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# Records of *Ixodes percavatus* sensu lato on Atlantic yellow-nosed albatrosses (*Thalassarche chlororhynchos*) on the Brazilian coast and offshore waters

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The *Ixodes percavatus* group is a complex of at least three valid species of ticks, currently classified as *Ixodes percavatus* sensu stricto (s.s.) Neumann, 1906, *Ixodes kerguelenensis* André & Colas-Belcour, 1942, and *Ixodes cornuae* Arthur, 1960 (Arthur 1960). Two taxa previously assigned to this group, *Ixodes zumpti* Arthur, 1960 and *Ixodes pterodromae* Arthur, 1960, have been synonymized with *I. kerguelenensis* (Wilson 1970). Morphologically, members of the *I. percavatus* group are unique in presenting the leg trochanters with spurs, and the palpal article I of the capitulum with an anteriorly directed spur and a mesodorsal spur (Arthur 1960).

The geographical distribution of *I. percavatus* s.s. has been restricted to Tristan da Cunha Archipelago (Arthur 1965), a group of four islands (Tristan da Cunha, Nightingale, Inaccessible and Gough) and several islets lying in the central South Atlantic Ocean, 2800 km distant from South Africa and 3200 km from South America (Hänel & Heyne 2008). In Tristan da Cunha Archipelago, females, nymphs and larvae of *I. percavatus* s.s. have been recorded feeding mostly on the Atlantic yellow-nosed albatross, *Thalassarche chlororhynchos* (Gmelin 1789) (Anastos 1954; Hänel & Heyne 2008). While there are earlier records of *I. percavatus* on several seabird species from the Kerguelen Islands in the Indian Ocean (Paulian 1953), some of these specimens were re-examined and confirmed as *I. kerguelenensis* (Wilson 1970). The geographical distribution of *I. kerguelenensis* is much broader, with records from several islands in the Indian, Pacific and Atlantic Oceans, always associated with seabirds (Roberts 1960; Wilson 1970). It has been reported in the Tristan da Cunha Archipelago (as either *I. zumpti* or *I. pterodromae*) in association with *T. chlororhynchos* and other seabird species (Arthur 1960; Hänel & Heyne 2008). On the other hand, the species *I. cornuae* is known only from two records, one on a Phasianidae bird in Ecuador, and another from an undetermined host in Chile (Arthur 1960). Herein, we report for the first time the parasitism of ticks of the *I. percavatus* group on *T. chlororhynchos* sampled on the Brazilian coast and offshore waters.

On 17 May 2018 and 07 May 2019, two adult specimens of *T. chlororhynchos* were received in a rehabilitation center at the coast of the Espírito Santo state, southeastern Brazil, ~20°S (Vanstreels *et al.* 2019). The first bird was rescued offshore (state of Rio de Janeiro) and the second on the beach of Linhares municipality, state of Espírito Santo. During clinical examinations, six ticks were found

attached to the body of the first bird and 17 ticks on the second bird. These ticks were collected and immediately preserved in a glass vial containing 70% ethanol. From 2016 to 2019, another five live and seven dead *T. chlororhynchos* were received at the same rehabilitation facility, but no ticks were found during clinical examination.

On September 2019, during a study of *T. chlororhynchos* off the Brazilian coast, approximately 50 km south of Rio de Janeiro city, several specimens were captured using a cast net after being attracted with fish offal (Bugoni *et al*. 2008a). A total of 13 birds [7 adults, 2 sub-adults (3rd year), 4 juveniles (1–2 years-old), aged based on plumage and bill color (Bugoni & Furness 2009)] were captured and examined for ticks, which were found on three birds (30%). From these three birds, a total of 14 ticks were collected and placed in vials containing 70% ethanol (Table 1).

**TABLE 1.** Records of *Ixodes percavatus* sensu lato from the host *Thalassarche chlororhynchos* on coastal waters of Brazil.

| Host | Date | Coordinates | Host age | Collected ticks | Remarks |
|---|---|---|---|---|---|
| A | 17 May 2018 | 21°51'50"S 41°01'05"W | Juvenile (1–2 yrs. old) | 3 larvae, 3 nymphs | Live bird found on a tugboat upon docking at Port of Açu, presumably having landed on the vessel while it was operational in the coastal waters of Rio de Janeiro state. |
| B | 07 May 2019 | 19°39'28"S 39°50'23"W | Juvenile (1–2 yrs. old) | 8 larvae, 9 nymphs | Dead bird found ashore. The carcass was relatively fresh [code 2 *sensu* Pugliares *et al.* (2007)]. |
| C | 03 Sep 2019 | 23°27'28.62"S 43°0'6.90"W | Sub-adult (3rd yr.) | 1 larvae, 3 nymphs | Live bird captured at sea (Band Number CEMAVE U63136). |
| D | 03 Sep 2019 | 23°27'28.62"S 43°0'6.90"W | Adult (definitive plumage) | 1 nymph | Live bird captured at sea (Band Number CEMAVE U63132). |
| E | 03 Sep 2019 | 23°27'28.62"S 43°0'6.90"W | Adult (definitive plumage) | 9 larvae | Live bird captured at sea (Band Number CEMAVE U63135) |

