Revista Brasileira de Entomologia 60 (2016) 275–283

SOCIEDADE BRASILEIRA
DE ENTOMOLOGIA
FUNDADA EM 1937

REVISTA BRASILEIRA DE
Entomologia
A Journal on Insect Diversity and Evolution

www.rbentomologia.com

Review

Extant diversity and estimated number of Gracillariidae (Lepidoptera) species yet to be discovered in the Neotropical region

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ARTICLE INFO

Article history:
Received 28 April 2016
Accepted 9 June 2016
Available online 28 June 2016
Associate Editor: Rodrigo Feitosa

Keywords:
Leaf miner moths
Microlepidoptera
Species richness
Phylogenetic diversification

ABSTRACT

Gracillariidae (Lepidoptera) are commonly known by the leaf miner habit found in the larval stage of most species. By using worldwide, public databases on species diversity and DNA sequences available for extant gracillariid species, we determined changes in the rate of taxonomic species descriptions through time, mapped their spatial distributions, examined their phylogenetic diversification, and estimated the number of species yet to be described for the family in the Neotropics. We recovered 185 species, a number that is smaller than that found in any other biogeographic region. However, it was estimated that at least 3875 additional species remain to be described in the region. Phylogenetic diversification showed a pattern of expanding diversity. A few entomologists have been involved with gracillariid taxonomy in the Neotropics, having 39% of the species been described by a single taxonomist. In most of such cases, descriptions were based on the adults only. A few species have been described from biomes known to have some of the greatest diversity on earth, such as the Atlantic Forest. Thus, such a scenario results from low sampling and scarce taxonomic activity that has prevailed for this family of moths in the Neotropics. It may also be associated with their small body size and to the fact that gracillariids do not seem to be attracted to light traps as much as other moths, which make their collection and identification by non experts difficult. We also suggested scientific and political actions that could be adopted to overcome such an unfavorable scenario.

Introduction

Since the pioneer, comprehensive inventories that have been conducted on the diversity of terrestrial arthropods in general, it became evident that the Lepidoptera are among the most diverse group of insects existing in tropical rainforests, including the Neotropical region (Erwin, 1982; Lawton et al., 1998; Basset et al., 2012). These studies also led to the prediction that the diversity of Lepidoptera in such areas is much greater in numbers than what we really know, and thus many either remain to be discovered and/or need to be taxonomically described. In fact, several surveys carried out a posteriori in such regions focusing on Lepidoptera have given support to such a prediction (e.g. Brehm et al., 2005; Janzen et al., 2005; Linares et al., 2009; Lees et al., 2014; Zenker et al., 2016). These studies, however, with the exception of Lees et al. (2014), have been conducted with primary attention on sampling of the macrolepidoptera. This artificial group that includes the Papilionoidea butterflies and the other more derived moth superfamilies, accounts for ca. 107,475 species (68.27%), and thus ca. 2/3 of the total (=154,424 species) extant Lepidoptera (van Nieukerken et al., 2011). However, it includes only 34 families in the Order; that is, 25.56% of the total (=133 families). From a species rank perspective, this would suggest phylogenetic diversification (sensu Morlon, 2014) of younger lineages has been proportionally greater in Lepidoptera, which is supported by the existence of a similar trend at the generic level; that is, from the total of 14,655 genera listed by van Nieukerken et al. (2011), 9713 (=66.28%) are macrolepidoptera, and the remaining 4942 (=33.72%) microlepidoptera. However, one cannot reject a priori the alternative hypothesis that this trend results at least in part from bias, associated to lower collection

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http://dx.doi.org/10.1016/j.rbe.2016.06.002

efforts of the older, microlepidoptera lineages, which is explored herein for the Gracillariidae, as a case study.

Gracillariidae (Figs. 1–8) is considered one the most diverse microlepidoptera families having an endophagous feeding habitat, accounting for 105 genera and more than 1950 species that are distributed worldwide, except Antarctica (De Prins and De Prins, 2016a). Although larvae of most species are leaf-miners, some mine either stems or fruits, and others are flower/fruit borers, leaf rollers, or gall inducers (e.g. Davis, 1987; Guillén et al., 2001; Vargas and Landry, 2005; Hu et al., 2011; Hanson et al., 2014; Kawakita and Kato, 2016). The family was proposed by Stainton (1854) to ascribe at that time the genera *Gracilaria* Zeller, 1839, *Coriscium* Zeller, 1839, and *Ornix* Kollar,1832. The former is now a synonym of *Gracillaria* Haworth, 1828, the type genus of the family. The latter two are synonyms of *Caloptilia* Hübner, 1825, at the present time also within Gracillariidae. Caloptiliidae Fletcher, Eucestidae Hampson, Lithocolletidae Stainton and Ornichidae Stainton are now considered synonyms of Gracillariidae (De Prins and De Prins, 2016b). Three subfamilies are commonly accepted for the family: Gracillariinae, Lithocolletinae and Phyllocnistinae (Davis and Robinson, 1998). However, others have been proposed, such as Oecophyllembiinae (Réal and Balachowsky, 1966; Kumata, 1998), Ornichinae (Kuznetzov and Stekolnikov, 1987) and Ornixolinae (Kuznetzov and Baryshnikova, 2001). Recently, the monophyly of Gracillariidae

