



BIOLOGICAL SCIENCES

## High turnover of Chrysomelidae (Coleoptera) species in semideciduous forest remnants in an agricultural landscape

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**Abstract:** Differences in species composition between sites ( $\beta$  diversity) may be the result of spatial species replacement (turnover) or nestedness (subgroups of species from a more diverse site). In fragmented landscapes, the environmental factors that lead to these differences may be spatially structured. Herein, our objective is to determine if the  $\beta$  diversity of Chrysomelidae (Coleoptera) is due to turnover or nestedness and whether the observed pattern is due to loss of forest cover or spatial processes in forest remnants immersed in a matrix dominated by intense agricultural practice. We used an incidence matrix of 99 species sampled from 16 forest remnants and found that the difference in species composition among the fragments is mostly determined by turnover and that this variation is not explained by forest cover or spatial variables. In regions where high habitat loss has generated landscapes containing small and isolated forest fragments, structural features, related both to habitat (area, isolation, shape, etc.) and landscape (land use, landscape heterogeneity, etc.) could predict diversity patterns.

**Key words:** beetles, beta diversity, fragmentation, herbivory, insects.

### INTRODUCTION

The land-use conversion is one of the major threats to biodiversity, owing to its association with habitat loss and fragmentation. Currently, about 70% of the tropical forest remnants are isolated from original forests and subject to deleterious effects of fragmentation (Haddad et al. 2015). In this anthropogenic process, the remaining native vegetation cover is generally fragmented into many small patches (Fahrig et al. 2019) and, as a result, many animal and plant populations are isolated in small, disconnected fragments surrounded by a matrix that is usually composed of agriculture and pasture, unsuitable for the survival of many native species (Fernández-Chacón et al. 2014). Isolation

and size of fragments can lead to changes in the composition and structure of animal and plant communities over time, which may generate local extinctions and consequently affect essential ecosystem functions, such as decomposition (Didham et al. 1996). Small fragments are often considered of low conservation priority because they do not support viable populations and are very vulnerable to local extinctions. However, studies show that small fragments can increase beta diversity by creating irregularities in spatial distribution of species and different local extinction dynamics between fragments (Haddad et al. 2015). In addition, a set of small habitat patches collectively may harbor more species than certain large patches with similar total area (Fahrig et al. 2019), resulting in high  $\beta$

diversity in fragmented landscapes (Tscharrntke et al. 2012).

Differences in species composition between assemblies, referred to as beta diversity ( $\beta$ ) by Whittaker (1960), can be generated by two distinct phenomena: species spatial replacement (turnover) or nestedness (Harrison et al. 1992, Williams 1996, Lennon et al. 2001). Turnover is described as spatial substitutions of some species by other species along an ecological or temporal gradient, which implies simultaneous gain and loss of species due to environmental filters, competition and historical events (Leprieur et al. 2011). On the other hand, nestedness describes a condition in which sites with fewer species are subgroups of a site with a greater number of species (Baselga 2010). Nestedness, can reflect the diversity of niches present in the region or other ecological processes such as physical barriers (Legendre 2014). In this way, partitioning the  $\beta$ -diversity among turnover and nestedness is important for the development of biodiversity conservation strategies, as well as for accurate understanding of processes driving the observed patterns (Felinks et al. 2011).

In the present study, our objective is to determine if turnover, nestedness or randomness would drive  $\beta$ -diversity of leaf beetle species (Chrysomelidae: Coleoptera) of forest remnants immersed in an agricultural matrix. We also aim to understand the contribution of spatial structures and forest cover on the variation of total beta diversity and its components turnover and nestedness.

