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Original article

The post-capping period of the tropical honey bee subspecies *Apis mellifera unicolor* in La Réuion

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Abstract – The honey bee *Apis mellifera* has shown a great intraspecific diversity, together with a variability of its life history traits. The development of the brood has been well documented in temperate but much less in tropical subspecies, but a limited number of studies focused on tropical subspecies. This study measures the post-capping period of *A. mellifera unicolor*, an African lineage subspecies living in a tropical and insular environment. The post-capping period was measured on 15 colonies distributed on two apiaries located at different altitudes (150 m and 900 m) in La Réunion. The mean post-capping period of *A. mellifera unicolor* was 280.1 ± 0.12 h and was significantly shorter in colonies at low altitude. A comparative analysis of the literature on the post-capping period for different subspecies showed that the post-capping period of the African lineage was significatively shorter than that of European lineage. *A. mellifera unicolor* post-capping period belonged to the hybrid cluster between them. Knowledge of such life history traits of *A. mellifera unicolor* may have implications for beekeeping practices and should be considered as one of the potential resistance traits to be evaluated in *Varroa destructor* breeding programmes.

***Apis mellifera unicolor* / post-capping period / tropical honey bee**

1. INTRODUCTION

There is a great variability in the life history traits of the honey bee *Apis mellifera,* reflecting its adaptation to a wide range of biotopes and climatic conditions. The species *A. mellifera* is divided into 33 subspecies belonging to five major evolutionary lineages (A, Y, C, M and O) (Ruttner 1988; Ilyasov et al. 2020). Some of the European subspecies have undergone anthropogenic selection for traits of interest in apiculture, in addition to natural selection in their native habitats. For instance, some subspecies, such as *A. mellifera ligustica* or *A. mellifera carnica* for their ability to produce more honey or to be more docile (Meixner et al. 2010; Cao et al. 2016; Carpenter and Harpur 2021; Wragg et al. 2022). While the life history traits of temperate subspecies have been extensively studied, the biology of tropical subspecies remains poorly known.

In temperate regions, which experience unfavourable winter conditions (cold temperatures

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and scarcity of resources), colonies perform overwinter with a significant reduction or even cessation of egg-laying and brood rearing (Döke et al. 2015, 2019). In tropical regions, where most of subspecies belong to lineage A, winters are warmer and associated with dry seasons, and colonies appear to raise brood permanently (Winston 1980; Feliciano-Cardona et al. 2020).

This brood dynamic in temperate areas is accompanied with an increase in pathogens load such as *Varroa destructor*, an ectoparasitic mite that reproduces inside capped brood cells during the honey bee nymphosis. The post-capping period is, therefore, a honey bee trait that could influence the parasite reproduction. For example, the post-capping period of *A. mellifera capensis*, a subspecies of lineage A, is much shorter than that of European subspecies, contributing to its natural resistance to *V. destructor* (Moritz and Hänel 1984; Moritz and Mautz 1990). A better knowledge of the life history traits of tropical honey bee subspecies, mostly of lineage A, would allow a better management of colonies in such climates and a better understanding of the dynamics of their pathogens. For example, treatment used to reduce *V. destructor*, such as oxalic acid, could be accompanied by a constrained brood-free period through the caging of the queen.

The present study focuses on a subspecies established in the South-West of the Indian Ocean, *Apis mellifera unicolor* (Latreille 1804). It is endemic to Madagascar and has spread to the islands of the surrounding archipelagos including La Réunion (Ruttner et al. 1978; Ruttner 1988; Rasolofoarivao et al. 2015; Techer et al. 2017). The honey bee populations of La Reunion were chosen because the island offers contrasted climatic areas. Reaching the highest point in the Indian Ocean (3070 m), its gradient gives rise to different microclimates and ecosystems, ranging from lowland tropical forest to subalpine vegetation (Cadet 1974). La Reunion has the highest proportion of original habitats compared to the other Mascarene islands, and 42% of the island is protected by a National Park classified as World Heritage (Baret and Strasberg 2005; Fenouillas et al. 2021). Honey bees can be found in all these micro-habitats where they contribute widely to the pollination of endemic plant species. In May 2017, the detection of the ectoparasitic mite *V. destructor* in La Reunion provoked significant colony losses, with an annual colony mortality increasing from 0.2 to 37%, and a disruption of beekeeping practices (Esnault, submitted). In this context, it was crucial to improve our knowledge on the post-capping period of a honey bee subspecies in a tropical environment. This study aimed to measure a trait of resistance to *V. destructor*, the post-capping period of worker brood, and to compare it with other periods found in the literature.

