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SHORT COMMUNICATION

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Sympatric and syntopic occurrence of cownose rays: Neonatal strategies for survival?

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1 | INTRODUCTION

Cownose rays (Rhinopteridae; Chondrichthyes) constitute a small taxonomic group, distributed along coastal and estuarine areas in temperate and tropical regions (Bigelow & Schroeder, 1953; McEachran & Carvalho, 2002). In Brazil, only two species occur, the cownose ray *Rhinoptera bonasus* (Mitchill, 1815) and the Brazilian cownose ray *R. brasiliensis* (Müller, 1836), recently validated (Gallo-da-Silva, Afonso, & Gomes, 1997; Menni & Stehmann, 2000; Vooren, Klippel, & Galina, 2005).

The current knowledge on *Rhinoptera* spp. in Brazil is restricted (Vooren et al., 2005), to neonates from *R. brasiliensis* (Domingues, Gonzalez, & Amorim, 2009), and its anatomy (Gallo-da-Silva et al., 1997). Differentiation between species occurring in Brazil can be puzzling and depend on the dental morphology (McEachran & Carvalho, 2002). This peculiar feature can become a problem, especially for ecological and biological surveys in Brazil (Cavalcanti, Gallo-da-Silva, & Afonso, 1997; Vooren et al., 2005).

The bentho-pelagic *Rhinoptera bonasus* is distributed from southern New England (EUA) to southern Brazil (Bigelow & Schroeder, 1953; McEachran & Carvalho, 2002). It is currently classified as "Near Threatened" on the IUCN Red List (International Union for Conservation of Nature; Barker, 2006).

There are few studies on the life history of *R. brasiliensis*, with individuals observed only in the Gulf of Mexico, Colombia (Caribbean Coast), Venezuela, and Brazil (Grijalba-Bendeck, Polo-Silva, & Acero, 2007; Lasso-Alcalá, Lasso, & Juan, 2009; McEachran & Carvalho, 2002). Specific reports on *R. brasiliensis* imply that its life history is similar to that of *R. bonasus*, with a single pup per pregnancy (McEachran & Carvalho, 2002; Vooren et al., 2005). The *R. brasiliensis* is currently classified as "Endangered" on the IUCN Red List. This suggests a priority in research for conservation, especially because fishing surveys in southern Brazil have shown a reduction in the local population in the 1980s (Vooren & Lamónaca, 2004). Although both species present an alarming population decline and extinction risk, studies on these species are rare.

Molecular techniques, such as diagnostic tools, are now commonly employed in species identification, often applied when traditional morphological and meristic-based analyses have proven to be challenging or unsatisfactory, as in *R. bonasus* and *R. brasiliensis*. DNA identification is based on the assumption that intra-specific genetic divergence is usually lower than inter-specific divergence (Meyer & Paulay, 2005). The present study examines the hypothesis that both species co-occur in the early stages of young-of-the-year (YOY), forming heterospecific groups (congener) that are tactic-adopted, which would increase their chances of survival during the first weeks/months of life. Thus, we believe that this work contributes to a better understanding of this species, which can be useful in others studies, providing data on patterns of migration and habitat use.

2 | MATERIALS AND METHODS

The individual cownose rays were collected on the northern coast of São Paulo State (Bertioga), Brazil (23°49′35.02″S; 46°5′41.69″W). The samples were accidental captures by fishermen using a beach seine method with the research permission n° 48572-1 (IBAMA—SISBIO). A 350 m long, 11 m high net was thrown at a distance of about 400–600 m onto the beach. Gathering was made manually. During the non-lethal samplings, the animals were removed from the fishing net and stored in water-filled plastic containers (50-L; two or three individuals per box) to reduce air exposure between samplings (tissue, blood, sex identification by the presence of claspers in males, plus morphological measurements) and released immediately after completing the sampling. Any animals that died were kept by the fisherman and also analyzed. The species were identified by morphological dental characteristics (Bigelow & Schroeder, 1953; Gallo-da-Silva et al., 1997; McEachran & Carvalho, 2002).

