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Short Communication

Twigs occupied by *Pheidole* Westwood, 1839: Is there a difference between species?

Suellen C. Barroso[1], Eduardo L. Longui[2], Tae T. Fernandes[1], Carla M. Oliveira[1], Alexandre C. Ferreira[3], Rogério R. Silva[4] & Maria Santina C. Morini[1]*

[1]Universidade de Mogi das Cruzes, Laboratório de Mirmecologia, Mogi das Cruzes, SP, Brasil.
[2]Instituto Florestal, São Paulo, SP, Brasil.
[3]Universidade Federal do Paraná, Laboratório de Sistemática e Biologia de Formigas, Curitiba, PR, Brasil.
[4]Museu Paraense Emílio Goeldi, Belém, PA, Brasil.
**Corresponding author: Maria Santina C. Morini, e-mail: mscmorini@gmail.com*

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Abstract: *Pheidole* is a genus with wide geographical distribution and diversity, especially in the leaf litter of neotropical forests, where nests are found at the soil-litter interface, in the soil and vegetation, among leaves, seeds, and twigs. Despite the availability of twigs and *Pheidole* species diversity in the leaf litter, most of this resource is not occupied, which suggests the existence of filters. This study analyzes whether twigs occupied by *Pheidole* species differ for the outer structure and anatomy of the wood. Twigs were collected from preserved Atlantic Forest fragments in southeastern Brazil. Twigs with *Pheidole* colonies were measured and the wood anatomy analyzed. We collected 224 twigs with *Pheidole* colonies, but the analysis was done at 41% due to wood decomposition. Five species were recorded in these twigs, which differ for the outer structure and anatomy of the wood. These results suggest the existence of preference in the occupation of twigs determined by wood structure.
Keywords: *ant; fiber length; dense ombrophilous forest; vessel length; wood.*

Galhos ocupados por *Pheidole* Westwood, 1839: há diferença entre espécies?

Resumo: *Pheidole* é um gênero com ampla distribuição geográfica e diversidade, especialmente na serapilheira das florestas da Região Neotropical, onde os ninhos são encontrados na interface solo-serapilheira, solo, vegetação, entre folhas, sementes e galhos. Apesar da disponibilidade de galhos e diversidade de espécies de *Pheidole* na serapilheira, a maior parte deste recurso não é ocupada, o que sugere a existência de filtros. Neste trabalho analisamos se galhos ocupados por espécies de *Pheidole* diferem em relação à estrutura externa e anatomia da madeira. A coleta de galhos foi realizada em fragmentos conservados de Mata Atlântica na região Sudeste do Brasil. Os galhos com colônias de *Pheidole* foram mensurados e a anatomia da madeira analisada. Foram coletados 224 galhos com colônias de *Pheidole*, mas a análise foi realizada em 41% devido à decomposição da madeira. Nestes galhos foram registradas cinco espécies, que diferem em relação à estrutura externa e anatomia da madeira. Estes resultados sugerem a existência de preferência na ocupação do galho determinada pela estrutura da madeira.
Palavras-chave: *formiga; comprimento de fibra; floresta ombrófila densa; comprimento de vaso; lenho.*

Introduction

Pheidole Westwood, 1839 is considered a hyperdiverse genus, for the 1,095 described species (Bolton 2019), with a set of morphological and behavioral characteristics that confer great adaptive success (Wilson 2003). The species distribution is wide, especially in habitats under warm and humid climate (Economo et al. 2015), as is the case in the Neotropical Region. Nests are built in the soil, leaf litter, and vegetation (Wilson 2003), from degraded areas to primary and continuous vegetation areas such as tropical forests (Delabie et al. 2000). *Pheidole* species are traditionally recognized by their generalist and detritivorous habit (Moreau 2008), consuming various soil invertebrates such as mites (Wilson 2005), but preferring seed consumption (Wilson 2003).

In the leaf litter, *Pheidole* colonies are found in seed remains, between leaves, under stones (Hölldobler & Wilson 1990), in fruits, associated or not with other invertebrates (Castaño-Meneses et al. 2015), and in living wood or wood under different stages of decomposition (Delabie et al. 1997; Carvalho & Vasconcelos 2002), such as in twigs.

