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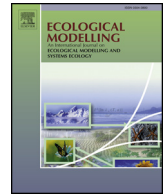
Demographic and performance effects of alternative host use in a Neotropical treehopper (Hemiptera: Membracidae)

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ABSTRACT

The mechanisms which promote divergence in populations of phytophagous insects using alternative hosts depend on the characterization of hosts as different selective scenarios and the modeling of the demographic dynamics of the insect on those hosts. In the Bolivian Yungas forests, the treehopper *Alchisme grossa* (Hemiptera: Membracidae) utilizes two sympatric Solanaceae hosts, *Brugmansia suaveolens* and *Solanum ursinum*. On both hosts, females take care of nymphs during their development and adults use almost exclusively their natal host species. We characterized: 1) the performance of families (mother and nymphs) on both hosts, 2) maternal care across families and hosts, 3) biotic (herbivory, predators and parasites) and abiotic (temperature, relative humidity and luminosity) conditions across hosts and 4) demographic parameters of cohorts living under natural conditions on both hosts using matrix population modelling. The life cycle was longer on *B. suaveolens* than on *S. ursinum*. Microenvironmental abiotic and biotic traits differed between hosts. Nevertheless, performance did not differ between hosts and no differences between hosts were found in demographic parameters. None of the environmental variables explained the demographic parameters. Females performed maternal care with the same intensity on both hosts. Taken together with published data on the system, these results suggest that individuals of *A. grossa* in this study belong to a single population. Colonization of one of these hosts might be a recent event and evolutionary processes that promote ecological adaptation and hence demographic or performance differences have not yet become noticeable.

1. Introduction

In phytophagous insects, patterns of host use as nutritional resources and habitats are shaped mainly by historical, physiological, morphological, chemical and ecological factors (Futuyma and Moreno, 1988; Jaenike, 1990; Bernays and Chapman, 1994; Schoonhoven et al., 2005; Fordyce, 2010; Richards et al., 2015; Glassmire et al., 2016). These factors may restrict host use and ecological specialization is more a rule than an exception (Futuyma and Moreno, 1988; Schoonhoven et al., 2005; Fordyce, 2010; Forister et al., 2015). Ecological and genetic mechanisms that lead to specialization of phytophagous insects on particular resources or habitats and the circumstances promoting divergence and speciation of populations have been extensively studied

(e.g., Berenbaum, 1996; Caillaud and Via, 2000; Schoonhoven et al., 2005; Fordyce, 2010; Forister et al., 2015; Nylin et al., 2015; Peterson et al., 2015; Richards et al., 2015; Harrison et al., 2016). Usually, the use of different hosts is a consequence of trade-offs in fitness; such trade-offs have been hypothesized to explain evolutionary specialization (Futuyma and Moreno, 1988; Agrawal, 2000; Gompert et al., 2015). This use of different hosts by phytophagous insects could lead to differences in traits associated with insect performance on those hosts, such as life cycle duration, mortality, and communication signal production and perception (Futuyma and Moreno, 1988; Jaenike, 1990; Agrawal, 2000; Nosil et al., 2007). However, differences in the fitness of individuals may not be apparent in the overall fitness of populations on different hosts. This may be due to host-to-host dispersal and gene flow

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from one potentially diversifying population to another and to the different host-plant based populations being at an early stage of the specialization and diversification process.

From the population viewpoint, performance could be addressed by means of demographic parameters estimated in the framework of Matrix Population Models (Caswell, 2001). When an insect species uses more than one host, variations may be expected in the reproductive output on each host, which are projected into the population dynamics of individuals developing on such hosts. These studies should be based on the assessment of biotic and abiotic environmental variables which characterize the hosts used and the estimation of the finite rate of increase (λ), an average proxy of fitness, through modelling the demographic dynamics of the insect on such hosts (Charlesworth, 1980; Caswell, 1983, 2001; Larsson et al., 2000; Awmack and Leather, 2002).