For DNA identification and using a sterile razor blade, a small piece of a foot or mantle tissue (40 mg) was excised, then fixed in ethanol 95%. DNA was extracted using an automated protocol (Ivanova, Dewaard, & Hebert, 2006). The 650 bp barcode region of COI was subsequently amplified according to Hebert, Ratnasingham, and Dewaard (2003). PCR amplicons were visualized on a 1% agarose gel E-Gels (Invitrogen) and bi-directionally sequenced using the BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Inc.) on an ABI 3730 capillary sequencer following the manufacturer's instructions. The sequences were aligned in Geneious 4.8.5 (Drummond et al., 2009), and submitted to the GenBank (Accession nos. 19950099). A maximum likelihood phylogenetic reconstruction was applied to construct a tree from the pairwise distances, which were estimated using the Tamura and Nei (1993) substitution model as implemented in MEGA version 6 (Kumar, Tamura, & Nei, 2004).

3 | RESULTS

We obtained a total of 40 YOY samples (10 January, and 23 and 24 March 2016). Nineteen specimens were released without species identification or tissue sampling (Figure 1). Both species showed similar sizes: *R. bonasus* 41.0–58.0 cm (disk width, DW) and 2,088 ± 551.4 g (total weight, *W*); *R. brasiliensis* 42.0–58.5 cm (DW) and total weight 2,470 ± 299.8 g (*W*).

The sequencing of samples resulted in 580 analyzable nucleotides of COI gene (18 samples–10 *R. bonasus*, eight *R. brasiliensis*). Insertions, deletions, and stop codons were not observed. Sequences showed higher levels of genetic variability between species when considering the composition of the COI gene (4.1% genetic distance), with 36 polymorphic sites. Two distinct clades representing *R. bonasus* and *R. brasiliensis* were recognized, with 100% bootstrap support (Figure 2).

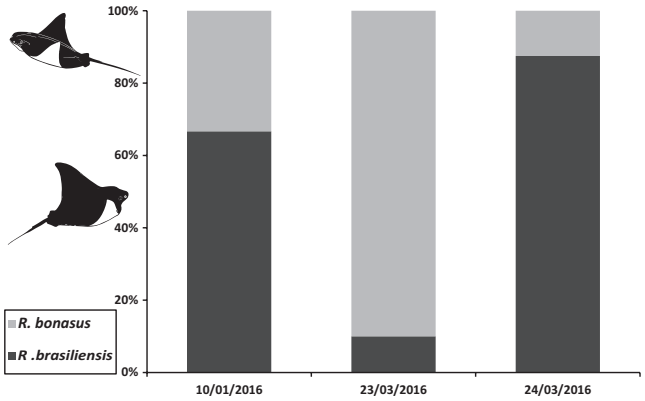

FIGURE 1 *Rhinoptera bonasus* and *R. brasiliensis* captures: 10 January (*R. bonasus n* = 1; *R. brasiliensis n* = 2), 23 March (*R. bonasus n* = 9; *R. brasiliensis n* = 1), 24 March 2016 (*R. bonasus n* = 1; *R. brasiliensis n* = 7), Bertioga, Brazil