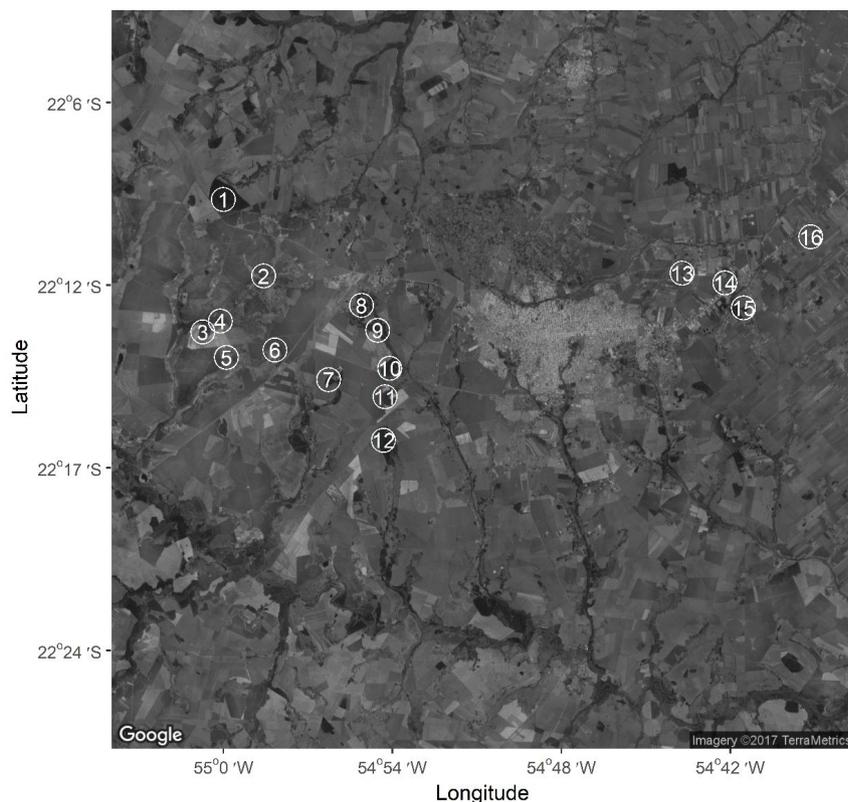
Chrysomelidae is a family of beetles with wide geographical distribution and enormous diversity, constituted by small beetles that represent a large part of the herbivorous insect fauna in diverse biomes (Andrew & Hughes 2004). More than 36,000 species are currently recognized in the family (Bouchard et

al. 2009), with estimates of more than 60,000 species (Jolivet 2015). In Brazil, more than 6,000 species are registered from more than 550 genera (Sekerka et al. 2017), however, in many states there is still a lack of knowledge about the diversity and ecological patterns of these insects, as in Mato Grosso do Sul. These insects are sensitive to environmental disturbances, respond to differences in habitat structural complexity (Linzmeier & Ribeiro-Costa 2009, Sandoval-Becerra et al. 2018, Teles et al. 2019) and have potential as bioindicators (Pimenta & De Marco 2015, Sánchez-Reyes et al. 2019).

## MATERIALS AND METHODS

The study was carried out in the Dourados municipality, located in the central-southern portion of the State of Mato Grosso do Sul, Brazil (22 ° 13'15 "S, 54 ° 48'21" W, 430 m altitude) (Fig. 1). The region is an ecotone with natural vegetation composed mostly of Alluvial Seasonal Forest and Cerrado, with influences of Atlantic Forest and Meridional Forest (Rizzini 1997), resulting in very diverse vegetation. However, the process of territorial occupation of the region was characterized by a lack of planning, causing extensive destruction of natural resources, leading to the replacement of native vegetation by agricultural crops and pastures for cattle raising (Martins 2001). The climate of the region, according to Köpen classification, is Cwa type, humid mesothermic, with wet summers and dry winters (Fietz & Fisch 2008) and a predominance of very clay-like Latosol (Amaral et al. 2000).

Between August 2012 and March 2013, we sampled 16 forest remnants with varying sizes (0.61-308ha) (Table I) using Malaise traps (Townes 1972). The sampling effort between the fragments was not the same, as it depended on the availability and access to privately



**Figure 1. Collection sites (16 forest remnants) of Chrysomelidae in Dourados, Mato Grosso do Sul.**

owned lands/properties. In total, we collected 60 samples, with each sample equivalent to 14 consecutive days of trap exposure. We separated the leaf beetles in morphotypes and identified through comparisons with specimens deposited at the “Coleção Entomológica Pe. Jesus Santiago Moure”, Universidade Federal do Paraná. We consider morphotypes those individuals that were not possible to identify at species level, but hereafter we name them species. The specimens vouchers are deposited in the “Museu da Biodiversidade da Faculdade de Ciências Biológicas e Ambientais”, Universidade Federal da Grande Dourados.

