



## Anatomy of a border: transition between Southwestern Atlantic tropical and Warm Temperate marine provinces as revealed by marine mites\*

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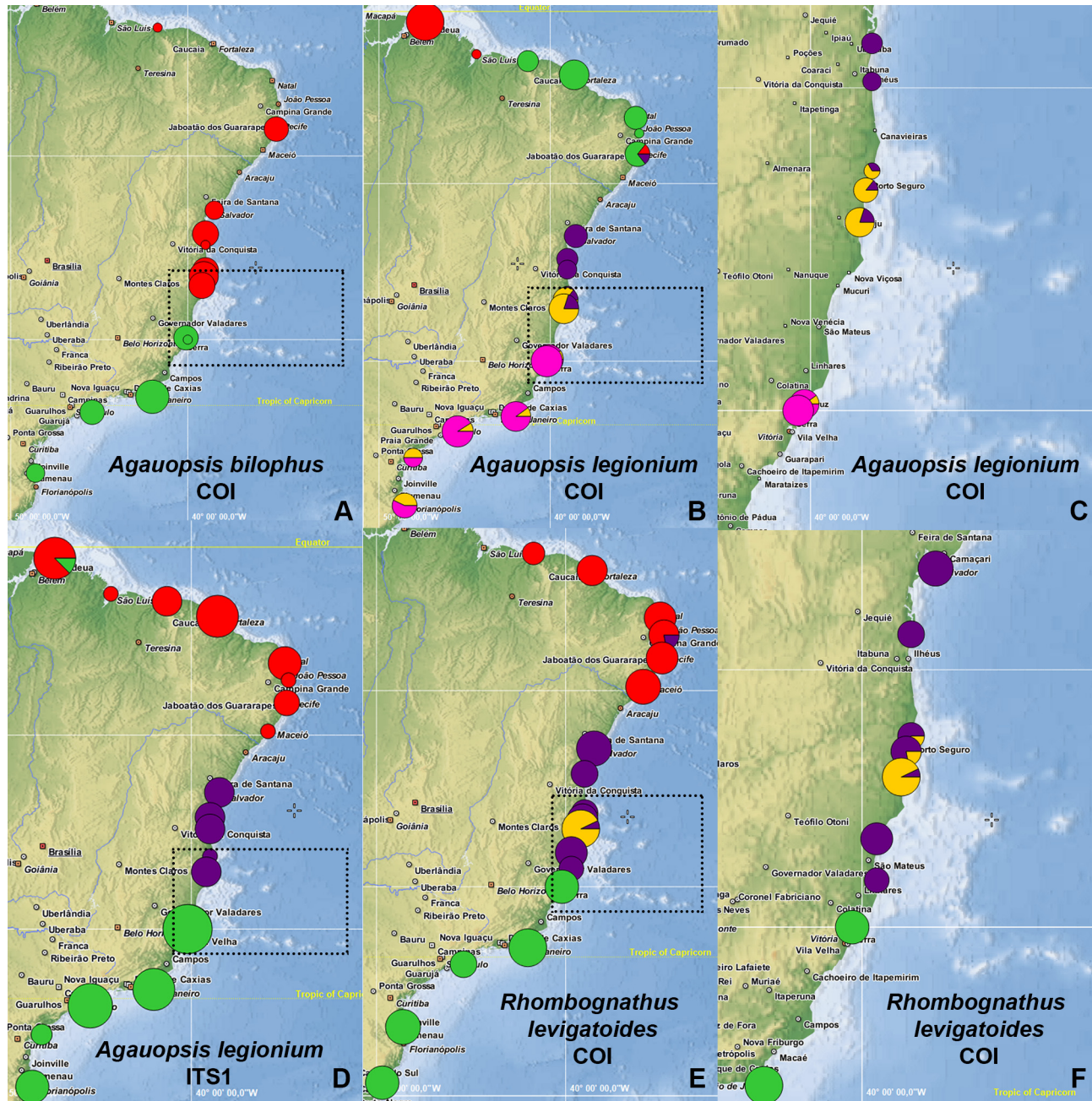
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A previous study found that the division between the Southwestern Atlantic Tropical and Warm Temperate Provinces for marine mites do not occur in Cabo Frio (~23°S), as usually assumed in marine biogeographical studies, but somewhere around the Abrolhos Plateau and the Vitória-Trindade Chain (~20–15°S). New sampling localities along this section of Brazilian coast and sequences from mitochondrial and nuclear genes of three species or species complexes of halacarids (*Agauopsis legionium*, *A. bilophus* and *Rhombognathus levigatoides*) deepened the understanding of this region by showing different scenarios for the different species. Individuals morphologically identified as *A. bilophus* had their *COI* (531, N=79), *28S D2 region* (~510 bp, N= 74), and *HSP70* (737 bp, N= 76) sequenced. All markers agreed in presenting two haplogroups, one occurring from ~2°S to ~17°S, and the other from 19°S to 26°S (Fig. 1A), recovered as cryptic species in species delimitation using a multi-loci multi-species coalescent approach. Similar to *A. bilophus*, *R. levigatoides* (*COI* 609 bp, N= 184; *28S D2* ~534 bp, N= 97; *HSP70* 721 bp, N= 53) had their individuals recovered as separated cryptic species, the northern clade ranging from ~2°S to ~19°S (Fig 1 E–F, red and purple), and the southern from ~16°S to ~29°S (Fig 1 E–F, yellow and green), note that distributions overlap along the Abrolhos Plateau. The northern species presented two *COI* haplogroups, the first occurring from ~2°S to ~10°S (Fig 1 E–F, red) and the second from ~13°S to ~19°S (Fig 1 E–F, purple), with a single isolated occurrence at ~7°S. The Southern clade was also divided in two haplogroups, one restricted to the Abrolhos Plateau region from ~16°S to 17°S (Fig 1 E–F, yellow), and the other from ~20°S to ~29°S (Fig 1 E–F, green). The nuclear markers agreed in distinguishing the two clades as separated species in multi-loci species delimitation analyses. Finally, *A. legionium* gene trees were shallow, all samples being recovered as members of the same species, according to analyses employing sequences from *COI* (609 bp, N=134), *ITS1* (509 bp, N=93), and *HSP70* (706 bp, N=55). It was possible to find five *COI* haplogroups: Haplogroup I occurring from ~ 0.6°S to ~2.5°S (Fig 1 B, red) and an isolated occurrence from 7.6°S; Haplogroup II, with occurrences from ~20°S to ~28°S (Fig 1 B–C, pink); Haplogroup III, predominant in the northeast Brazil, from ~ 3°S to ~17°S (Fig 1 B–C, green); Haplogroup IV with occurrences from ~16°S to ~28°S (Fig 1 B–C, yellow); and Haplogroup V with haplotypes from Abrolhos Plateau region and Bahia State coast (~13–17°S) and an occurrence at ~7°S (Fig 1 B–C, purple). Sequences from *ITS1* were divided in three haplogroups: Haplogroup I from ~ 0.6°S to 7.6°S (Fig. 1 D, red); Haplogroup II predominant in Southeast Brazil from ~20°S to ~28°S with a single occurrence at ~ 0.6°S (Fig. 1 D, green); and Haplogroup III with sequences from ~13°S to ~17°S (Fig. 1 D, purple). The new data support previous conclusions that the direction of superficial currents and environmental parameters related to the presence of nutrient rich and colder South Atlantic Central Waters shape the division between the bio-geographical provinces, but also that (i) the Southwestern Atlantic Tropical and Warm Temperate provinces along Brazilian littoral have its transitional area corresponding to the Abrolhos Plateau, with its southern border corresponding to Doce River mouth (~20°S), and that (ii) it is characterized by the co-occurrence of cryptic species or haplogroups from northern and southern regions in two out of three studied halacarid species, making this small region genetically more diverse than other areas and this section of Brazilian littoral meriting special concern when formulating environmental policies.

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**FIGURE 1.** Distribution of marine mite haplogroups along Brazilian coast (A–B, D–E, Abrolhos plateau area indicated by a dotted line rectangle) or along Abrolhos plateau (C and F) (Colors are distinct haplogroups). *Agauopsis bilophus* complex haplogroups were recovered as distinct species as well as *Rhombognathus levigatoides* complex haplotypes in red and purple grouped in a species distinct to yellow and green in multiloci coalescent species delimitation methods. Figures produced in PopART 1.7 (<https://popart.maths.otago.ac.nz/>).

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