Figs. 1–8. Leaf mines (left) and adults (right) from putative species of Neotropical gracillariids: (1, 2) *Spinivalva gaucha* Moreira & Vargas (Gracillariinae) on *Passiflora actinia* Hook (Passifloraceae); (3, 4) *Porphyrosela minuta* Clarke (Lithocolletinae) on *Trifolium repens* Linnaeus (Fabaceae); (5, 6) *Angelabella tecomae* Vargas & Parra (Oecophyllembiinae) on *Tecoma fulva* (Cav.) G. Don (Bignoniaceae); (7, 8) *Phyllocnistis* sp. (Phyllocnistinae) on *Baccharis anomala* DC. (Asteraceae). Scale bars = 10, 1, 10, 1, 5, 1, 5, 1 mm, respectively.

was supported by analyses based on molecular data, including four subfamilies, which are adopted in the present study: Gracillariinae, Lithocolletinae, Phyllocnistinae and Oecophyllembiinae (Kawahara et al., 2011). Hypermetamorphosis, a dramatic change in morphology during larval onthogeny, from sap-drinking to tissue-feeding and/or spinning (see Kumata, 1978), has been proposed as an apomorphy for Gracillariidae (Davis, 1987). However, recently Brito et al. (2013) demonstrated that the gracillariine *Spinivalva gaucha* Moreira & Vargas, described from Southern Brazil does not have sap-feeding larval instars. On the other hand, Kuznetzov and Stekolnikov (1987) suggested Gracillariidae are unique within Lepidoptera by having male genitalia with only four pairs of muscles and without a gnathos, and female genitalia, with a short, laterally flattened ovipositor, which should be further explored. Although it has been accepted that there are many species of Gracillariidae still to be described in the Neotropical region (Davis and Wagner, 2011; Brito et al., 2012; Lees et al., 2014), as far as we are concerned no data available supporting such a prediction has been ever comparatively explored.

By using data existing in the literature and public databases available as electronic publications in the World Wide Web, we herein retrieved taxonomic data on Neotropical gracillariids, listing the valid species by author and year of description, and map the distribution of types according to corresponding biogeographic regions and political borders. Then, we adjusted the annual variation of the described species to a logistic model, predicting the number of gracillariid species still to be described in the Neotropics, as well as the time needed to achieve that goal given the present time rate of species descriptions in the region. Also, by using DNA sequences available in public databases we estimated the phylogenetic diversification of Gracillariidae in the Neotropics throughout time. These and additional findings such as on host–plant use are discussed in comparison to other biogeographic regions. By reviewing the status of the known diversity, evaluating their phylogenetic diversification and predicting the number of gracillariid species yet to be described in the Neotropics, we aim to establish a corresponding basis for future taxonomic studies in the region, suggesting research priorities and scientific actions, as well as the existence of potential difficulties to be surpassed regarding taxonomic impediment, if any. We hope our findings will stimulate further scientific interest in Neotropical gracillariids, not only from a taxonomic perspective but also their use as models on evolutionary ecology based studies, particularly those focusing on host-plant interaction, and also on conservation biology.

Material and methods

Data on species diversity were compiled from the catalog on Neotropical Gracillariidae provided by Davis and Miller (1984) and the public database on World Gracillariidae maintained by De Prins and De Prins (2016a). The global database of Gracillariidae (Lepidoptera) was launched in 2006 as an online searchable catalog of all known Gracillariidae species. The emphasis is focused on the following aspects: i) completeness (taxonomic information on all 1956 species, 413 synonyms of species names, 8 subspecies and 309 unavailable species-group names, 105 genera and 42 synonyms of genus-group names and 17 family-group names are found in a searchable format), ii) regular updates, i.e. twice per year, iii) presentation of cross-referenced verified data (data on gracillariid taxonomy, 9054 distribution records, 7641 host plants records and 4199 parasitoid records are presented online linked with the publication reference and citation page), iv) generation of data with user-targeted multi-searchable function (e.g. regional or country catalogs, detailed species checklists in association with their host plant species/genera/families; v) documentation of tri-trophic interactions of host(s)–gracillariid moth species and their parasitoid(s); vi) a robust gallery of 2188 images with the aim to document visually every gracillariid species including internal diagnostic characters of the last abdominal segments, morphology of pre-imaginal stages, species biotopes, as well as biological and ecological characteristics; vii) automatically generated country-linked species distribution maps; viii) type specimens and their depository; ix) robust reference list of 4806 publications; x) fossil species and species originally described as gracillariids but later transferred to other families.