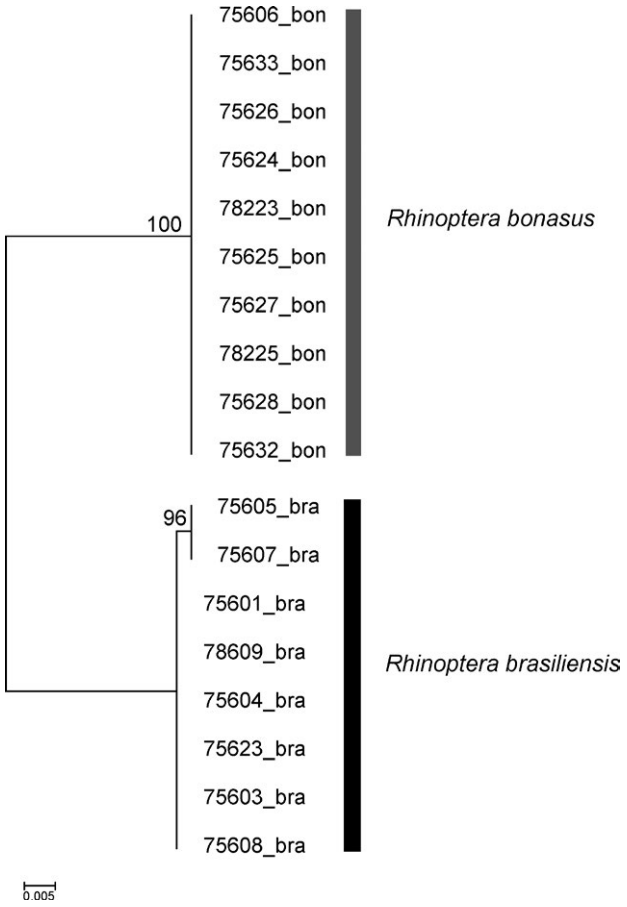

FIGURE 2 Maximum likelihood phylogenetic reconstructions, *Rhinoptera bonasus* and *R. brasiliensis*. A combined analysis of COI was conducted. Branch support values are maximum likelihood bootstrap values

4 | DISCUSSION

The data from this study confirm the geographically sympatric and ecologically syntopic occurrence of two YOY cownose ray species,

R. brasiliensis and *R. bonasus*, on the São Paulo coast, orroborating the hypothesis that both species co-occur, at least on certain occasions in their lives (Menni & Stehmann, 2000; Vooren & Lamónaca, 2004). Identification at the species level is imperative not only for proper attribution of life history and ecological traits but also for the correct identification of subgroups, as described in this study, being a robust indicator of sympatric distribution. Despite the limitations of this study, it is intended to call attention to the formation of heterospecific groups (congener), for future research on biological and fishing aspects, and enhance the need for proper fishing monitoring.

Despite the ontogenetic and seasonal partition observed in neonates and YOY shoals, which may be an adaptation to reduce intraspecific competition between life stages (Ajemian & Powers, 2016), heterospecific groups (congener) were thus far never reported. The formation of heterospecific groups does occur in some species of stingrays, namely, *Pastinachus sephen*, *Himantura uarnak* (Semeniuk & Dill, 2006), *H. fai*, *Dasyatis microps*, *Taeniurops meyeni* (Meekan, Trevitt, Simpfendorfer, & White, 2016). Postulated benefits of groupings are the likely reduction in predation rates and/or enhanced foraging success, or simply chance encounters (Meekan et al., 2016; Semeniuk & Dill, 2006).

Thus, grouping could work either as a defense strategy molded by evolution or a short-term learned behavior to minimize the predatory effect on neonates and YOY (Heithaus, 2004). The cownose ray exhibits a cooperative feeding strategy that improves behavior and individual capacity in their search for prey (Bedore, Harris, & Kajiura, 2014). Thus, a heterospecific grouping between *R. bonasus* and *R. brasiliensis* could enhance foraging efficiency. The species develop strategies to maximize the growth of their YOY in the first stages of life, keeping them in protected areas, such as estuarine and coastal regions, before performing energetically demanding activities, such as migration (Ajemian & Powers, 2016; Fisher, Call, & Grubbs, 2013) and prey search (Neer, Carlson, & Thompson, 2006).

The new information imparted in this study can be an indicator that *R. brasiliensis* has a behavioral plasticity. Also implied is that the heterospecific group formation is a tactic adopted by the species to increase the chances of survival and feeding success during the first weeks/months of life. However, more studies, especially behavioral analyses, are critical to better understanding the patterns of migration and habitat use of both species.

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