All analyzes were performed in the R program (R Core Team 2019) with different statistical packages (see details below). In order to determine if the differences in species composition found in fragments were due to turnover (spatial replacement) or nestedness,

we used a presence and absence matrix containing the 99 leaf beetle species. With this matrix, we calculated the total diversity, as well as its turnover and nestedness components, using the “beta.multi” function of the betapart R package (Baselga et al. 2018). We used the Sørensen dissimilarity index ( $\beta_{SOR}$ ), which was partitioned into the turnover components, calculated by the Simpson dissimilarity index ( $\beta_{SIM}$ ), and nestedness, expressed by the difference of the Sørensen dissimilarity index minus the dissimilarity index of Simpson ( $\beta_{SNE}$ ) (Baselga 2010). We tested the null hypothesis that leaf beetle diversity is randomly spatially distributed between the fragments by comparing the observed data against the results obtained by null models with the “oecosimu” function of the vegan package (Oksanen et al. 2018) using the model construction method “r1” with 1000 permutations.

**Table I.** Forest fragments of Chrysomelidae (Coleoptera) sampling in Dourados, Mato Grosso do Sul State, Brazil, with Malaise traps. S = species richness; N = abundance; Buffer (%) = percentage of forest cover in buffers of 250m and 1500m radius from the centroid of each fragment.

| Fragments | Area (ha) | N.samples | S  | N   | Buffer (%) |       |
|-----------|-----------|-----------|----|-----|------------|-------|
|           |           |           |    |     | 250m       | 1500m |
| 1         | 4.09      | 1         | 5  | 18  | 35.19      | 2.86  |
| 2         | 66.25     | 3         | 6  | 7   | 100        | 36.73 |
| 3         | 10.70     | 1         | 2  | 2   | 56.22      | 3.21  |
| 4         | 44.29     | 4         | 9  | 11  | 100        | 19.80 |
| 5         | 13.97     | 1         | 3  | 3   | 58.96      | 15.96 |
| 6         | 80.55     | 4         | 13 | 18  | 99.64      | 22.44 |
| 7         | 32.01     | 4         | 8  | 15  | 92.32      | 29.41 |
| 8         | 0.61      | 1         | 13 | 23  | 2.29       | 5.78  |
| 9         | 308       | 9         | 32 | 104 | 100        | 52.03 |
| 10        | 1.59      | 4         | 7  | 44  | 12.34      | 3.15  |
| 11        | 4.23      | 6         | 12 | 30  | 20.11      | 1.50  |
| 12        | 11.95     | 6         | 17 | 50  | 57.59      | 17.70 |
| 13        | 5.62      | 4         | 16 | 50  | 36.11      | 14.44 |
| 14        | 58.51     | 6         | 6  | 9   | 96.89      | 13.78 |
| 15        | 84.36     | 3         | 10 | 46  | 92.32      | 23.44 |
| 16        | 202.93    | 3         | 8  | 20  | 87.75      | 27.11 |

We used the percentage of forest cover in 250 and 1500 m radius buffers for each of the fragments as environmental variables. These variables were obtained through Landsat 8 images via the USGS EarthExplorer database (<http://earthexplorer.usgs.gov/>) in the ArcGis Desktop 10.5 program. The spatial variables were generated using the distance-based Moran Eigenvector Maps (dbMEM) method through the “dbmem” function of the adespatial package (Dray et al. 2019). This analysis creates spatial variables (axes) with the geographic coordinates of the centroids of the fragments, that represent spatial structures at different scales. We selected the first four axes that presented positive spatial correlation, so that the first two axes generated by dbMEM represent spatial variables referring

to a broad scale, while the latter two represent a fine scale.

In order to determine the contribution of spatial variables and forest cover (environmental component) in the variation of beta diversity and its components, we first calculated three dissimilarities matrices that represent the variation of the total beta diversity, the turnover component and the nestedness component. We calculated these matrices using the “beta.pair” function of the betapart package (Baselga et al. 2018). We performed a distance-based redundancy analysis (db-RDA) with variation partitioning for each response matrix (Dray et al. 2012, Legendre et al. 2012) to understand the importance of spatial and environmental variables in the variation of these matrices.

This analysis divides the variation in the response matrix into four components: [a] pure environmental component; [b] component shared between environment and space; [c] pure spatial component; and [d] unexplained variation.

