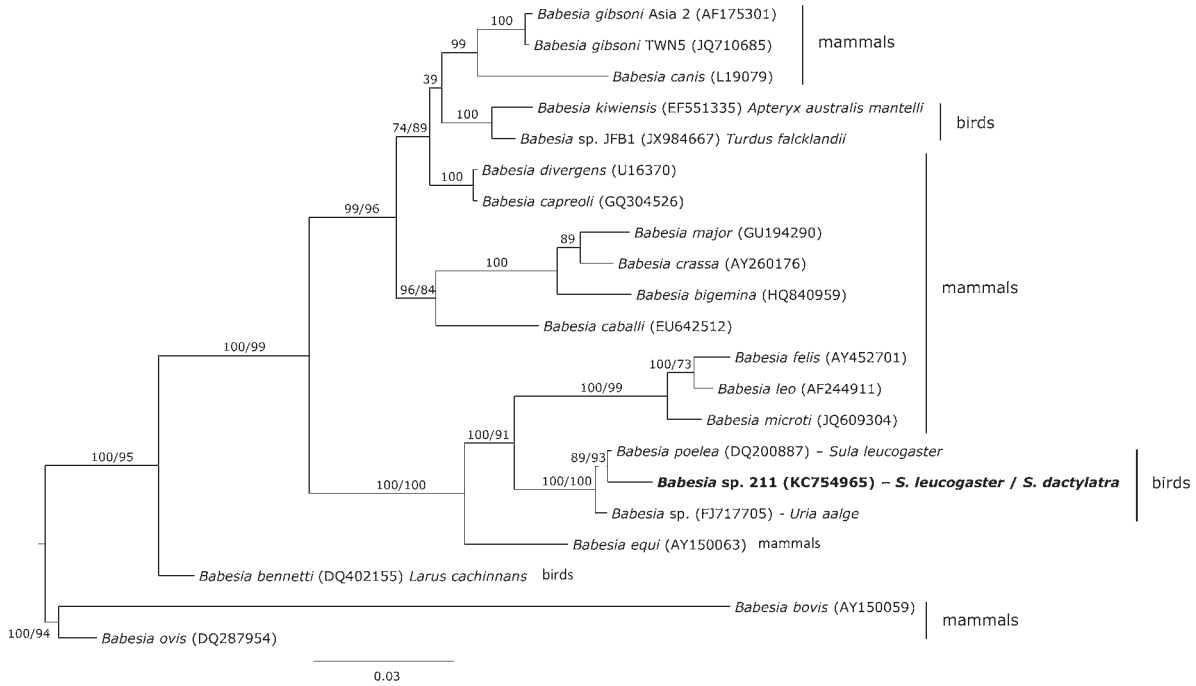

Fig. 3. Phylogenetic inference of the *Babesia* haplotype found in brown and masked boobies. Phylogenetic tree was obtained with the program MrBayes v3.2 using the substitution model GTR + I + G. When clades present two support values, the first one corresponds with the Bayesian support and the second one with that achieved by maximum likelihood inference. The *Babesia* haplotype isolated in the present study is marked in bold.

Noddies as hosts of Haemoproteus

Haemoproteus sp. are globally distributed in birds with about 100 named species (Peirce, 2005). Within seabirds, *Haemoproteus* parasites are especially common in frigatebirds, Fregatidae (Quillfeldt *et al*. 2011; Merino *et al*. 2012) and gulls and terns, Laridae (Quillfeldt *et al*. 2011).

Thus, our present finding of a *Haemoproteus* infection in a noddy, belonging to, or with close affinity to the Laridae, supports this pattern. The only previous record of a *Haemoproteus* infection in a noddy was detected on Aldabra Atoll in the Indian Ocean, where one of 24 adult brown noddies was infected by an unknown species of *Haemoproteus* (Lowery, 1971). The phylogenetic analysis showed that the *Haemoproteus* haplotype isolated from noddies on Brazilian islands are close to others isolated from seabirds including gulls. The lack of haematic stages in the blood smears indicated that the infection was chronic and not acute (e.g. Madsen *et al*. 2007). The fact that only adult individuals were found parasitized further suggested that risk of infection is related to their permanence in areas inhabited by suitable vectors.

In general, *Haemoproteus* parasites are considered relatively benign in birds. Two seabird species affected by *Haemoproteus* have been studied in detail, through correlative analyses. In magnificent frigatebirds, 16% of males were infected with *H. iwa*, but all infections were light (< 1% of erythrocytes) and were thus classified as chronic (Madsen *et al*. 2007). In yellow-legged gulls, *Larus cachinnans*, Martínez-Abraín *et al*. (2002) found significant differences in *Haemoproteus lari* prevalence between two breeding colonies, which were explained with differences in the vector abundance. The birds of both colonies were in equally good body condition and had similar clutch sizes. Further, the intensity of *H. lari* infection was not correlated with body condition or egg volume (Martínez-Abraín *et al*. 2002), suggesting that *H. lari* parasites had little effect on the health of the gulls under normal conditions.