The collected ticks were brought to the laboratory, where they were initially identified as 21 larvae and 16 nymphs of the genus *Ixodes*. All ticks were fully engorged. The 16 nymphs were morphologically identified as belonging to the *I. percavatus* group, following the descriptions of the *I. percavatus* s.s. nymph by Anastos (1954) and the *I. kerguelenensis* nymph by Roberts (1960) and Wilson (1970). The key morphological features to sustain this taxonomic identification were: basis capituli with broad cornua directed postero-laterally, palpal article I with short upcurved dorsal spur directly anteriorly, and strong meso-dorsal spur; coxae I–IV each with external and internal spurs (internal spur as a salient ridge in coxa IV); trochanters I–IV with ventral spurs (Fig. 1) and trochanter I with dorsal spur. Collected ticks were deposited in the tick collection "Coleção Nacional de Carrapatos Danilo Gonçalves Saraiva" (CNC) of the University of São Paulo, São Paulo, Brazil, under accession numbers CNC-3978, -4050 and -4088.

Based on the above nymphal descriptions of *I. percavatus* s.s. and *I. kerguelenensis*, we were unable to discern to which of the two species corresponded the nymphs collected in the current study. In contrast to adults, which can be separated by their external morphology (Arthur 1960, 1965), nymphs of these two species are highly similar, and to our knowledge, literature providing characters to separate them based on morphological characters is unavailable. Since both *I. percavatus* s.s. and *I. kerguelenensis* have been reported on *T. chlororhynchos*, we have retained our taxonomic identification as *I. percavatus* sensu lato (s.l.).


**FIGURE 1.** Nymph of *Ixodes percavatus* sensu lato collected on an Atlantic yellow-nosed albatross (*Thalassarche chlororhynchos*) for the present study. A. Dorsal gnathosoma, showing palpal article I with short, upcurved dorsal horn directly anteriorly (arrow head), and strong meso-dorsal spur (arrow). Bar: 0.2 µm. B. Coxae I–IV and trochanters with spurs. Bar: 0.2 µm.

All 21 engorged larvae had the same morphotype, compatible with the brief morphological descriptions available for *I. percavatus* s.s. and *I. kerguelenensis* (Anastos 1954; Wilson 1970). In order to confirm conspecificity of nymphs and larvae, one nymph and one larva from the *T. chlororhynchos* sampled on May 2019 were individually submitted to DNA extraction (Sangioni *et al.* 2005) and PCR targeting a ≈460 base pair-fragment of the tick mitochondrial 16S rRNA gene (Mangold *et al.* 1998). PCR products were sequenced and yielded two identical sequences, confirming their conspecificity. By BLASTn analyses (http://blast.ncbi.nlm.nih.gov/Blast.cgi), the obtained 16S rDNA sequence of *I. percavatus* s.l. was closest (96% identity) to sequences of *Ixodes auritulus* Neumann, 1904 (AF549845, AF113928, MH183252) and a sequence of an *Ixodes* sp. from Costa Rica (KF702352). There were no publicly-available DNA sequences of any species of the *I. percavatus* group in GenBank prior to this study, and our sequence was deposited in Genbank under the accession code MT072697.

To assess the phylogenetic position of *I. percavatus* s.l., an alignment including our sequence and 52 sequences of *Ixodes* spp. retrieved from GenBank was constructed with MAFFT (Katoh *et al.* 2002). The General Time Reversible model was selected as the best nucleotide substitution with MEGA (Tamura *et al.* 2011), and a phylogenetic tree using the maximum likelihood inference was implemented in PhyML (Guindon & Gascuel 2003) using the Nearest Neighbor Interchange distance method (Li *et al.* 1996), and 1000 bootstrap replicates. *Ixodes percavatus* s.l. clustered with an *Ixodes* sp. from Costa Rica, within a highly supported monophyletic clade that also comprised *Ixodes downsi* Kohls, 1957 and four distinct haplotypes of *I. auritulus* (Fig. 2).