Treehoppers (Hemiptera: Membracidae) are sap-feeding insects that depend entirely on their host for feeding, development, mating and oviposition. In various genera of treehoppers, this dependence is reflected in host specialization leading to a synchrony between the insect's life cycle and host phenology (Wood and Olmstead, 1984; Wood, 1993b; Sattman and Cocroft, 2003). Some treehopper species are solitary and immature individuals do not receive parental care, while others display subsocial behaviors such as maternal care and feeding facilitation by the female (Godoy et al., 2006; Lin, 2006; Torrico-Bazoberry et al., 2014), which play a key role in the survival of the offspring (Tallamy and Schaefer, 1997); hence, variation in maternal care across hosts may be part of what determines differences in fitness or group performance.

Alchisme grossa Fairmaire (Membracidae) shows a Neotropical distribution and belongs to the tribe Hoplophorionini whose members display maternal care and nymphal aggregation without mutualistic interactions with ants (Camacho et al., 2014; Torrico-Bazoberry et al., 2014). In general, the genus *Alchisme* uses solanaceous plants as hosts, a fact which has been linked to plant chemistry (McKamey and Deitz, 1996; Pinto et al., 2016). In the cloud forests of the Bolivian Yungas, *A. grossa* utilizes two hosts occurring in sympatry, *Brugmansia suaveolens* (Humb. and Bonpl. ex Wild) Bercht. and C. Presl and *Solanum ursinum* (Rusby) (both Solanaceae), which represent phenotypically and probably ecologically different substrates for the development of the insects (Pinto, 2015; Pinto et al., 2016; Torrico-Bazoberry et al., 2014, 2016). Females of *A. grossa* ovipositing on these two hosts exhibit maternal care with egg and nymph guarding, active antidepredatory defense and feeding facilitation. Cohorts on a given host show low dispersion and tend to move within their natal host, as shown by mark-recapture and female oviposition studies (Torrico-Bazoberry et al., 2014; Pinto, 2015). We hypothesize that maternal care should be more important on hosts with higher predation risk for the offspring. Different intensities of maternal care may involve trade-offs reflected in the performance of groups living on a given host. These interactions might be illustrated by an MPM approach. To evaluate whether each host represents a unique selective context where two populations of *A. grossa* are potentially experiencing a differentiation process, we documented biotic and abiotic microenvironmental variables on each host and characterized family performance, behavioral traits related to subsociality, and demographic parameters of cohorts estimated for the MPM.

2. Materials and methods

2.1. Study area and data collection

We collected data from *A. grossa* on *B. suaveolens* (BS) and *S. ursinum* (SU) during the rainy season at Incachaca (Cochabamba, Bolivia, 17°13′S - 65°49′W; 2450 m a.s.l.), within the Yungas biogeographical region. Twenty two field trips were performed from December 12, 2012 to April 24, 2013. During the first field trip, we chose recently oviposited cohorts and tagged families on each plant with a plant code and the petiole of leaves containing treehopper families with a leaf code.

The number of families on each plant varied from 1 to 10. We momentarily removed the adult female from each family to measure the length and width of her egg mass and to mark her on the right side of the pronotum with a permanent marker. We measured egg masses with a GMC-190 Goldtool® digital caliper (0.1 mm precision) and estimated the number of eggs in each mass based on its size, following the model described by Torrico-Bazoberry et al. (2016).

2.2. Life cycle duration

Life cycle of *A. grossa* involves seven stages: egg, 5 nymphal instars, and adult. To characterize life cycle parameters of *A. grossa*, we monitored all tagged families once every 5 days (between 11:00 and 14:00 h) following Torrico-Bazoberry et al. (2016) and recorded the number and development stage (instar) of nymphs. We compared the total duration of immature stages (*i.e.*, duration in days after the egg mass was marked until offspring reached the adult stage) of those families that successfully completed preimaginal development ($n = 22$ on BS; $n = 18$ on SU) between hosts, using the Student's *t*-test. We also compared the duration of each developmental stage between hosts, using the Mann–Whitney test.

2.3. Microenvironmental variables

We evaluated the following microenvironmental biotic variables: a) number of parasite mites on mothers and nymphs, b) abundance and richness of predators and c) herbivory, using a herbivory index (Dirzo and Domínguez, 1995) with data obtained on a single day in February (midway in the study period) on 10 randomly chosen leaves of each marked plant. The *herbivory index* (HI) was based on the categorization of the leaves by a single person in one of the following categories of herbivory (foliar damage): 0 = no herbivory; 1 = 1–5% leaf area removal; 2 = 6–12%; 3 = 13–25%; 4 = 26–50%; 5 = 51–100% (Domínguez and Dirzo, 1995). HI was estimated as $\Sigma (X_i\ n_i)/N$, where, X_i = herbivory category, n_i = number of leaves on the category X and N = total number of leaves studied. HI was considered as an indirect measure of competition between *A. grossa* and other phytophagous insects (*i.e.*, folivores). We compared the HI per plant between hosts using the Student's *t*-test. We compared the abundance and diversity of predators per plant, jointly expressed as the Brillouin index and the mean number of parasitic mites per family (*i.e.*, on the female and on the nymphs) observed during all the observation periods between hosts, using the Mann–Whitney test.

