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THAÍSSA BROGLIATO JUNQUEIRA ENGEL

MACROEVOLUTIONARY STUDIES IN MAXILLARIINAE (ORCHIDACEAE)

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Thesis presented to the Institute of Biology of the University of Campinas in partial fulfillment of the requirements for the degree of Doctor in Plant Biology.

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Orientadora: Eliana Regina Forni Martins

Co-orientadora: Ana Paula de Moraes

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RESUMO

Mudanças em número cromossômico e tamanho de genoma podem implicar alterações genômicas estruturais e culminar em efeitos imediatos na aptidão e no fenótipo dos organismos, estando frequentemente associadas a diferenciação de nicho. Uma vez que traços genômicos e morfológicos são herdáveis e taxa mais proximamente relacionados têm maiores chances de compartilhar distribuição geográfica, as possíveis associações entre mudanças genômicas, variáveis ecológicas e condições ambientais devem ser avaliadas sob uma abordagem filogenética. A subtribo Maxillariinae (Orchidaceae) constitui um excelente modelo para o estudo dessa temática, pois apresenta ampla distribuição espacial e grande diversidade morfológica e cariotípica. No entanto, as relações dentro da subtribo ainda não estão bem resolvidas, e sua diversa morfologia vegetativa, somada aos traços florais uniformes, impulsiona discussões sobre o melhor tratamento taxonômico para representar adequadamente a evolução do clado. O objetivo do presente estudo foi avaliar as correlações entre mudanças genômicas e as variáveis ecológicas e ambientais associadas à distribuição de orquídeas neotropicais, utilizando Maxillariinae como modelo. Para tal, uma hipótese filogenética utilizando os marcadores nrITS, mtak-trnk e atpB-rblc foi desenvolvida para a subtribo por Inferência Bayesiana a fim de entender relações entre gêneros da subtribo e para análises macroecológicas. Para entender como as espécies respondem ao ambiente, produzimos Modelos de Distribuição de Espécies (SDMs). Também compilamos informações sobre hábitos, número cromossômico e tamanho de genoma. Para avaliar as relações entre as variações nas características genômicas e as variáveis ecológicas e ambientais em uma abordagem filogenética, foram feitas regressões múltiplas por Mínimos Quadrados Generalizados Filogenéticos (PGLS). A hipótese filogenética obtida foi majoritariamente bem resolvida, recuperando a maioria dos gêneros como monofiléticos e com alto suporte, com exceção de *Nitidobulbon* e *Heterotaxis*. O core Maxillariinae também teve alto suporte, e as relações entre gêneros estão majoritariamente de acordo com a literatura, apoiando a divisão do antigo mega gênero *Maxillaria* em 17 gêneros menores. Em relação às respostas ambientais, observamos que temperatura, precipitação e medidas de variação de temperatura afetam a adequação ambiental para a ocorrência de espécies, sugerindo que a ocorrência de Maxillariinae pode ser favorecida em locais mais úmidos, com

temperaturas estáveis e invernos relativamente amenos. Sobre as regressões, ao excluir os poliplóides da amostra, o aumento do tamanho do genoma foi associado à deserção do epifitismo como único hábito possível e a ambientes mais secos. Essas mesmas condições foram observadas associadas ao aumento do número cromossômico, mas apenas quando os poliplóides são incluídos na amostra. Nossos resultados sugerem que a poliploidia pode estar permitindo que as espécies ocorram em ambientes mais adversos, possivelmente devido aos benefícios conferidos pela maior diversidade genômica. Por outro lado, a correlação de genomas maiores com os ambientes secos pode estar relacionada à deserção do epifitismo, uma vez que espécies com grandes genomas não podem ser epífitas, e espécies não epifíticas são capazes de acessar a umidade do solo. Mediando as relações entre hábitos e tolerâncias ambientais, as mudanças genômicas parecem ser um componente central para a distribuição das espécies, com a poliploidia permitindo que os organismos cresçam em condições mais adversas.

Palavras-chave: *Maxillaria*, Mínimos Quadrados Generalizados Filogenéticos, Modelos de Distribuição de Espécies, Poliploidia, Tamanho do Genoma, Nichos Ecológicos.