2. MATERIAL AND METHODS

2.1. Post-capping period experiment

The post-capping period was monitored in May 2021 on 15 *A. mellifera unicolor* colonies distributed in two different apiaries. Five colonies were located at high altitude (21° 17′ 3.426″S, 55° 33′ 41.819″E, 900 m), and 10 colonies located at low altitude (21° 19′ 19.107″S, 55° 29′ 20.156″E, 150 m).

The first day at 8 a.m., one frame containing at least one hundred uncapped cells with fifth instar worker larvae was extracted and photographed for each colony. The frames were photographed again at 12 a.m. and 4 p.m. to estimate the time of capping. From day 9 to day 14, the frames were photographed every 4 h until all imagoes emerged, *i.e.* at 8, 12, 16, 20, 0, 4 o'clock every day. Any manipulation of the frame did not exceed 4 min to minimize thermal stress. Honey bee brood is maintained by workers between 32 and 36 °C inside the hive and beyond this range, fitness losses have been reported (Stabentheiner et al. 2010). However, it has been shown that up to 12 h of exposure to 20 °C, causes less than 5% mortality in honey bee pupae (Wang et al. 2016). Brood frame manipulation time was therefore not found to cause thermal stress in our case. Images of the same frame were visualised in Fiji, an extension of the ImageJ software (Abràmoff 2004; Schindelin et al. 2012), and overlaid using the

"Register Virtual Stack Slices" plugin (Arganda-Carreras et al. 2006). The post-capping period of worker brood cells was calculated by subtracting the capping time from the emergence time. Values were expressed as mean ± confidence interval ($\alpha = 0.05$).

Statistical analyses were performed using the R Studio software (version 1.4.1106) (Team 2015). As the post-capping duration was asymmetrically distributed, a Box-Cox transformation (lambda = 11.6) was performed, using the "MASS" package, to correct the heteroscedasticity of the residuals. A linear model was then fitted using the "lme4" package, with the apiary and the colony as explanatory variables, followed by a multiple means comparison with the "emmeans" package to determine significant differences between groups.

2.2. Post-capping period subspecies comparison

Data on post-capping periods from the literature review were selected for comparison using means, standard deviations, and total number of brood cells. When the standard error was available, corresponding standard deviation was calculated as follows.

$$sd = se * \sqrt{n}$$

where sd = standard deviation; se = standard error; n = total number of brood cells

When these did not exist, the minimum and maximum observed post-capping periods were used. Considering the normal distribution of post-capping periods. We could say that 99.7% of the values were in the interval $[\bar{x} - 3sd; \bar{x} + 3sd]$ where $\bar{x}$ = the average post-capping period. The estimate of the standard deviation could be calculated as follows:

$$sd_{min} = \frac{\bar{x} - x_{min}}{3}$$

where sd_{min} = minimum standard deviation estimation; x_{min} = minimum observed post-capping period.

$$sd_{max} = \frac{\bar{x} - x_{max}}{3}$$

where sd_{max} = maximum standard deviation estimation; x_{max} = maximum observed post-capping period.

$$sd = \frac{sd_{min} + sd_{max}}{2}$$

The statistical analysis of the post-capping periods was performed using the R Studio software (version 1.4.1106) and the package "nparcomp" (Team 2021). In order to compare our data with those found in the literature, the Student's *t*-test was calculated for each combination of means. Benjamin and Hochberg (1995) correction was applied to the *p*-values and the package "multcompView" allowed the visualisation of the pairwise comparisons.

Each subspecies was associated with its evolutionary lineage (Garnery et al. 1992; Arias and Sheppard 1996).When data from the literature showed crosses between honey bee lineages, these were considered as "hybrid". Buckfast honey bee was classified within European lineages (Okuyama et al. 2018; Soares et al. 2019).