We evaluated microenvironmental abiotic variables (luminosity, temperature and relative humidity) using a light meter (Lutron® LX-101) and a thermocouple (EXTECH®) positioned 2 cm below the underside of tagged leaves to characterize the microenvironment around each insect family. We performed two different measurements per leaf between 11:00 and 14:00 h throughout the study. We characterized the abiotic microenvironment of each family by the mean values of temperature, relative humidity and luminosity using this data. We compared microenvironmental parameters between families on the two hosts using Mann–Whitney tests. All analyses were performed with SigmaPlot 12.0 (Systat Software, Inc., 2012).

2.4. Insect performance

We compared the performance of *A. grossa* between hosts through the following parameters, using each individual plant as the experimental unit: a) the proportion of families that completed development (successful families), *i.e.*, the number of families whose nymphs completed development / number of initial marked families, b) the total number of adult offspring per initial family, *i.e.*, the total number of adult offspring counted per plant / initial number of reproductive females marked and c) the total number of adult offspring per family that had completed development. We performed the comparisons using

Mann–Whitney tests for the first two variables and a Student's *t*-test for the last one.

2.5. Demographic analysis

Since the first and second nymphal instars are not mobile and depend completely on maternal care for survival (Torrico-Bazoberry et al., 2014), we combined them into a single stage for modelling population dynamics by means of MPMs. Additionally, we combined third, fourth and fifth nymphal instars into a single stage since they are mobile stages which do not completely depend on maternal care for survival but complete their life cycle on the same individual plant where they were born (Torrico-Bazoberry et al., 2014). Thus, we modelled the life cycle of *A. grossa* as a 4-stage *Lefkovitch* matrix: egg (*E*), nymphs 1 (*N1*: first and second nymphal instars), nymphs 2 (*N2*: third, fourth and fifth nymphal instars) and adult (*A*) (Caceres-Sanchez et al., 2017).

We only considered the female component of the population, following Caswell (2001) and multiplying the number of eggs, nymphs 1, nymphs 2 and adults by the female proportion on each host (0.56 on BS and 0.54 on SU; Pinto, 2015; Caceres-Sanchez et al., 2017). We constructed 29 four-stage demographic matrixes (13 for BS and 16 for SU), one for each individual plant with data from all marked families on that plant (Caswell, 2001; Caceres-Sanchez et al., 2017). These matrixes included the following demographic parameters: fecundity of adults (FA), the survival rates or probabilities of surviving and remaining at the same stage (stasis) for an egg, nymphs 1, nymphs 2 and adult (SE, SN1, SN2 and SA, respectively), and the growth rates or probabilities of surviving and reaching the next stage (growth) for an egg, nymphs 1 and nymphs 2 (GE, GN1 and GN2, respectively). We determined adult fecundity (FA) as the average number of eggs deposited by the females on each individual plant. We estimated stasis and growth parameters following Caswell (2001) and compared female fecundity between hosts using the Student's *t*-test.

We constructed an individual MPM for each plant. We conducted an analysis using Poptools (Hood, 2010) to obtain λ, a value calculated as the dominant eigenvalue, $λ_1$, of the *population projection matrix* (Caswell, 2001; Hood, 2010). We calculated the mean λ values for *A. grossa* ovipositing on BS and SU using each plant as a replicate. We then performed comparisons of λ values between hosts using the Student's *t*-test.

To evaluate if λ values could be explained by any of the abiotic microenvironmental (*i.e.*, temperature, relative humidity and luminosity) or biotic [*i.e.*, predator diversity (Brillouin's index), parasitism (number of parasitic mites per family) or indirect competition (herbivory index)] variables at the plant level, we performed 12 general linear model analyses (GLM) considering λ as the dependent variable and each of the environmental variables as the predictor variable for each host separately (*i.e.*, 12 GLM arise from 6 variables in 2 hosts). We performed GLM analyses using Statistica 10.0 (StatSoft, Inc., 2001).