Twenty-one species were recorded in twigs (Fernandes et al. 2012; Souza-Campana et al. 2017), but it is a low number compared to the total diversity of *Pheidole* in the leaf litter (Suguituru et al. 2015). Thus, despite this species diversity and availability of twigs in the leaf litter of the rainforests (Sagata et al. 2010), few *Pheidole* species occupy them.

Working in areas of native forest and *Eucalyptus* plantations with a developed understory, Fernandes et al. (2018) showed that *P. sospes* Forel, 1908 occupies twigs with the same diameter, regardless of the diversity of twigs in the native forest. However, the same was not observed for *P. sarcina* Forel, 1912. Given this, we start from the premise of the existence of filters during occupation of twigs by *Pheidole* species. Thus, the present study investigates the structure of twigs occupied by *Pheidole* species in the Atlantic Forest leaf litter. We have hypothesized that twigs occupied by *Pheidole* differ for the external (length, diameter, and number of perforations) and internal structure of the wood (vessel diameter, vessel length, and fiber length), as it avoids competition between species.

Material and Methods

Collections were made in 2015, from 9 am to 1 pm to maintain the standardization used by our group in all collections, on days not preceded by rain to prevent the twigs from being too humid, in preserved Atlantic Forest fragments in southeastern Brazil. In total there were six collection sites – "Cachoeira Pedra Bonita"; "Lago da Mata"; "Pousada Rural Sítio Matsuo"; "Sítio Cantos da Mata"; "Parque das Neblinas - Trilha da Cetesp"; and "Parque das Neblinas" (Figure 1), with altitudes between 600 to 850 m and average annual temperature approximately 20 ºC (Tomasulo & Cordeiro 2000). According to the Köppen classification, the climate of the region is mesothermic with a dry winter (Cwb), with an annual rainfall accumulation of 1,500 mm (Cptec-Inpe 2019).