As these data were not normally distributed and the residuals were not homoscedastic, a non-parametric Kruskal-Wallis test was performed on the ranks of the post-capping periods to assess the effect of the evolutionary lineage on the post-capping period.

3. RESULTS

3.1. The post-capping period of *A. mellifera unicolor* varied with altitude

The post-capping period of *A. mellifera unicolor* was assessed on 853 and 1190 worker brood cells ($n_{total} = 2043$) for the low- and high-altitude apiaries, respectively. The post-capping period was significantly shorter at low altitude (279.00 ± 0.30 h) than at high altitude (281.40 ± 0.38, $F = 133.530$, $p < 0.001$). Significant differences were also observed between colonies ($F = 30.924$, $p < 0.001$),

with almost 10 h separating the colonies with the shortest (273.93 ± 1.80 h) and the longest (283.55 ± 0.82 h) post-capping periods (Figure 1).

3.2. *Apis mellifera unicolor* has a post-capping period similar to that of African/European hybrid and longer than that other African subspecies

The mean post-capping period of *A. mellifera unicolor* (280.1 ± 0.12 h) was analysed together with other periods found in the literature (Supplementary materials), representing 11 *A. mellifera* subspecies, with 46 values from European C and M lineages, 15 values from African A and Z lineages and 15 values from European/African hybrids (Figure 2).

The post-capping periods of subspecies from the African lineage were significantly shorter than post-capping of subspecies from the European lineages ($p < 0.05$). Hybrid post-capping periods were significantly shorter than European periods ($p < 0.001$) but not significantly different from African ones ($p = 0.0502$) (Figure 2). Post-capping periods of ten out of 15 European/African hybrids and two out of 46 European were not significantly different from *A. mellifera unicolor*. Post-capping periods of all other African subspecies showed a significant difference with *A. mellifera unicolor*.

4. DISCUSSION/CONCLUSION

The differences in the average post-capping period between colonies from the two apiaries could be explained by the ambient temperature of the apiaries and the ability of the colonies to thermoregulate. The post-capping period was 2.4 h longer at 900 m (with a mean temperature of 18.5 °C) than at 150 m of altitude (mean temperature of 23.9 °C). This could be explained by the longer development time for honey bee brood in a colder environment (Büchler and Drescher 1990).

Genetic variability may partly explain the variation observed between colonies from the same sites. Duration of capping in African and European subspecies is a highly genetically determined trait, with a heritability value of $h^2 = 0.87$ (Moritz 1985). Within the European subspecies, where the genetic variance is lower than between African and European subspecies, the lower heritability of $h^2 = 0.23$ is high enough to consider the trait as heritable and to establish a breeding program for honey bees with a reduced post-capping period (Büchler and Drescher 1990; Le Conte et al. 1994). The variability of post-capping period is a maternally and paternally influenced trait (Moritz and Jordan 1992). The post-capping period of *A. mellifera unicolor* was not significantly different from that of honey bees crossbred between A and European (M and C) lineages but was significantly different from that of other African subspecies within the same lineage (Figure 2). Analysis of COI-COII partial mitochondrial sequences of honey bee populations in the South-Western Indian Ocean (SWIO) showed different genetic origins. In La Reunion, the mtDNA COI-COII of *A. mellifera unicolor* was 95.4% from lineage A, 3.8% from lineage C and 0.8% from lineage M (Techer et al. 2017). Nuclear genome analysis revealed that individuals belonged to lineage A with introgression of European genetic background (Wragg et al. 2017). Past European introgression might therefore also partly explain the in-between status of the post-capping period in *A. mellifera unicolor* from La Réunion. Although this composition is widely represented by lineage A and due to the high genetic determination of post-capping period, differences in this trait should be observed in the SWIO.