To map the gracillariid species diversity, we adopted the regionalization of the Neotropical region as proposed by Morrone (2014), using the corresponding shape file that was provided by Löwenberg-Neto (2014) to plot the data. These authors adopted a restrictive definition of the Neotropical region, excluding the southern portion of the Andean area and also northern Mexico. To conceal this with the database provided by De Prins and De Prins (2016a), who adopted a broader view, these areas were herein included in the analyses, and thus treated in conjunction with the remaining subregions (sensu Cox, 2001). The idea was to have representative subdivisions from a biogeographic perspective, without necessarily taking into account the ranks proposed by Morrone (2014).

We estimate the potential number of gracillariid species still to be described in the Neotropical region by adjusting the cumulative number of described species through time to a three-parameter logistic model (Ratkowsky, 1990), by using the software SigmaPlot 11.0 (Systat Software, Inc.). Thus, we assumed there is a finite number of species existing on earth. Also, that as the number of recorded species nears the total number of species in existence for a given area it becomes increasingly difficult to discover new species in that area (Diamond, 1985; Cabrero-Sañudo and Lobo, 2003). This model showed a satisfactory fit when used recently by Cardoso et al. (2015) to predict the number of still undescribed mayfly species existing in Brazil. As these authors, the function was adjusted herein by using the Quasi-Newton method and criterion of convergence 0.0001. Also, the same default values adopted by them were used: that is, starting value of 0.1 and sept-width of 0.5, for all parameters. The adjustment was based on 200 interactions, using the number of 173 species considered as valid at present time, 1867 as the year of the first species description, and 2013, as the last one.

We calculate the expectation for the number of lineages of Neotropical gracillariids in a reconstructed phylogeny using 171 extant operational taxonomic units (OTUs), by conditioning on the age of the tree as well as with assuming a uniform prior for the age of the tree. We used the barcode index number (BIN), a system that clusters barcode sequences algorithmically, as a proxy of lineages, since clusters show high concordance with species. We employed this approach to document the diversity in the absence of taxonomic information for most of the available data. Sequences of the Cytochrome oxidase I (COI) gene (i.e., barcodes) were used to reconstruct the taxonomic position of species and genera, mainly from the BOLD and GenBank databases, but also incorporating novel OTUs sequenced by our research group (Tab. S1). *Bucculatrix* was used to root the tree (based on Kawahara et al., 2011; Wahlberg et al., 2013). Sequences were aligned using Clustal X in MEGA v6 (Tamura et al., 2013), according to default parameters. The best fit model of sequence evolution (GTR) was implemented according to the Akaike Information Criterion scores for substitution models evaluated using jModeltest v.0.1.1 (Darriba et al., 2012). BEAST v.2.1.3 (Bouckaert et al., 2014) was used to estimate the divergence time under a Bayesian approach (Drummond et al., 2006). An uncorrelated lognormal model of rate change was implemented. To test whether the diversification rates have changed over time (i.e. increased lineage speciation) we contrasted the likelihood values of the data (branching times derived from the trees) under two models of constant speciation rate (λ). Two independent analyses using as tree prior a pure birth model with a $\lambda = 0$ and extinction rate $\mu = 0$ (the Yule process), and a birth-death model with $\lambda > 0$ and $\mu > 0$, were run using 100 million generations sampled every 10,000 generations. Convergence was confirmed by effective sample sizes over 200 for all parameters in Tracer 1.6 (Rambaut et al., 2014). The resulting trees and divergence times estimated under different tree priors were similar. Bayes Factor analysis was performed under the smoothed marginal likelihood estimate and with 1000 bootstrap replicates computed in Tracer v.1.6, but it did not provide strong support for selecting any of the priors (BF < 2). We chose to use the trees reconstructed under the birth–death process tree prior as it accounts for both speciation and extinction (Gernhard, 2008). The time calibration (in millions of years [Ma]) was calculated using a second order date for the group Gracillariidae + Bucculatricidae, proposed by Wahlberg et al. (2013). We applied a normal distribution for the prior with a mean of 115.34 Ma (108.34–122.34; 99% CI). To examine (visually) the temporal pattern of lineage diversification, mean semi-logarithmic lineage through time (LTT) plots were constructed using Tracer v1.6 with 95% confidence intervals generated on the final BEAST trees.