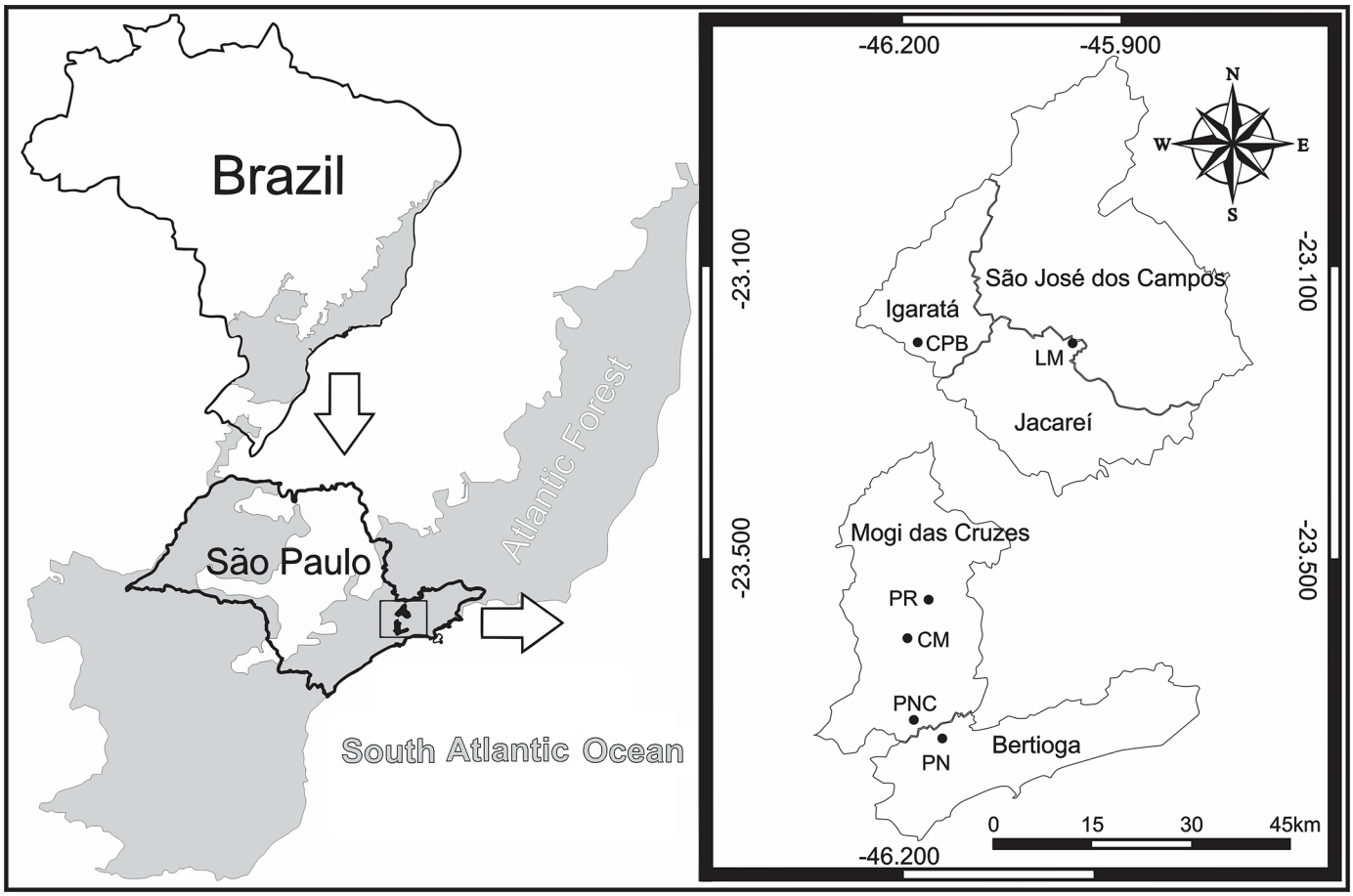

Figure 1. Geographic location of collection areas in the São Paulo State, Brazil. Legend: CPB – "Cachoeira Pedra Bonita" (23°12'28"S; 46°10'39"W); LM – "Lago da Mata" (23°12'33"S; 45°58'02"W); PR – "Pousada Rural Sítio Matsuo" (23°33'31"S; 46°09'45"W); CM – "Sítio Cantos da Mata" (23°36'40"S; 46°11'28"W); PNC – "Parque das Neblinas - Trilha da Cetesp" (23°43'21"S; 46°10'57"W); PN – "Parque das Neblinas" (23°44'51"S; 46°08'39"W).

A linear transect with seven plots distanced 10 m apart was established in each area. Each plot measured 16 m where all twigs (= nests) were collected. The twigs were individually wrapped in plastic bags. In total, 42 plots and 672 m of leaf litter were analyzed. In the laboratory, each twig was checked for ant colonies, but only twigs with *Pheidole* colonies had the outer structure and wood anatomy analyzed.

We classified twigs as occupied if they contained ≥ 10 workers; if fewer than 10 workers were present, twigs were considered occupied if they contained immatures, queens, or males (Fernandes et al. 2012). *Pheidole* species were identified following the key proposed by Wilson (2003). Moreover, an interactive key was used through the Lucid platform elaborated by Dr. John T. Longino (available at https://sites.google.com/site/newworldpheidole/). The vouchers are deposited in the collection of the Alto Tietê Myrmecology Laboratory at the University of Mogi das Cruzes (SP), and in the Padre Jesus Santiago Moure Entomological Collection (DZUP) at the Federal University of Paraná.

We tried to identify wood species based on the literature and on samples from the Forest Institute Xilaryum (SPSFw), but due to wood conditions such as natural degradation, we could not determine the species. To analyze the outer structure of twigs we measured the length using a ruler, and the diameter (three measurements: two at the ends and one at the center) with a digital caliper. The entire surface of the twig was examined under a Motic SMZ-168 stereoscope, and carefully inspected for holes using a rotating support and an entomological pin (Nº 0).

Then the twigs were opened and the number of adults (workers, queens, and males) and immatures were counted. To analyze the internal structure (wood anatomy), we used wood fragments to prepare macerations according to the modified Franklin method (Berlyn & Miksche 1976). The wood fragments were stained with aqueous safranin and mounted in a solution of water and glycerin (1:1). Fiber and vessel measurements (mm) (Figure 2) were performed on an Olympus CX31 microscope equipped with a camera (Olympus Evolt E330) and a computer with image analyzer software (Image-Pro 6.3). Terminology followed the IAWA list (Iawa 1989).