Comparison of post-capping periods between species confirmed that lineage A and hybrid subspecies have shorter post-capping periods than European honey bees (Moritz and Hänel 1984; Moritz 1985; Calderón et al. 2010). However, there was no statistical difference between hybrids and African honey bees ($p = 0.0502$) (Figure 2). It is worth noting that the lack of statistical significance was related to the populations of *A. mellifera lamarckii* belonging to

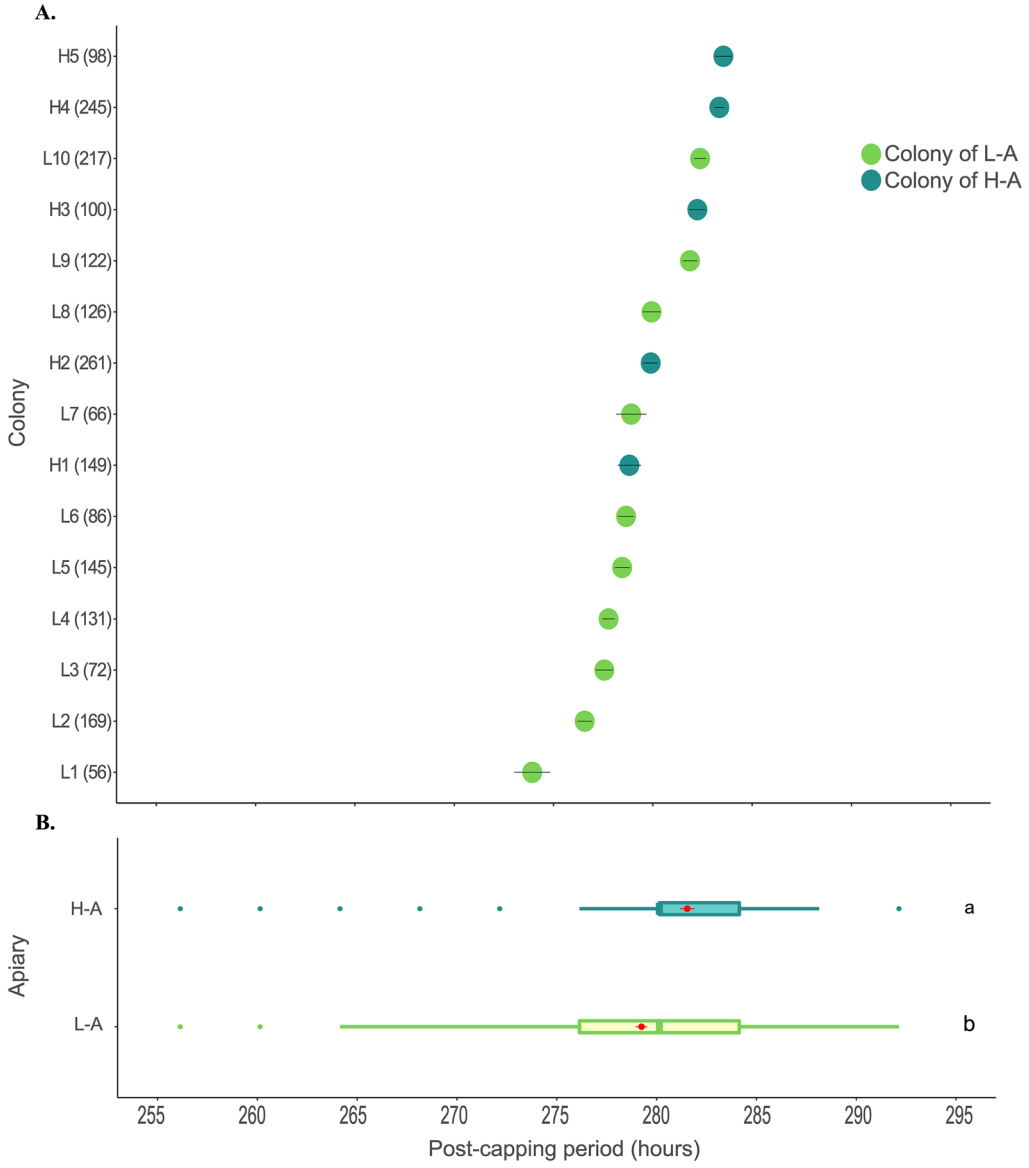
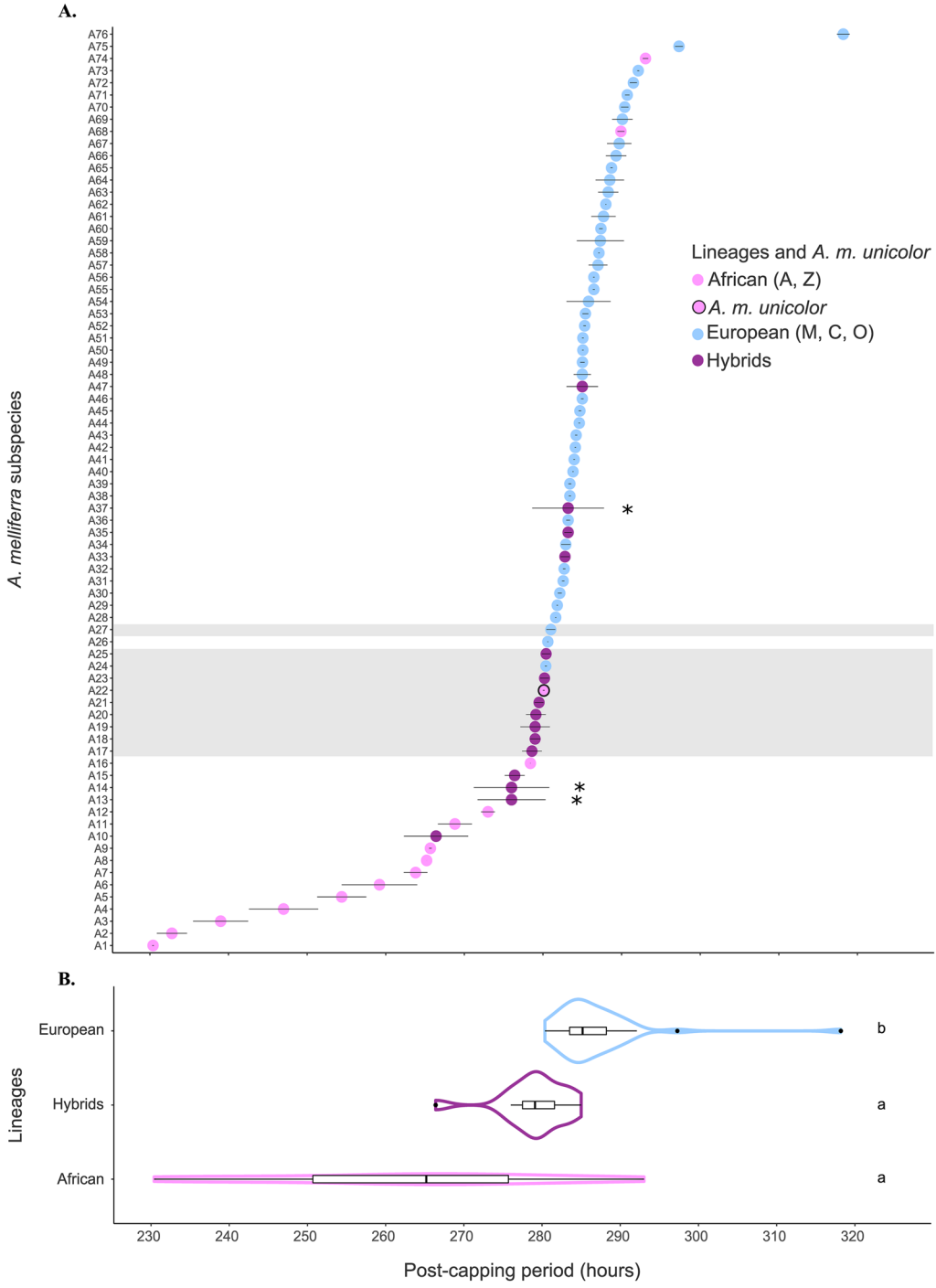