ABSTRACT

Changes in chromosome number and genome size imply structural changes to the genome and may cause effects on the fitness and the phenotype of organisms, being often associated with niche differentiation. Since genomic and morphological traits are heritable, and related taxa have greater chances of sharing spatial distribution, relationships among chromosomal changes, ecological variables and environmental conditions should be appraised under a phylogenetic background. Maxillariinae, a neotropical Orchidaceae subtribe, constitute an excellent model for studying this matter, since it presents a wide distribution and huge morphological and karyotypic diversity. However, relationships within the subtribe are not well resolved, and its diverse vegetative morphology, added to uniform floral traits, drives discussions on the best taxonomic treatment. In the present study we aimed to appraise the correlations of genomic changes to the ecological and environmental variables associated with the distribution of neotropical orchids, using Maxillariinae as a study group. To do so, we sequenced three DNA regions, nrITS, mtak-trnk and atpB-rblc, of species from genera underrepresented in previous phylogenetic studies, and adding species from databases to the matrix, we produced a new phylogenetic hypothesis through Bayesian Inference, to serve both the understanding of relationships in the subtribe, and the macroecological analyses. To understand how species respond to the environment, we produced Species Distribution Models (SDMs) using a maximum entropy algorithm. We also compiled from the literature information species habits and on chromosome numbers and genome sizes, which were added to new data produced through chromosome countings and flow cytometry. To assess the relationships between the variations in the genomic traits and the ecological and environmental variables we performed Phylogenetic Generalized Least Squares (PGLS) regressions. The phylogenetic hypothesis from our data was mostly well resolved, recovering most of the genera as monophyletic and well resolved, exceptions being *Nitidobulbon* and *Heterotaxis*. The core Maxillariinae was also strongly supported, and relationships within are in accordance to the literature, supporting the splitting of the former megagenus *Maxillaria* into 17 smaller genera. Regarding environmental responses, we found temperature and precipitation, as well as measures of temperature variation, to affect suitability for species distribution, suggesting Maxillariinae orchids occurrence might be favoured in humid and

stable sites, with relatively mild winters. When excluding polyploids from the sample, genome size increase was associated with desertion of epiphytism as only possible habit and to dryer environments, and these same conditions were observed associated with chromosome number increase, but only when polyploids were included in the sample. Our results suggested that, for chromosome number, polyploidy might be allowing species to occur at more disadvantageous environments, possibly owing to benefits from enhanced genomic diversity. Meanwhile, the correlation of bigger genome sizes with the dryes environments might be due to the desertion of epiphytism, since species with big genomes cannot be epiphytes, and non epiphytic species are able to access soil moisture. Mediating relationships among habit and environmental tolerances, genomic changes were demonstrated to be a central component to species distribution, with polyploidy allowing for organisms to grow under adverse conditions.

Keywords: *Maxillaria,* Phylogenetic Generalized Least Squares, Species Distribution Models, Polyploidy, Genome Size, ecological niches.

SUMMARY

INTRODUCTION

A century ago geneticists described polyploidy, not by observing meiotic behavior or karyotypic traits, but by perceiving outwardly distinctive phenotypic characteristics, morphologic intermediacy compared to progenitors, quantitative differences in growth and traits, differential spatial distributions and environmental tolerances, all putative indicatives of ecological divergences (Ramsey & Ramsey, 2014). At that time, a lot of that, although particularly foresighting, was circunstancial or rather speculative, but set the basis for the study of ecology of genomic changes. Up to nowadays, cytogenetics, nuclear and cytoplasmic genetic markers, flow cytometry, genomic sequencing, massive database on genome size and chromosome numbers, phylogenetic comparative methods and dozens of cutting edge technologies (Levin, 2002; Madlung, 2013; Mayrose & Lysak, 2020) have been applied in the understanding of chromosomal changes (Levin, 2002; Madlung, 2013; Mayrose & Lysak, 2020). Supported by such powerful tools, ecological studies have been assessing relationships between genetic traits such as chromosome number (Manzaneda *et al.*, 2012; Thompson *et al.*, 2014; Pimentel *et al.*, 2017) and genome size (Knight & Ackerly, 2002; Díez *et al.*, 2013; Souza *et al.*, 2019; Paule *et al.*, 2020) and ecological niches, seeking to identify potential factors underlying species distributions.

The species realized range and niche are primarily determined by species inherent abiotic requirements due to physiological constraints, by resource availability and biotic interaction partners, and by dispersal ability (Soberón, 2010). The above mentioned niche drivers are evolvable, and may hence be constrained by genomic traits, making chromosomal changes a potentially useful tool to understand species distributions. Nonetheless, these ecological investigations should be carried out with a phylogenetic background, since related taxa have a greater chance of sharing present or past spatial distribution, environmental conditions and selective pressures (Anacker & Strauss, 2014), and genomic traits. Yet, the comprehension of such entangled relationships still requires more studies, remaining an open question and a central subject in modern ecology.