Results

A total of 185 Gracillariidae species for the Neotropical region (Fig. S1) were recovered, belonging to four subfamilies and 26 genera (Tab. S2). The Gracillariinae were the most represented (145 species), followed by the Lithocolletinae (21), Phyllocnistinae (17) and Oecophyllembiinae (02) (Fig. S2). Corresponding type localities are scattered distributed in space, varying in number from zero species in the South-eastern Amazonian dominion to 42 in the Pacific dominion (Fig. 9). Most Neotropical countries hold at least one type locality; Brazil accounted for the greatest number of types, followed by Ecuador and Peru (Fig. S3).

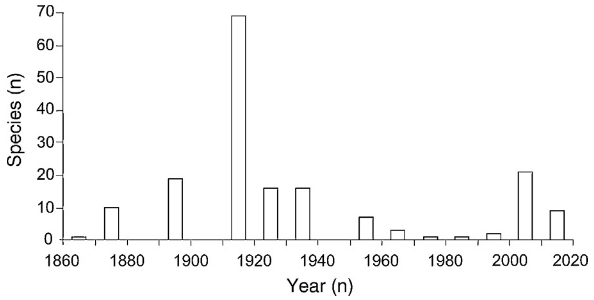

Fig. 10. Annual variation in number of gracillariid species described for the Neotropical region.

Species description-through time plot showed an erratic pattern (Fig. 10), starting in 1867 with the first described species, and reaching a peak between 1911 and 1920 when 69 species were described. A total of 24 authors accounted for the corresponding descriptions. The British taxonomist Edward Meyrick produced the greatest number of contributions in this regard (78 species; Fig. 11). Types were designated in the original description for most cases. They were deposited among 16 museums that are located mostly abroad, mainly in the Natural History Museum,

Fig. 9. Diversity of extant gracillariid species (Arabic numbers) in the Neotropics, according to biogeographical regionalization proposed by Morrone (2014). Asterisks indicate areas not contemplated in his restricted definition of the Neotropical region (see text for further description).

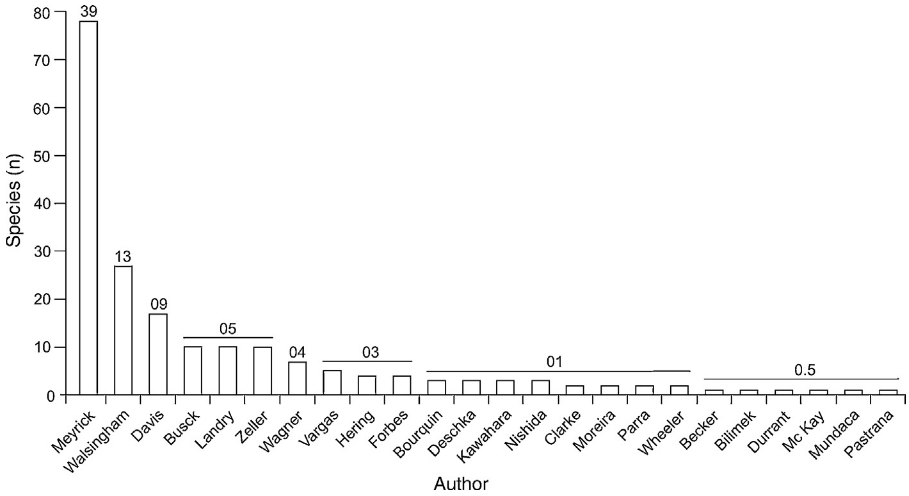

Fig. 11. Numerical contributions by author on description of gracillarid species for the Neotropical region. Numbers above bars represent percentages in relation to the total number of species ($n = 175$).

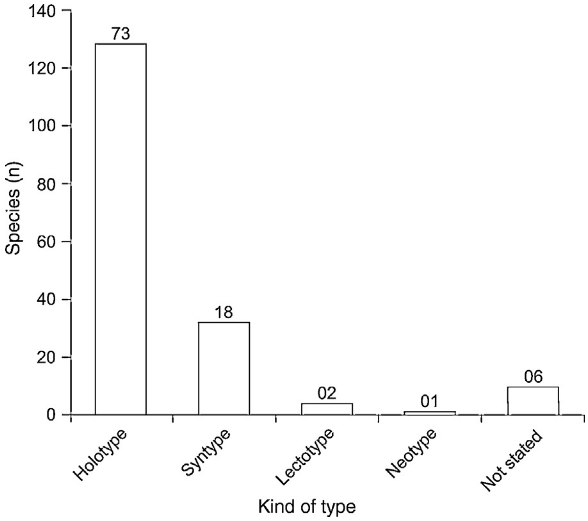

Fig. 12. Relative representation of type specimens for gracillariid species in the Neotropical region. Numbers above bars represent percentages in relation to total number of extant species ($n = 175$).