Figure 1. **A** Post-capping period of *A. ellifera unicolor* worker brood estimated in May 2021 at the low altitude (light green) and the high altitude (dark green) apiaries in La Reunion. Numbers in brackets on the y-axis correspond to the number of brood cells used to determine the post-capping period of each colony. Black lines indicate the standard deviation of the mean of each colony. **B** Post-capping period in low altitude (light green) and high altitude (dark green) apiaries. For each plot, the bold line indicates the median, the red dot and red line indicate the mean and standard error, the lower and higher boundaries of the box indicate the 25th and 75th percentiles respectively, the whiskers below and above the box indicate the 10th and 90th percentiles respectively and the dots indicate values outside those percentiles. The star indicates significant difference.



the evolutionary sub lineage Z (A16, A68 and A74) (Figure 2). Indeed, removing *A. mellifera lamarckii* from the analysis revealed significant difference between hybrids and African honey bees ($p = 0.0083$). Thus, the three African, European and hybrid clusters would be distinct, the

Figure 2. Post-capping period of *A. mellifera* subspecies. **A** Comparison of *A. mellifera unicolor* post-capping period with other subspecies. Subspecies belonging to the African lineage are in pink, the black circled pink point representing *A. mellifera unicolor*. Subspecies belonging to European lineages are in blue and hybrid honey bees are in purple. Black lines indicate the estimated standard error. Post-capping periods located in the grey areas were not significantly different from that of *A. mellifera unicolor*. Stars indicate unsignificant difference from *A. mellifera unicolor* due to large standard errors. **B** Post-capping period according to European (blue), hybrid (purple) or African (pink) lineages For each plot, the black line indicates the median, the lower and higher box boundaries indicate the 25th and 75th percentiles respectively, the whiskers below and above the box indicate the 10th and 90th percentiles respectively, and the dots indicate values outside those percentiles. The letters indicate significant difference between lineages.

latter being an intermediate between the two founders. However, within the African cluster, the post-capping periods of *A. mellifera capensis* are over-represented compared to the other subspecies considered in this cluster (six out of 15 for 5 subspecies diversity). The subspecific diversity of data should be increased to get a better idea of the differences between these clusters.

There are strong genetic similarities between *A. mellifera capensis* and *A. mellifera scutellata* and clear differences between *A. mellifera lamarckii* and other African subspecies (Eimanifar et al. 2017; Abou-Shaara 2019). The post-capping period ranks studied here support this trend, with low post-capping period ranks shared by *A. mellifera capensis* and *A. mellifera scutellata* and high ranks represented by *A. mellifera lamarckii* within the African group. The *A. mellifera lamarckii* individuals considered in this study originated from three different localities: Southern Egypt, with the shortest post-capping period (A16, 278.4 + 0.017 h), and Northern Egypt (A68, 289.92 + 0.45 h and A74, 293.04 + 0.35 h) (Figure 2) (Kaschef 1959). Imports of *A. mellifera carnica* into Egypt occurred between 1934 and 1962, resulting in different levels of hybridisation with *A. mellifera lamarckii* (Kamel 1991; Sheppard et al. 1996; Kamel et al. 2003), and possibly variations in post-capping period if these hybridizations could have been conserved. In conclusion, the wide range of post-capping periods observed in the African lineage may be due to the greater genetic diversity within African subspecies compared with European populations (Franck et al. 2001).

The reduction of the post-capping period is a known resistance mechanism against *V. destructor* (Ritter and Jong 1984; Camazine 1986). For instance, reducing the post-capping period by an hour led to an 8.7% decrease in mite infestation in European honey bees (Büchler and Drescher 1990; Sammataro 1996). The inter-colony variability in post-capping period observed in *A. mellifera unicolor* in La Réunion presents interesting opportunities for the selection of naturally mite-resistant colonies. Further investigation of other resistance mechanisms, such as MNR or VSH mechanisms, could be investigated on *A. mellifera unicolor* populations in La Réunion.

SUPPLEMENTARY INFORMATION

The online version contains supplementary material available at https://doi.org/10.1007/s13592-023-01032-w.

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AUTHOR CONTRIBUTION

All authors contributed to the study conception and design. BJ, GL, DD performed experiments and analyses. All authors contributed to the analysis and to the writing of the paper.

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DATA AVAILABILITY

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

CODE AVAILABILITY

R software custom codes for data analyses for the study are available from the corresponding author on reasonable request.

DECLARATIONS

Consent for publication All authors have read and approved the manuscript.

Competing interests The authors declare no competing interests.

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