Likewise, *Haemoproteus* infection in the present study was not correlated to the body condition of noddies. Studies in other birds also suggest that *Haemoproteus* numbers are normally kept low by natural immunity and only tend to multiply under stress and other diseases. It has therefore been suggested that increasing *Haemoproteus* parasitaemia (percentage of red cells infected) can serve as a valuable indicator of an underlying disease (e.g. Remple, 2004) or of stress (Valkiūnas, 2005). However, experimental studies suggest important detrimental effects of *Haemoproteus* blood parasites on bird fitness (Merino *et al*. 2000; Marzal *et al*. 2005). In magnificent frigatebirds, males infected with *H. iwa* had a less intensely coloured red inflatable gular pouch (Madsen *et al*. 2007), which is an important ornament used in mate choice (Dearborn *et al*. 2001). This suggests that even light infections can influence the reproductive success

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of individuals and thus, be subject to intense selection.

Boobies as hosts of B. poelea

Babesia spp. are tick-transmitted protozoan haemoparasites that infect mammals and birds. Currently, over 100 *Babesia* species are known, and together with *Theileria* spp. they are referred to as piroplasmids or piroplasms (Piroplasmida). Of 14–18 *Babesia* species recognized in birds (Jefferies *et al*. 2008; Votýpka, 2011; Peirce and Parsons, 2012), five infect different seabird groups: *B. poelea* (boobies), *Babesia peircei* (2 penguin species), *B. bennetti* (1 gull species), *Babesia uriae* (1 auk species) and *Babesia ugwidiensis* (5 cormorant species). While the majority of species infecting domestic mammals cause disease, only two avian species, *Babesia shortti* and *B. uriae*, are known to be pathogenic (Samour and Peirce, 1996; Yabsley *et al*. 2009; Votýpka, 2011).

Babesia had been found previously in two studies in boobies (Table 2). The first record applied to two of nine nestling masked boobies (22%) that were infected at Desnoeufs, Amirantes, Western Indian Ocean (Peirce and Feare, 1978), and *B. poelea* that was then described in Brown Boobies at Johnston Atoll (Work and Rameyer, 1997). There, *B. poelea* was found in blood smears from 54% of the chicks, and 13% of the adults. While the prevalence was high, mean parasitaemia in adults and chicks was less than 1% (Work and Rameyer, 1997), similar to our findings. *Babesia* infection in the present study was not correlated to the body condition of brown booby juveniles, but masked booby juveniles with a *Babesia* infection were lighter than those not infected, indicating a slight effect on the health of these birds.

It has been established that once birds become infected with haemosporidian parasites, they remain infected either for life or many years (Garnham, 1966; Valkiūnas, 2005), with infections tending to be dynamic, with relapses occurring. In contrast, juveniles in our study were more susceptible to *Babesia* infections than adults, and infections apparently decrease in intensity or disappear with increasing age and acquisition of immunity. A higher prevalence of infection in chicks also had been noted in previous studies in boobies (Work and Rameyer, 1997). Similarly, 20% of juvenile prairie falcons *Falco mexicanus* were infected with *Babesia moshkovskii* (Croft and Kingston, 1975) and *Babesia* infections in birds are commonly reported from undernourished young individuals (see Merino, 1998; Peirce, 2000; Merino *et al*. 2002). Boobies and other birds would therefore make interesting models to study mechanisms of condition- or age-related acquired immunity.

Babesia poelea was originally described as an endemic avian haemoparasite in seabirds from the central Pacific (Work and Rameyer, 1997). The *Babesia* haplotype isolated in the present study was closely related with *B. poelea*, the genetic distance between them being 0·3%. Furthermore, the lack of haematic stages in the smears does not make it possible to identify the species. Peirce (2000) suggested that *B. peircei* could be a synonym of *B. poelea* and a molecular study is currently being conducted to solve this problem (see Peirce and Parsons, 2012). The result of the latter study could also help to identify the *Babesia* species reported here. However, the most relevant issue achieved from the phylogenetic analysis was the lack of monophyly for avian *Babesia*, as previously reported by Yabsley *et al*. (2009).

The *Babesia* life cycle typically consists of a sexual phase that takes place in Ixodid ticks, and asexual reproduction inside erythrocytes of vertebrate hosts (Schnittger *et al*. 2012 and references therein). Given the abundance of Ixodid ticks in many seabird colonies, the scarcity of piroplasms is surprising. However, the avian piroplasms remain an understudied group of protozoans (Jefferies *et al*. 2008), and targeted studies are expected to uncover more avian piroplasms in the future and also to resolve the current taxonomic confusion. There is also debate whether *Babesia* species are highly adapted to specific vertebrate hosts. Most species of *Babesia* infecting mammals or birds are host-specific at least to the family level (Votýpka, 2011). However, some recent studies suggest relatively low host specificity and host switching, such as occasional human infections by *Babesia microti, B. divergens, B. duncani* (see also Criado *et al*. 2006; Yabsley and Shock, 2013). In the present study, sympatrically breeding seabirds such as noddies were not infected with *Babesia*, while boobies were infected with *B. poelea* in far separated sites in the Pacific as well as the Atlantic, thus suggesting high host specificity of this piroplasm.

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