London (UK) and the National Museum of Natural History – Smithsonian Institution (USA) (Figs. 12 and 13). Original descriptions were based primarily on the adult stage, particularly on wing venation and color pattern. Data on the genitalia of either male or female were provided for only 46 (25%) in the original descriptions of species. Description of the immature stages (either egg, larva or pupa) are presented in 11% of the articles containing species descriptions and, regarding molecular data (DNA sequences), in only 4% (Fig. 14). Host–plant species were recorded in 29 families of angiosperms, Fabaceae being the most commonly used (Table S3, Fig. 15).

Cumulative species description-through time data adjusted well ($p < 0.05$) to the Logistic equation (Fig. 16). Annual rate of species description was 1.18 per year. The model predicted the existence of 4048 species in the Neotropical region. Corresponding standard error was 91,004 ($t = 44.487$, $p < 0.001$). Description of the remaining 3875 species still to be described in the Neotropical region would be completed in the year 2174.

Phylogenetic diversification revealed by the LTT plot for the Gracillariidae in the Neotropical region suggests a pattern of even speciation rates, with expanding diversity throughout the evolutionary time, with progressive, exponential accumulation of lineages until the last 25 Mya, and a nearly constant, linear rate from that to the present time (Fig. 17).

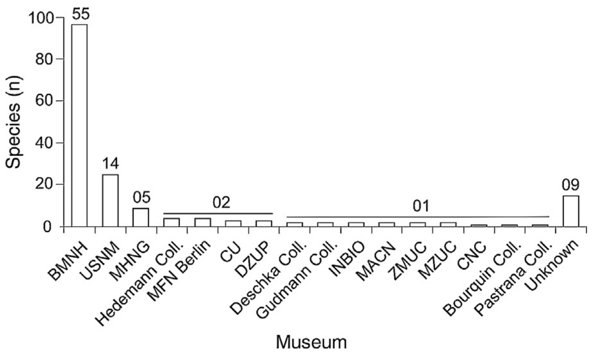

Fig. 13. Variation in number of type specimens available among museum collections for Neotropical gracillarids. Numbers above bars represent percentages in relation to total number of species ($n = 175$). See Tab S3 for description of museum acronyms.

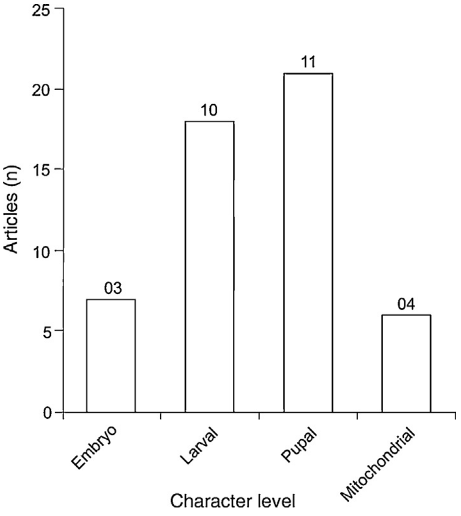

Fig. 14. Variation in number of articles regarding original descriptions on Neotropical gracillariids that were based on adults but that included also data either on gross morphology of immature stages or DNA sequences.