2.6. Sociality

We estimated the duration of maternal care by each marked *A. grossa* female as the total number of days that the female was observed next to her offspring. Then, we performed Spearman correlation tests on families of *A. grossa* on each host to assess the relationship between the duration of maternal care, as a proxy of subsociality and the number of nymphs at each stage (hatched nymphs, second instar nymphs and third instar nymphs) as proxies of offspring survival. Also, we compared the duration of maternal care between hosts using Mann–Whitney tests considering: a) the total duration of maternal care (as described above) and b) the duration of maternal care minus the duration of the egg stage, thus considering only maternal care duration after the eggs hatched. The latter comparison was performed because the total duration of maternal care could be biased by the development stage of the egg mass when the family was marked. We repeated correlation

Table 1

Stage duration in *Alchisme grossa* on *Brugmansia suaveolens* and on *Solanum ursinum*. Total duration of immature stages was compared using the Student's *t*-test. Duration of each developmental stage between host plants were compared using the Mann–Whitney test. $N = 22$ for *B. suaveolens* and $N = 18$ for *S. ursinum*. * shows statistical difference between hosts at $p < 0.05$. t = Student's *t*-test, U = Mann–Whitney test.

Stage	Mean ± SD duration in days on *B. suaveolens*	Mean ± SD duration in days on *S. ursinum*	Statistical parameter	*p*
Egg*	33.4 ± 5.5	25.3 ± 10.8	$U = 103.5$	0.01
First instar*	11.8 ± 3.0	9.7 ± 3.3	$U = 124.5$	0.043
Second instar	10.4 ± 3.3	9.1 ± 3.8	$U = 151.5$	0.271
Third instar	12.0 ± 4.5	10.5 ± 3.9	$U = 147$	0.259
Fourth instar	15.2 ± 4.0	16.0 ± 3.9	$U = 175.5$	0.546
Fifth instar	24.7 ± 7.6	24.1 ± 5.0	$U = 189.5$	0.827
Total duration of immature stages*	107.5 ± 12.3	94.8 ± 13.7	$t = 0.380$	0.004

analyses taking into consideration the absence of egg mass duration to evaluate if patterns were maintained.

3. Results

3.1. Life cycle duration

We marked 120 families (69 on BS and 51 on SU). Total duration of immature stages of successful families differed between hosts ($t = 3.08$, d.f. = 38, $p = 0.0038$) and was longer on BS (Table 1). Duration of the egg stage and first nymphal instar differed between hosts ($U = 103.5$, $p = 0.01$ and $U = 124.5$, $p = 0.043$ respectively), both being longer on BS.

3.2. Insect performance

There was no significant difference between hosts in the proportion of successful families (Table 2). There was no significant difference between hosts in the total number of adult offspring per initial family (*i.e.*, total number of adult offspring/initial number of marked females) or in the total number of adult offspring per family (*i.e.*, total number of adult offspring/number of families whose offspring survived to produce adult offspring). Adult fecundity (number of eggs/female), number of eggs per female of successful families and number of hatched nymphs per female did not differ between hosts (Table 2). The number of eggs per female of successful families and the number of hatched nymphs per female did not differ significantly. This indicates that the starting condition (*i.e.*, eggs laid or first instar nymphs) did not differ between hosts, thus excluding a possible bias in the other performance parameters.

3.3. Microenvironmental variables

Abiotic (luminosity, temperature and relative humidity) and biotic (abundance and diversity of predators per plant and herbivory index) traits differed between hosts, except for mean abundance of mites per family (Table 3). Eight predator morphospecies were found on both hosts: four species of spiders (Aranae) and four insect species: the assassin bug (Hemiptera; Reduviidae), the lady beetle (Coleoptera; Coccinellidae), the elaterid (Coleoptera; Elateridae; *Semiotus sp.*) and the wasp (Hymenoptera; Vespidae). None of these species were exclusive to a given host; however, predator diversity and abundance were higher on BS (Table 3). None of the 12 GLM analyses were statistically significant ($p > 0.05$), suggesting that none of these microenvironmental variables explained λ values.