Twig dimensions, number of perforations, and wood anatomical features were assessed by parametric analysis of variance (one-way analysis of variance). When a significant difference was observed, the Tukey test was used to identify pairs of significantly different means.

Results and Discussion

Five hundred and seventy-five twigs with ants were collected and 224 (39%) had *Pheidole* species. Wood anatomy was analyzed on 92 twigs out of a total of 224, since the wood of the other twigs with *Pheidole* colonies was in an advanced stage of decomposition, preventing analysis.

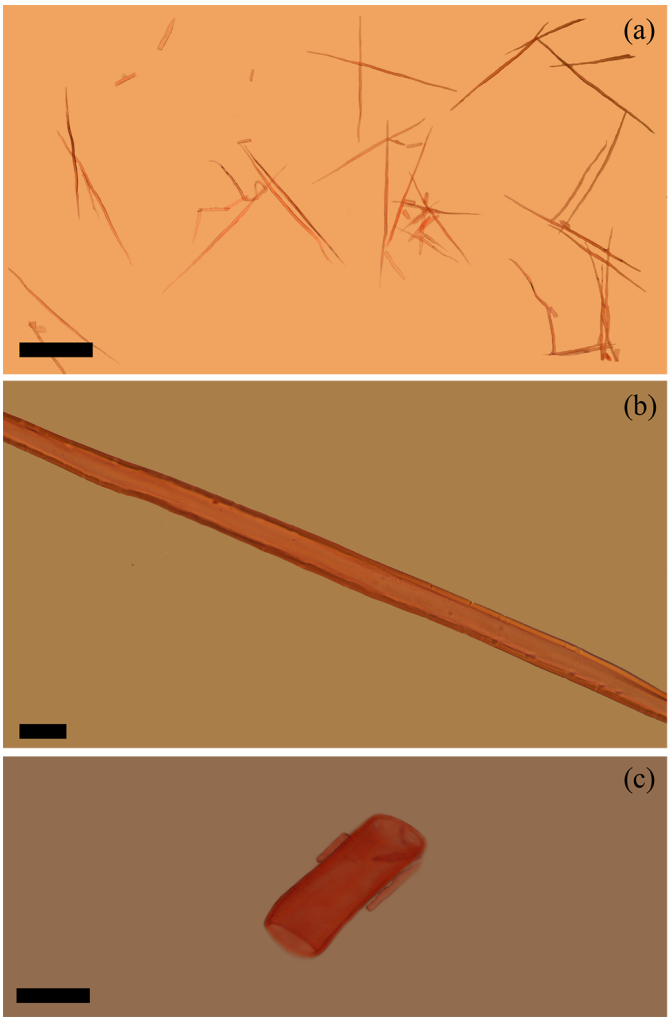

Figure 2. Cell dimensions in wood colonized by *Pheidole* species. A. Vessel element length (scale bar = 250 µm); B. Vessel diameter (scale bar = 10 µm). C. Fiber length (scale bar = 100 µm).

In the twigs with appropriate characteristics for wood anatomy analysis, we identified *P. flavens* Roger, 1863; *P. sarcina*; *P. sigillata* Wilson, 2003; *P. sospes* and *Pheidole* gr. *tristis* sp. (Table 1). All these species are considered common inhabitants of twigs, because they are ant that colonized 10 or more twigs in a given leaf litter area (Fernandes et al. 2018). Our results show also that *P. sarcina* is the species that most uses twigs in the leaf litter as a resource, which corroborates the results of Fernandes et al. (2018).

Pheidole species differ for the outer structure (length, diameter, and number of perforations) of the occupied twig (Table 1). The species

Table 1. Number of occupied twigs, twig dimensions, and number of perforations by *Pheidole* species.