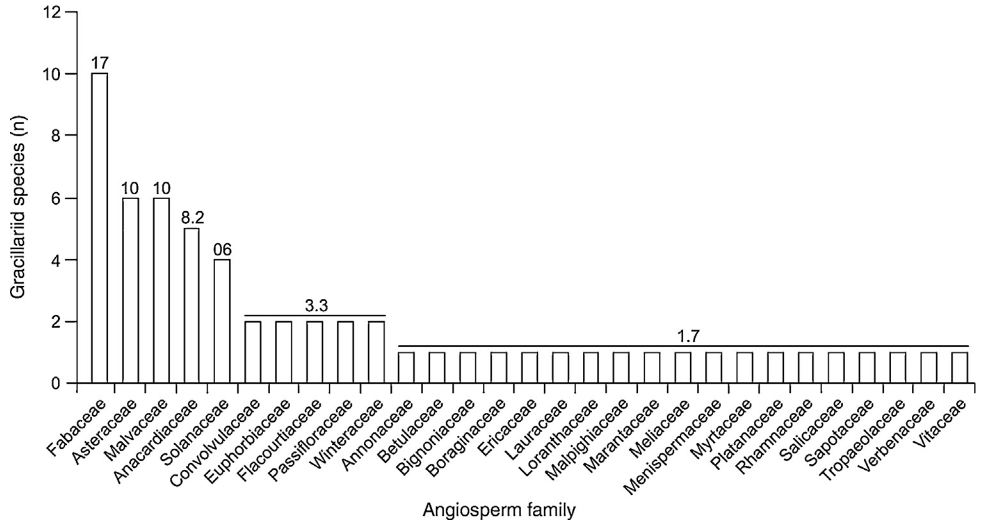

Fig. 15. Variation in number of records for angiosperm families used as hosts for gracillariid species in the Neotropical region. Numbers above bars represent percentages in relation to total number of plant families.

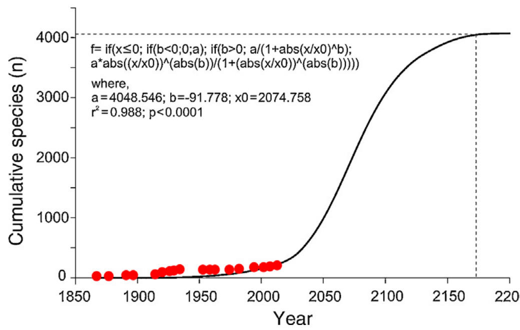

Fig. 16. Predictive future cumulative species description curve for the Gracillariidae in the Neotropical region, based on the Logistic model.

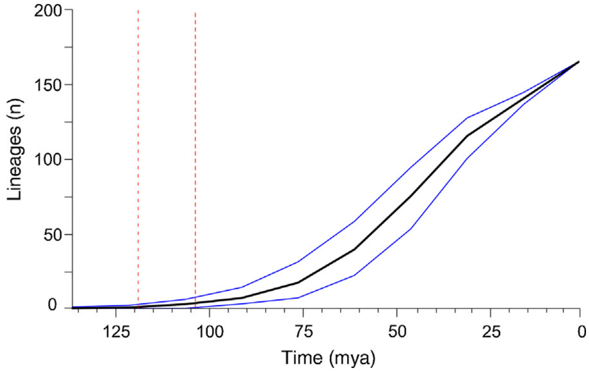

Fig. 17. Lineage-Through-Time (LTT) plot of Neotropical Gracillariidae. LTT was plotted using 1000 trees from the COI dataset analyses. The number of lineages (*y* axis) is plotted against time (*x* axis) in such way that each increase on the number of lineages represents a cladogenesis (a node) in one of the 1000 phylogenetic trees. 2. Dashed red and blue lines represent 95%-confidence intervals for time of starting diversification and LLT plot, respectively.

Discussion and conclusions

Findings in the present study provided strong evidence that the small number of known Gracillariidae for the Neotropical region results from under sampling, associated with low taxonomic activity on the family throughout history in the region. It calls attention to the fact that such a number is the smallest (8% of the total) found for any other biogeographic region in the world (Fig. S1). This is not expected given the size and diversity of plants known to exist in the Neotropical region. In other words, this trend does not find support on what is observed for other Lepidoptera families whose worldwide diversity is better known. For example, if we consider Geometridae, Pyralidae and Nymphalidae, this relation in species diversity does not sustain; to the contrary, for these families diversity is greater in the Neotropics compared to the Palaearctic and Nearctic (Fig S5), the two greatest biogeographic regions in terms of area on earth. It is important to mention that the larval stage of many macrolepidopteran families are external feeders, and many are either oligophagous or polyphagous. As already mentioned, larvae of gracillariids in general are internal feeders, having a specialized feeding apparatus and thus are highly specific in terms of host–plant use, many being monophagous (Davis, 1987). Thus, for a given area, comparatively to such lepidopteran families, one would expect the diversity of Gracillariidae to be more influenced by that of plants, and in consequence should be greater. This trend would not be expected only in case of differences in age and variation of historical diversification rates due to any reason, which remains to be tested for these macrolepidopteran families in the Neotropics. However, Gracillariidae are known to be much older than these families (Davis and Robinson, 1998), and we provided evidence herein that its diversification in the Neotropics has progressively expanded at an exponential rate until 25 Mya, and at a constant rate thereafter until the present. This pattern of speciation rate over time has often been interpreted as a diversity-dependent effect resulting from a saturation of niche space following adaptive radiations (Phillimore and Price, 2008; Rabosky and Lovette, 2008; Etienne and Haegeman, 2012). In addition, LTT plot results are sensitive to incomplete taxon sampling (Nee et al., 1994; Ricklefs, 2007), which likely affected the estimates in the case of gracillariids. In spite of this, as a first phylogenetic diversification analysis on gracillariids, we were able to recover even rates of speciation throughout the evolution, indicating that Neotropical lineages have been the target of the diversification process for a long time in evolutionary history. Thus, we found no indication and hence refute the hypothesis for occurrence of a greater rate of diversification early in the gracillariid phylogeny. In other words, there is no indication for the existence of a negative balance between speciation and extinction rates that would lead to the existence of low diversity of gracillariids in the Neotropical region.