Table 2

Insect performance parameters (mean ± SD) of *Alchisme grossa* on two hosts, *Brugmansia suaveolens* and *Solanum ursinum*. Each individual plant was used as experimental unit. *t* = Student's *t*-test, *U* = Mann–Whitney test.

Insect performance parameters	B. suaveolens	S. ursinum	Statistical parameter	p
Number of eggs / female	79.1 ± 17.3	79.0 ± 20.5	t = −1.65	0.11
Proportion of successful families	0.7 ± 0.3	0.9 ± 0.2	t = 1.43	0.14
Number of eggs per female of successful families	81.0 ± 12.4	89.3 ± 20.2	t = 0.18	0.86
Number of hatched nymphs per female	64.0 ± 22.0	62.5 ± 24.3	t = 0.57	0.57
Total number of adult offspring per initial family	14.8 ± 13.3	18.2 ± 14.9	U = 89.5	0.54
Total number of adult offspring per family that had completed development	23.8 ± 15.1	21.1 ± 16.5	t = −0.18	0.86

3.4. Demographic analysis

Finite rates of increase (λ) were 1.013 ± 0.031 (mean ± SD) on BS and 1.021 ± 0.039 (mean ± SD) on SU. There was no statistical difference in λ between hosts (t = 0.56, d.f. = 27, p = 0.58).

3.5. Sociality

Significant correlations were observed between maternal care duration and the number of hatched nymphs (BS: $R = 0.79$, $p < 0.01$, n = 69; SU: $R = 0.45$, $p < 0.01$, n = 51), second instar nymphs (BS: $R = 0.78$, $p < 0.01$, n = 69; SU: $R = 0.57$, $p < 0.01$, n = 51) and third instar nymphs (BS: $R = 0.73$, $p < 0.01$, n = 69; SU: $R = 0.49$, $p < 0.01$, n = 51). Similar statistical patterns were obtained after repeating the analyses using maternal care duration after egg hatching on both hosts; the correlation coefficients (R) increased in all cases: hatched nymphs (BS: $R = 0.79$, $p < 0.01$, n = 69; SU: $R = 0.58$, $p < 0.01$, n = 51), second instar nymphs (BS: $R = 0.87$, $p < 0.01$, n = 69; SU: $R = 0.66$, $p < 0.01$, n = 51) and third instar nymphs (BS: $R = 0.82$, $p < 0.01$, n = 69; SU: $R = 0.60$, $p < 0.01$, n = 51).

There were no significant differences in the duration of maternal care between hosts considering either total duration ($U = 1393$, $p = 0.051$) or only data after egg hatching ($U = 1617$, $p = 0.42$). Maternal care extended from the first to third nymphal stages in both hosts. After this point, nymphs formed mixed aggregations along the main stem of the plants. Several mothers practically abandoned the host plant after the third nymphal stage of their families.

4. Discussion

The mean duration of the life cycle in cohorts of *A. grossa* was higher on BS than on SU. This could be due to the differences observed in microenvironmental traits evaluated on both hosts or to the effect of host features (e.g., chemical traits) affecting insect fitness, ecology and biology as has been demonstrated in several phytophagous insects (Futuyma and Moreno, 1988; Jaenike, 1990; Wood, 1993a). Microenvironmental temperatures were higher on SU than on BS. Since the rate of development of ectothermic organisms increases with temperature at their breeding site, the total time to reach maturity is reduced (Charnov and Gillooly, 2003; Kingsolver and Huey, 2008). A faster development time would be expected for *A. grossa* on SU than on

BS. The biotic environment around each host also suggests that SU is a better host than BS, since diversity and abundance of *A. grossa* predators differed between hosts and were around three and five times, respectively, lower on SU than on BS. A potential factor counteracting these costs for *A. grossa* living on BS is the higher competition for resources on SU, which had a higher herbivory index.