Species	Colonized twigs (%)	Length (cm)	Diameter (cm)	Perforations (Nº)
Pheidole flavens	8	12 -35 (26^{b})	0.7 -1.8 (1.2^{c})	0 -3 (1^{c})
Pheidole sarcina	40	9 -237 (49^{ab})	0.6 - 8.6 (1.7^{b})	0 -10 (2^{bc})
Pheidole sigillata	19	11 -671 (86^{a})	0.4 - 4.9 (1.6^{bc})	0 - 5 (2^{bc})
Pheidole sospes	22	19 -195 (63^{ab})	0.9 -8.5 (1.3^{c})	0 - 8 (3^{b})
Pheidole gr. *tristis* sp.	10	24 -198 (71^{ab})	0.7 - 5.3 (3.1^{a})	1 – 13 (5^{a})

Minimum and maximum (mean) values for twig length, diameter, and number of perforations. Distinct letters in the same row differ statistically ($P < 0.05$) by the Tukey test.

recorded in the present study have different strategies, that is, while *P. flavens* houses their small colonies in smaller diameter twigs, *P.* gr. *tristis* sp. occupies larger diameter twigs and its colonies are comparatively larger (Figure 3).

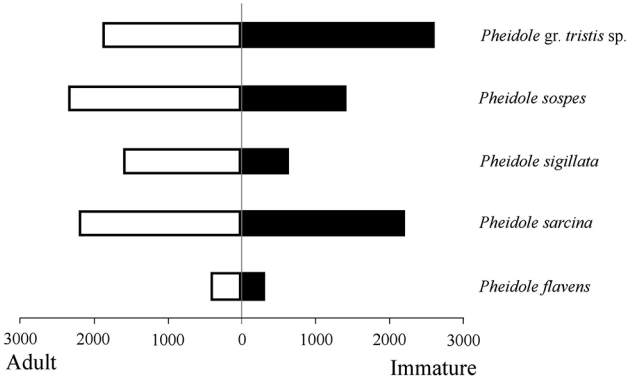

Figure 3. Abundance of adults (workers, winged males, and queens) and immatures in twigs according to *Pheidole* species.

As *Pheidole* is a very diverse group in the leaf litter of tropical forests (Delabie et al. 2000; Silva & Brandão 2010), possibly the different twig occupation strategies are related to the competition for this resource. Our results suggest that ant occupation of the twig may be related to the specific attributes of the wood.

Longer vessels occurred in twigs occupied by *P.* gr. *tristis* sp. when compared to other species. Vessel diameter differed between woods: larger vessels occurred in woods occupied by *P.* gr. *tristis* sp., and narrower vessels in woods occupied by *P. flavens* and *P. sarcina*. Vessel diameter in woods occupied by *P. sigillata* and *P. sospes* did not differ from that of other woods. Longer fibers occurred in woods occupied by *P.* gr. *tristis* sp. and *P. sospes* (Figure 4).

In general, the larger cell sizes found in woods colonized by *P.* gr. *tristis* sp. (in larger twigs) may be related to radial development of anatomical features. During growth in trunk diameter, twigs or roots, there is an increase in vessel and fiber length, and in vessel diameter (Lachenbruch et al. 2011). Hence, because they have a larger diameter (Table 1), we suggest that twigs occupied by *P.* gr. *tristis* are older than other twigs occupied by other ant species.

Apparently there is no relationship between wood species and ant species occupation, as also observed by Armbrecht et al. (2004). Thus, there is an association between twig diameter and ant species, in which *P.* gr. *tristis* sp. seeks to nest on wider twigs compared to the other four ant species studied.

Our results suggest that from five species, only two differ in their twigs: *P. sarcina* occupies smaller and larger diameter twigs, and *Pheidole* gr. *tristis* sp. occupies twigs with the largest diameter, but in less proportion. Possibly *P. sarcina* is the most generalist in the twig occupation, which corroborates the results found by Fernandes et al. (2018). These authors have shown that this species occupies most of the twigs in Atlantic forest areas.