## RESULTS

We found 450 leaf beetles belonging to 99 species (Table II). The most abundant and speciose subfamily was Galerucinae with 273 individuals and 43 species. Fifty-one species

(51.5% of total) presented only one individual and 10 species had 10 or more individual. The most abundant species ( $\geq 10$  individual) were Galerucinae sp.1 (53 individuals), followed by *Wanderbiltiana sejuncta* (Harold, 1880) (36 individuals), *Wanderbiltiana* sp.1 (35 individuals), Galerucinae sp.4 (32 individuals), *Systema* sp.1 (32 individuals) and *Costalimaita ferruginea* (Fabricius, 1801) (24 individuals) (Table II). Only four species were distributed in more than five fragments. *Wanderbiltiana* sp.1 and *W. sejuncta* were the most frequent species, found in nine of

**Table II.** Frequency and abundance (number of leaf beetles) of most frequent (> 5 fragments) and abundant ( $\geq 10$  leaf-beetles) Chrysomelidae (morpho)species\* sampled with Malaise traps in 16 remnants of semi-deciduous forest (sites) in Dourados, Mato Grosso do Sul, Brazil.

| Taxa   | Frequency (%) |          | Abundance |
|--|---------------|----------|-----------|
|  | on sites      | on traps |           |
| <b>Galerucinae</b>                               |               |          |           |
| <i>Systema</i> sp.1                              | 31            | 15       | 32        |
| <i>Wanderbiltiana sejuncta</i> (Harold, 1880)    | 56            | 28       | 36        |
| <i>Wanderbiltiana</i> sp.                        | 56            | 32       | 35        |
| Galerucinae sp.1                                 | 25            | 7        | 53        |
| Galerucinae sp.2                                 | 12.5          | 7        | 14        |
| Galerucinae sp.4                                 | 19            | 7        | 32        |
| <b>Eumolpinae</b>                                |               |          |           |
| <i>Costalimaita ferruginea</i> (Fabricius, 1801) | 19            | 5        | 24        |
| <i>Endocephalus</i> sp.1                         | 6             | 3.33     | 11        |
| <i>Maecolaspis</i> sp.1                          | 31            | 12       | 15        |
| Eumolpinae sp.11                                 | 12.5          | 3        | 19        |

\*For complete list access <[https://github.com/Tsteles/Chrysomelidae-Beta-Diversity-/raw/master/Complete\\_list\\_Chrysomelidae\\_Teles-et-al.pdf](https://github.com/Tsteles/Chrysomelidae-Beta-Diversity-/raw/master/Complete_list_Chrysomelidae_Teles-et-al.pdf)>

16 fragments, and *Maecolaspis* sp.1 and *Systema* sp.1 found in five fragments.

The beta diversity found in fragments was high ( $\beta_{SOR} = 0.94$ ) and due almost exclusively to turnover ( $\beta_{SIM} = 0.90$ ), compared to nestedness ( $\beta_{SNE} = 0.04$ ). This pattern differs significantly from what would be expected by randomness, indicating that leaf beetle species are not randomly distributed among the remaining forest fragments (Table III).

The variation partitioning showed that the variation of general beta diversity ( $\beta_{SOR}$ ) is explained almost exclusively by forest cover, however this explanation is low (2%) and insignificant (df = 2, F = 1.1872, P = 0.158; Fig. 2a). In the same way, forest cover explained little (3.8%) and was insignificant (df = 2, F = 1.2315, P = 0.214) for the variation in spatial replacement (turnover component -  $\beta_{SIM}$ ; Fig. 2b). Regarding the variation of the nestedness component ( $\beta_{SNE}$ ), forest cover and spatial component have no influence. On the other hand, unlike  $\beta_{SOR}$  and  $\beta_{SIM}$ , the shared fraction between forest cover and spatial component accounts for about 21% of the variation in nestedness (Fig. 2c). The variation explained by the spatial variables in  $\beta_{SOR}$ ,  $\beta_{SIM}$  and  $\beta_{SNE}$ , as well as the shared fraction between forest cover and spatial variables in  $\beta_{SIM}$

presented negative values and are interpreted as zero (Legendre 2008), as they indicate that the explanatory variables explain less variation than the normal random variables.