The difference in life cycle duration could potentially affect the reproductive isolation of groups living on each host. Some treehoppers which use alternative hosts show differences in traits such as life cycle duration and signals involved in mate recognition, which can interfere with a free interchange or breeding of individuals from such alternative hosts (Wood et al., 1999; Lin and Wood, 2002; Cocroft et al., 2008, 2010). *Alchisme grossa* living on alternative hosts have low vagility, *i.e.*, they show a tendency to stay on the same plant where they were born (Pinto, 2015) and developmental asynchrony (Torrico-Bazoberry et al., 2014;). Although this could be a consequence of reproductive isolation barriers, it may also represent a phenotypically plastic behavior in the development of this treehopper species. In this latter case, the effects of assortative mating may easily break down when even just a small number of individuals move between hosts. This was observed in mark-recapture studies, particularly with individuals which developed on BS (Pinto, 2015), and is reinforced by the lack of genetic structure in insects using both hosts (Pinto, 2015).

The proxies of performance of *A. grossa* evaluated in this study did not differ between hosts, consistent with the absence of differences in demographic parameters. Since host quality affects diverse traits of phytophagous insects (e.g., fecundity, survivorship), when an insect species uses more than one host, variations may be expected in the reproductive output on each host; such variations may in turn project into the population dynamics of individuals developing on such different hosts. Studies of these interactions under natural conditions are relatively scarce, especially in the Neotropical region. Thus, this work becomes one of the few examples of population dynamics in phytophagous insects developing on different hosts (Larsson et al., 2000; Awmack and Leather, 2002; García-Robledo and Horvitz, 2011).

Species within the Hoplophorionini have the highest degree of maternal investment among treehoppers (McKamey and Deitz, 1996). Maternal care has been shown to be crucial to offspring survival in the Hoplophorionini species *Umbonia crassicornis* Amyot & Serville. The absence of females led to a four-fold increase in predator success (Cocroft, 2002). In *A. grossa*, female removal led to 100 % offspring

Table 3

Mean values ± SD and comparison of abiotic and biotic environments between *Brugmansia suaveolens* and *Solanum ursinum*. Predator diversity is represented as the Brillouin's index. *t* = Student's *t*-test, *U* = Mann–Whitney test.

Parameter	B. suaveolens	S. ursinum	Statistical parameter	p
Luminosity (lux)	85.3 ± 54.4	131.5 ± 97.6	U = 1297	0.014
Temperature (°C)	19.0 ± 1.2	20.5 ± 2.2	U = 946.5	< 0.001
Relative humidity (%)	82.9 ± 4.6	77.9 ± 6.2	U = 917.5	< 0.001
Predator abundance per plant	3.5 ± 3.7	0.7 ± 1.3	U = 225.5	< 0.001
Predator diversity per plant	0.37 ± 0.4	0.06 ± 0.1	t = 4.063	< 0.001
Parasitic mites per family	0.8 ± 1.4	2.9 ± 8.2	U = 1632.5	0.444
Herbivory index	2.2 ± 0.7	3.3 ± 0.6	t = −5.09	< 0.001

mortality (Torrico-Bazoberry et al., 2014). In the present study, significant, positive correlations were found between maternal care duration and the number of eggs and of two nymphal stages on both hosts, thus reinforcing the idea that maternal protection may have evolved as a means of decreasing offspring mortality and enhancing female fitness (Tallamy and Schaefer, 1997; Torrico-Bazoberry et al., 2016).

Three different lines of arguments suggest that individuals of *A. grossa* used in these studies constitute a single treehopper population that uses two different sympatric hosts: i) *A. grossa* individuals accept both hosts and feed on any of them (Pinto, 2015), ii) evolutionary processes such as natural selection are operating in the same direction on both hosts (Torrico-Bazoberry et al., 2016) and iii) no genetic structure has been detected on insects living and developing on each host (Pinto, 2015). Nevertheless, this single population of *A. grossa* showed a difference in life cycle duration between hosts. It is possible that colonization of one of these hosts (probably *B. suaveolens*; Pinto, 2015) might be a recent event and evolutionary processes that promote ecological adaptation and hence demographic or performance differences have not yet become noticeable. This situation contrasts with that in the *Enchenopa binotata* complex in which population divergence has been shown (Wood et al., 1999) and host-insect associations are of long duration (Hsu et al., 2018).

5. Contribution of authors

CFP, DTB, LFP, ROB and HMN conceived and designed the study. DTB conducted fieldwork, DTB and CFP analyzed the data. CFP, DTB and HMN wrote the paper with contributions from LFP and ROB. All authors read and improved the manuscript and agreed to its final content.

Declaration of Competing Interest

None.

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