In fact our data shows that the number of gracillariid species described up to now from the Neotropical region is far from realistic. For example, the Paraná dominion, within which the Atlantic forest is located, accounted only for 7 species. The Atlantic forest is known as one of the areas with the greatest diversity of plants and animals on earth, being extremely rich in endemics (Myers et al., 2000; Carnaval et al., 2009; Freitas et al., 2011). Approximately 50% of almost seven thousand species of plants existing in the area are endemics (Stemann et al., 2009). As already mentioned, leaf-miner moths are highly host–plant specific, and based on this, Brito et al. (2012, 2013) predicted that hundreds of undescribed, endemic gracillariid species should exist in the Atlantic forest. Indeed, several gracillariid species reared from leaves collected in the southern part of the Atlantic forest, most belonging to angiosperm families not recovered as host plant of gracillariids in the present survey for the Neotropics, await for description in our laboratory. Other areas in Brazil, such as the central and northeastern portions of the Chacoan dominion (Cerrado and Caatinga biomes), and the occidental Amazon area have been traditionally misrepresented in Lepidoptera collections in general, due to low sampling efforts applied for these insects in such areas (Santos et al., 2008a,b). Recently, in a survey carried out in small areas of French Guiana and Ecuador, Lees et al. (2014) estimate the corresponding gracillariid richness to be 240 species, from which approximately 85% are believed to represent new species to science. While surveying gracillariids assigned to the genus *Phyllocnistis* Zeller in a localized area in Costa Rica, Davis and Wagner (2011) came to the conclusion that the richness for this genus alone should account for hundreds of species in the Neotropical region. This trend may well be extended to the temperate portions of the Neotropical region. The gracillariid fauna of the Southern Cone of South America in particular is largely unexplored, and this region accounts for at least 18,139 species of plants, among which ca. 43% are endemics (Zuloaga and Belgrano, 2015).

Thus, it is not surprising that the prediction made in this study suggests that the number of gracillariid species in the Neotropical region is circa 20 times greater. It is also important to note that the taxonomic descriptions of such unknown species would be completed only about two centuries from now. This estimate, however, was based on the low collection intensity and scarce taxonomy activity on gracillariids that have historically prevailed in the region, as already mentioned. Thus, such time may be altered substantially in the future, depending upon changes in priorities and the investment in field and laboratory research. Intensive surveys for gracillariid species when carried out in rich biomes (e.g. Lees et al., 2014) would substantially speed up the curve, reducing the time to reach such a plateau. This would be particularly true if modern molecular biology techniques, such as DNA barcoding (sensu Hebert et al., 2003) are used in conjunction with traditional alpha taxonomic methods. Use of DNA sequences in this case would facilitate not only the identification of adult specimens that may be collected by using any kind of trap, but particularly, in the association with their immature stages collected directly from host–plants, and vice versa.

Our data showed clearly that the underlying causes for paucity in the knowledge of gracillariid diversity in the Neotropics fit into the typical issues involved in what taxonomists called lately the "taxonomic impediment" (for discussion, see Lipscomb et al., 2003; Scotland et al., 2003; Wheeler, 2004; Carvalho et al., 2007). In other words, what has obstructed progress of gracillariid taxonomy in the Neotropics is primarily the scarce activity of specialists on such a fauna throughout history. Corresponding species descriptions have been low and erratic in time, and were provided in a superior number by foreign taxonomists that eventually worked with Neotropical gracillariids, mostly in the first half of the last century. Consequently, corresponding types were deposited mostly abroad, and more importantly, no major, well curated collection of gracillariids has ever been compiled for the region. There was no indication in this study that such a situation varies much among Neotropical countries. Just to give one example, the Serra Bonita collection (Camacãn, Bahia, Brazil), one of the greatest collections on microlepidoptera existing in the Neotropical region, has circa 1200 pinned-dried preserved adults of gracillariids that are placed into one drawer (V.O. Becker, pers. comm.). In our opinion, however, additional issues inherent to these leaf miner moths in particular have contributed to the maintenance of a poor knowledge about their taxonomy in the region, and should be taken into account when establishing policies needed to overcome such an undesirable scenario. First, as typical microlepidopterans, gracillariids are not easy to work with due to their small size, requiring extra ability from taxonomists to mount them, and especially during dissections needed for taxonomic descriptions, as for example their genitalia. Thus, the existence of appropriate equipment, such as high resolution light stereomicroscopes and scanning electron microscope facilities, and corresponding training, are important in this case. Second, they do not seem to be much attracted to light (Vári, 1961), and thus the use of light traps may be less efficient to capture them as adults compared to nocturnal macrolepidopterans. This means that other methods of capture, including rearing the adults from host–plant mines, are desirable, leading to better results in many cases. Searching for mines, however, is a time consuming activity, and sometimes the larvae occur under low densities and are difficult to access, as for example in the canopy. Third, recent studies have indicated that for some gracillariid lineages, as for example in the genus *Phyllocnistis* Zeller, specific variation is greater in the immature stages compared to the adults (Davis and Wagner, 2011; Brito et al., 2012). And thus, location of host–plants and rearing of these stages are not only desirable but required for an accurate identification in such cases.