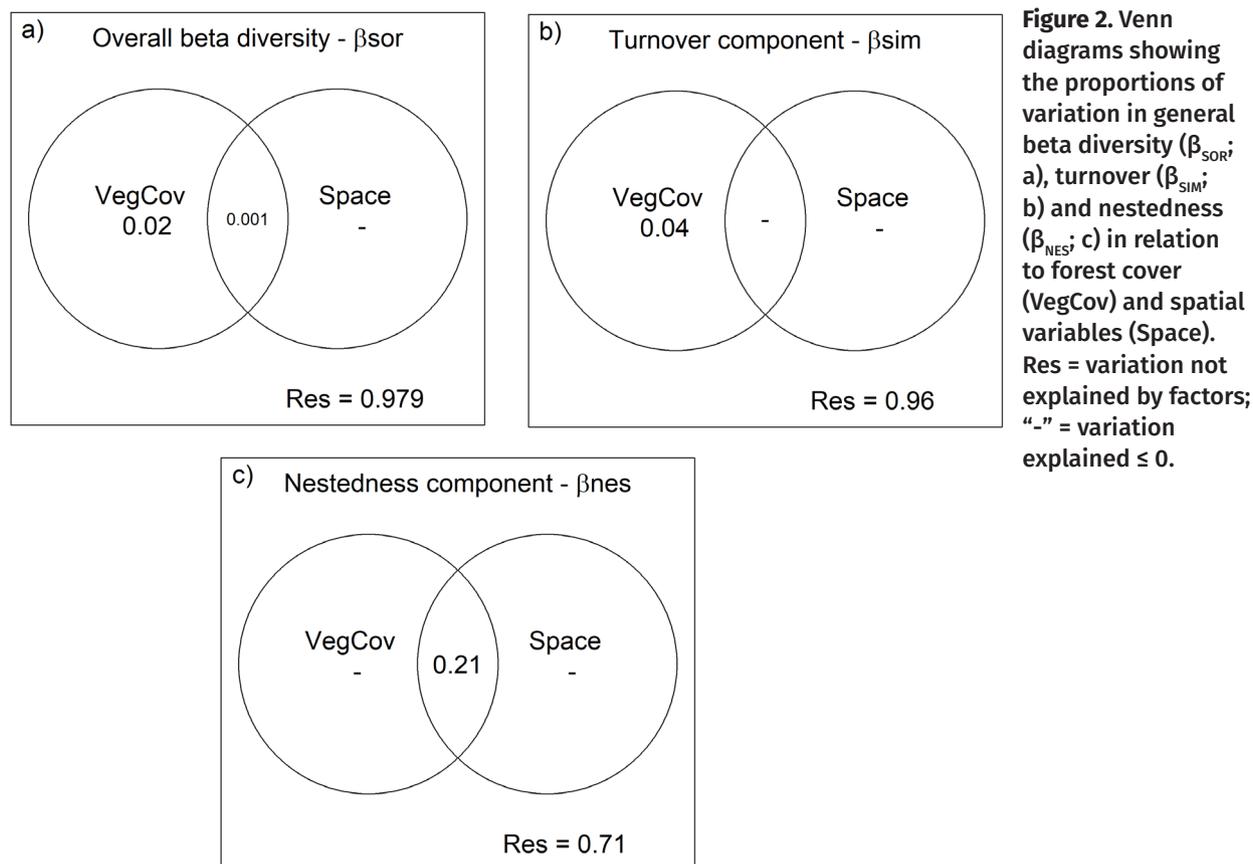
## DISCUSSION

Our results show that in forest remnants immersed in an agricultural matrix, the beta diversity of Chrysomelidae is high and differs significantly from what would be expected by randomness. This fact indicates that species are not randomly distributed among the fragments. We also observed that the difference in species composition between fragments is almost exclusively due to the turnover process, with great spatial substitution of species between the areas and a low contribution of the nestedness to this difference. However, although we see that species are not randomly distributed among fragments, this variation is neither caused by vegetation cover (environmental variables) nor by spatial variables.