To understand whether there are associations between ant species and wood species, in the next studies we will seek to identify wood from knowledge of tree species that are close to leaf litter collection

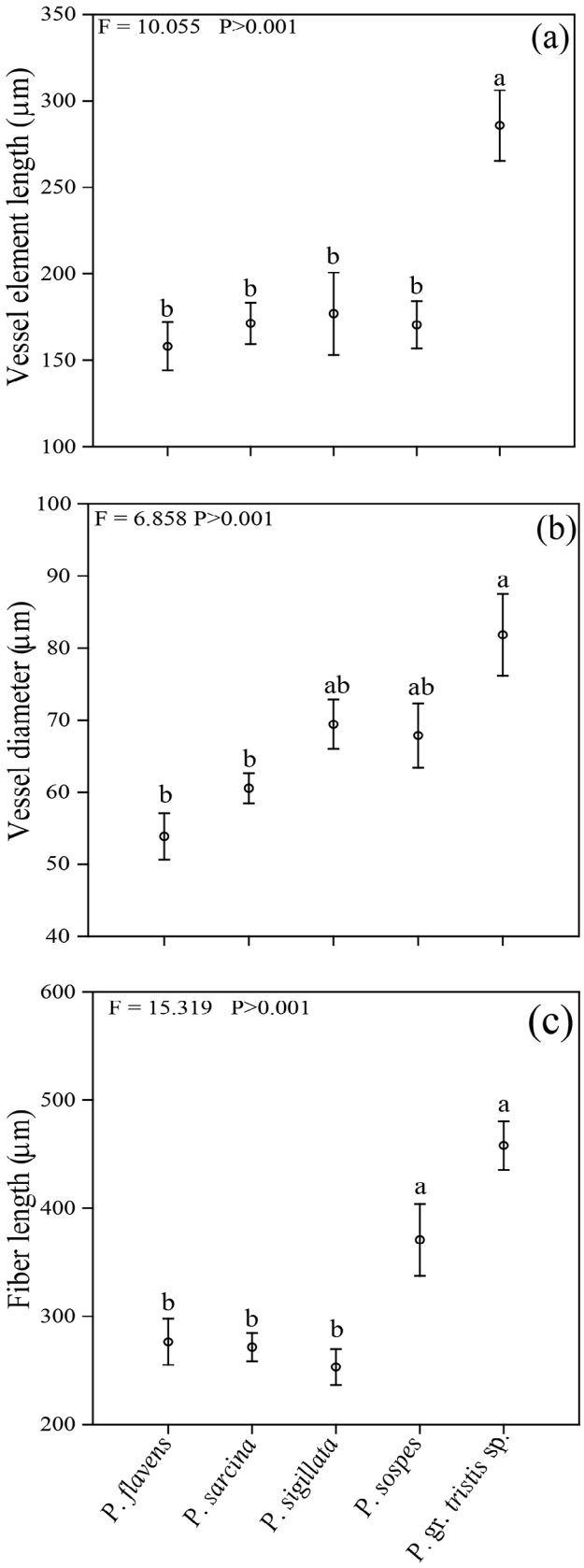

Figure 4. Wood anatomical features occupied by *Pheidole* species. Distinct letters differ statistically ($P < 0.05$) by the Tukey test.

sites. Therefore, we will have some indicative and a known number of species to compare with the literature information and also with the samples from the xilaryum.

We emphasize that there are no other studies relating ant species to wood anatomy comparing and discussing our information. Thus, we understand that our study is pioneering in this regard, so the delineation of how to develop this investigation more appropriately is being developed as difficulties arise. Overall, our results contribute to understanding the natural history of a hyperdiverse genus, mainly involving characteristics related to the choice of the nesting site.

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Author Contributions

Suellen C. Barroso: contribution to data collection; contribution to data analysis and interpretation and manuscript preparation.

Eduardo L. Longui: substantial contribution to manuscript preparation; contribution to data analysis and critical revision, adding intellectual content.

Tae T. Fernandes: substantial contribution in the concept and design of the study; contribution to data collection and manuscript preparation.

Carla M. Oliveira: contribution to data collection and manuscript preparation.

Alexandre C. Ferreira: contribution to species identification.

Rogério R. Silva: substantial contribution to manuscript preparation and contribution to critical revision, adding intellectual content.

Maria Santina C. Morini: substantial contribution in the concept and design of the study; contribution to manuscript preparation and critical revision, adding intellectual content.

Conflicts of interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

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