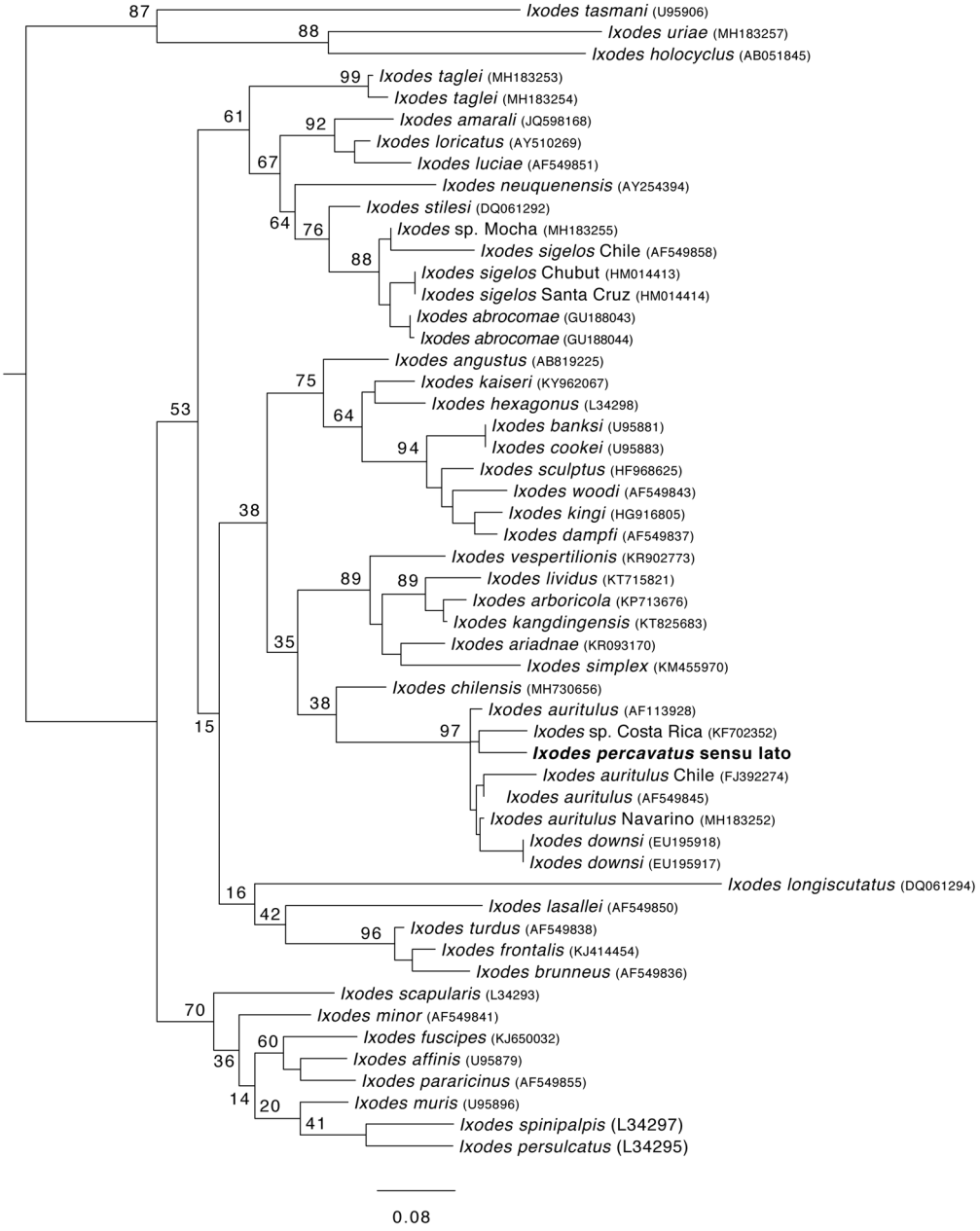


**FIGURE 2.** Maximum likelihood phylogenetic tree of *Ixodes* spp. Bootstrap values are shown above or below each branch. The tree is drawn to scale with the scale bar indicating nucleotide substitutions per site. The position of *Ixodes percavatus* sensu lato is highlighted in bold.

The Atlantic yellow-nosed albatross is endemic to the Tristan da Cunha Archipelago, mid-Atlantic Ocean (BirdLife International 2019). The species occurs at sea from southern Africa (Makhado *et al.* 2018) to South America (Carneiro *et al.* 2020), where it is one of the most frequent seabird attending fishing vessels for discards (Bugoni *et al.* 2008b). This species of albatross occurs along the Brazilian coast on the continental shelf and offshore waters regularly, during the non-breeding and breeding seasons, when it commutes between Tristan da Cunha and foraging areas on

the Brazilian south and southeastern continental shelf, as demonstrated by satellite-tracked birds (C.D. Gabani & L. Bugoni, FURG, unpub. data).

The fact that all collected ticks of the present study were fully engorged is compatible with a probable scenario in which the birds acquired the ticks when they were still on Tristan da Cunha Archipelago, where both *I. kerguelenensis* and *I. percavatus* s.s. are known to occur (Arthur 1965). In this case, engorged ticks might remain attached to the host until it reaches a suitable place that would favor their survival upon drop off from the host. Such behavior has been demonstrated for immature stages of *Ixodes arboricola* Schulze & Schlottke, 1929, another bird-associated tick species (White *et al.* 2012).

Our findings of engorged *I. percavatus* s.l. ticks on *T. chlororhynchos* along the Brazilian coast suggest that ticks of this group might have been repeatedly introduced into Brazil via infested *T. chlororhynchos*. However, there is yet no evidence that *I. percavatus* s.l. is established in Brazil, a condition supported by the fact that *T. chlororhynchos* stays at sea, reaching land only when debilitated or dead (Faria *et al.* 2014; Vanstreels *et al.* 2019). Finally, further studies are necessary to determine to which species (i.e., *I. percavatus* s.s. or *I. kerguelenensis*) the ticks of the present study belong. Since we have generated DNA sequences, this issue shall be clarified when new 16S rDNA sequences, obtained upon adult ticks, become available.

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