The pattern of spatial species replacement (turnover) has been reported for several insect groups (e.g. Tenebrionidae, Fattorini & Baselga 2012, Carabidae, Gañán et al. 2008, Zygoptera, Brasil et al. 2018) including leaf beetles (Freijeiro

**Table III. Values of beta diversity ( $\beta_{SOR}$ ) with turnover ( $\beta_{SIM}$ ) and nestedness ( $\beta_{NES}$ ), calculated according to Baselga (2010), of Chrysomelidae (Coleoptera) from forest remnants of Dourados, Mato Grosso do Sul State, Brazil. Obs = observed value; SES = standardized effect size; Est = average of the estimated values based on 1000 simulations of null models ("r1" method); 2.5%, 50% and 97.5% = percentiles of the distribution of the estimated values; P = probability based on the comparisons between the observed values and the mean of the estimated values.**

|  | Obs. | SES   | Est. | 2.5% | 50%  | 97.5% | P            |
|--|------|-------|------|------|------|-------|--------------|
| Turnover ( $\beta_{SIM}$ )               | 0.90 | 5.01  | 0.87 | 0.86 | 0.87 | 0.88  | <b>0.001</b> |
| Nestedness ( $\beta_{NES}$ )             | 0.04 | -4.11 | 0.05 | 0.04 | 0.05 | 0.06  | <b>0.001</b> |
| Overall beta diversity ( $\beta_{SOR}$ ) | 0.94 | 5.76  | 0.92 | 0.92 | 0.92 | 0.93  | <b>0.001</b> |



& Baselga 2016) and different factors have been attributed as drivers of such patterns. For example, environmental factors, such as geographic constraints, indicate that they have played an important role in the compositional gradient of tenebrionids throughout the European continent (Fattorini & Baselga 2012). Environmental filters coupled with spatially structured processes are responsible for the spatial substitution of Cryptocephalinae and Chrysomelinae (Freijeiro & Baselga 2016) throughout the European continent. However, these patterns have been observed in studies at wide spatial scales and that present structural gradients of the entire landscape, encompassing wide structural variation and land use.

In fragmented landscapes, where the fragmentation process was preceded by intense

habitat loss, the spatial substitution of species could be the result of several factors such as decreased quantity and quality of habitat and the isolation of remaining habitat patches. Habitat loss and fragmentation may lead to the replacement of original fauna, where initially specialist species are replaced by more generalist species (Morante-Filho et al. 2016). In degraded landscapes, more specialist species tend to respond negatively to fragmentation and disturbance (Devictor et al. 2008). Regarding the isolation of areas, this fact can lead to a difference in the composition of local species due to differences in the dispersal capacity of different species. Isolation may also lead to low genetic variability within populations and to the local extinction of some species.

In our study system, fragments of forest remnants are immersed in a matrix dominated largely by agriculture and livestock, and thus constantly suffer pressures and disturbances caused by these production systems. In addition to the pressure generated by the intense use of agricultural pesticides, these fragments present small areas, are isolated from each other and suffer intense and uncontrolled extirpation of their trees (for wood) and fauna (hunting). Such historical factors may have contributed to the species substitution patterns that we found, since they have significantly altered the landscape overall, both structurally and in terms of land use. Thus, local factors more restricted to the habitat patch, such as structural factors (size, shape, insulation, etc.) and wider ones related to landscape characteristics (amount of vegetation cover, number of patches, spatial heterogeneity, different soil uses etc.) may be causing observed patterns. Different landscape patterns can interfere in both ecosystem services (Duarte et al. 2018) and insect communities (Rösch et al. 2013).

Based on our results, we speculate that other variables are causing such high species replacement among fragments. These variables could be related to structural factors of the habitat, such as insulation, shape, amount of edge, etc., as well as characteristics related to landscape characteristics such as habitat quantity, soil use, etc. Thus, in order to elucidate the processes that are structuring Chrysomelidae communities in forest fragments, we suggest that variables related to the local structure at the habitat patch level and variables related to broader scales such as composition and heterogeneity of the surrounding landscape should be considered.

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#### Author contributions

Thiago Silva Teles conceptualized and performed the study, analyzed the data and prepared figures and tables, identified the species, wrote and revised the versions of the paper. Francisco Valente-Neto and Danilo Bandini Ribeiro wrote and revised the versions of the paper. Josué Raizer assisted in the conceptualization and performance of the study, data analysis and preparation of figures and tables, and wrote and revised the versions of the paper. Adelita Maria Linzmeier assisted in the conceptualization and performance of the study, data analysis and preparation of figures and tables, species identification, and wrote and revised the versions of the paper.