Corresponding knowledge about gracillariid biology in general is also at the infancy level in the Neotropics, except for species of economic importance, as for example those with the potential for use as biological control agents of invasive plants (e.g. Davis et al., 1991; McKay et al., 2012). *Phyllocnistis citrella* Stainton, a cosmopolitan gracillariid species recently introduced into the region, becoming an important pest of *Citrus* orchards in many Neotropical countries, has been the focus of several studies (e.g. Vargas et al., 1998; Chagas and Parra, 2000; Jahnke et al., 2005; Santos et al., 2008a,b). The biology of the leaf miner *Porphyrosela minuta* Clarke, which causes damage on *Trifolium repens* in Uruguay was explored by Bentancourt and Scatoni (2007). Additional studies that do not focus on taxonomic descriptions are uncommon for Neotropical gracillariid species without any apparent economic interest (but see Storey-Palma et al., 2012, 2014; Maita-Maita et al., 2015). On the other hand, these leaf miner moths have been studied abroad from several biological perspectives, and they have been suggested to be used as models in studies involving the evolution of insect host–plant interactions. This is particularly due to the dramatic changes in larva morphology (hypermetamorphosis) found in most lineages (see Davis, 1987) that are associated with very specialized endophagous feeding habits (Wagner et al., 2000; Body et al., 2015). The existence of host shifts has been reported, with strong evidence for the existence of co-speciation with host–plants in some cases (Ohshima and Yoshizawa, 2006; Ohshima, 2008). Also, some gracillariid species have a mutualistic association with plants by acting as pollinators in the adult stage and feeding as larvae on the reproductive structures of such plants (Kawakita et al., 2004; Kawakita and Kato, 2006; Zhang et al., 2012). All these pervasive avenues in research, which must be preceded by a solid taxonomic background, remain to be explored for the Neotropical gracillariid fauna in general.

In summary, it was herein clearly demonstrated that the diversity and biology of Neotropical gracillariids remain largely unknown, which is attributed particularly to the existence of a taxonomic impediment, prevailing throughout the region. Thus, the establishment of scientific policies by governments of all countries is urgent in order to speed up gracillariid taxonomy and thus to overcome such an undesirable scenario in the Neotropics (for an example of such policies, see Aguiar et al., 2009). Finally, it is also important to mention that as far as we are concerned, no Neotropical gracillariid species has been taken into account up to now from a conservation biology perspective. As already mentioned, there are many species in this family throughout different geographic dominions in the Neotropical region that are specialists on rare and/or endemic plants (e.g. Davis, 1994; Vargas and Landry, 2005; Vargas and Parra, 2005; Mundaca et al., 2013; Brito et al., 2013). By being dependent on endemic host plants at a regional scale, these species in particular are under comparatively greater threat, since such plants are usually localized in distribution (Lewinsohn et al., 2005). To uncover further such kinds of hidden diversity of gracillariid species, and thus to set the basis for their conservation before extinction, we suggest endemic plants in particular should be prioritized on host-focused inventories for their associated leaf miners in the Neotropics.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

We are grateful to Carlos Lopez Vaamonde (INRA) for allowing us to access barcode data in the BOLD System Gracillariidae projects. Thanks are also due two anonymous reviewers for important suggestions made to the final version of the manuscript. R. Brito and G.L. Gonçalves were supported, respectively by a CNPq Doctoral Scholarship and a Postdoctoral CAPES Fellowship (PNPD), and O. H.H. Mielke and G.R.P. Moreira by grants from CNPq. This article is dedicated to Héctor A. Vargas (UTA) who early in this decade kindly introduced G.R.P. Moreira and R. Brito to the study of Neotropical gracillariids.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.rbe.2016.06.002.

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