LITERATURE REVIEW

1. A brief history of Maxillariinae

The family name Orchidaceae was used to group orchid species for the first time in 1737, in Linnaeus *Genera Plantarum (Linnaeus, 1737),* and became fully established as a family name when Jussieu published his own *Genera Plantarum* in 1789 (de Jussieu, 1789). At that time many orchid species were described, starting from 69 species in *Species Plantarum (Linnaeus, 1753)*. For species known today under the Maxillariinae subtribe, history officially began with the description of *Maxillaria* and *Anguloa* by Ruiz and Pavón (1794) in a comprehensive study of the Peruvian and Chilean flora. Following that, many other studies have described hundreds of neotropical species under many genera, and as species descriptions accumulated, a very diverse set of morphological traits drove botanists to attempt many classifications, creating subtribes, tribes, alliances, subfamilies and divisions, and constantly transferring species between them (Lindley, 1830-1840, 1826, 1843; e.g. Swartz, 1800; Brown, 1810; Richard, 1818; Bentham, 1881b; Bentham & Hooker, 1883; Pfitzer, 1887, 1889; Dressler & Dodson, 1960; Dressler, 1979, 1981, 1993; Senghas, 1993), wich included the description of the subtribe Maxillariinae by Bentham (Bentham, 1881a) in his *Genera Plantarum* (Bentham & Hooker, 1862-1883), a 27 years study in collaboration with George Hooker, comprising more than 92,000 species.

With the advent of molecular studies and DNA based phylogenies a progressively deeper comprehension of orchids taxonomy was allowed (Chase & Hills, 1992; Chase *et al.*, 1994; Neyland, 1995; Neyland & Urbatsch, 1996; Cameron *et al.*, 1999; Williams & Mark Whitten, 2003), shedding light on the relationships within the subtribe Maxillariinae. Reviews on orchids taxonomy supported a broadened subtribe (Whitten *et al.*, 2000; Chase *et al.*, 2003), including taxa formerly placed in the former Lycastinae Schltr., making Maxillariinae then encompass the genera *Anguloa* Ruiz & Pav., *Ida* A. Ryan & Oakeley (now accepted as *Sudamerlycaste* Archilla), *Lycaste* Lindl., *Neomoorea* Rolfe, *Xylobium* Lindl., *Bifrenaria* Lindl., *Guanchezia* G.A. Romero & Carnevali, *Horvatia* Garay, *Hylaeorchis* Carnevali & G.A. Romero, *Rudolfiella* Hoehne, *Scuticaria* Lindl., *Teuscheria* Garay, and the core Maxillariinae, *Anthosiphon* Schltr., *Brasiliorchis* R. Singer, S. Koehler & Carnevali, *Cryptocentrum* Benth.,

Chrysocycnis Linden & Rchb.f., *Cyrtidiorchis* Rauschert, *Maxillaria* Ruiz & Pavon, *Mormolyca* Fenzl, *Pityphyllum* Schltr., and *Trigonidium* Lindl.

In the early 2000's intra and intergeneric studies based on morphology and DNA were enhancing the comprehension of relationships within the subtribe and its genera, such as for *Cryptocentrum (Carnevali, 1997, 2001)*, *Anguloa* and *Lycaste* (Ryan, 2001), *Bifrenaria (Koehler et al., 2002; Koehler & do Amaral, 2004)*, *Heterotaxis (Ojeda et al., 2003, 2005)*, *Pityphyllum* (Whitten *et al.*, 2006) and *Brasiliorchis (Singer et al., 2007)*, but the relationships within the subtribe remained unclear, owing specially to the intricate *Maxillaria* (*sensu* Ruiz and Pavón). As originally circumscribed, *Maxillaria* had about 570 species (Christenson, 2013) widely distributed in the Neotropics, presenting a great diversity of vegetative morphologies, but flowers with relatively uniform structure (Szlachetko *et al.*, 2006).

A few initial attempts of classification of *Maxillaria* were made with morphological data (Carnevali, 1991; Senghas, 1993, 2000; Dressler, 1993; Christenson, 2002), but with support from DNA data, the genus *Maxillaria s.l.* was shown to be polyphyletic (Whitten *et al.*, 2000; Dathe & Dietrich, 2006). The first comprehensive phylogeny for *Maxillaria s.l.* and related genera analyzed four DNA regions of 618 specimens (354 species) looking for a molecular support that, associated with possible morphological synapomorphies, could uphold a better clade design (Whitten *et al.*, 2007). The authors observed that clades often recognized as genera, such as *Anthosiphon* Schltr. and *Chrysocycnis* Linden & Rchb.f., and even clades easily diagnosed by their distinct morphologies, such as *Cryptocentrum* and *Trigonidium*, were included in *Maxillaria s.l.* Since *Maxillaria sensu* Ruiz & Pavón would not be morphologically diagnosable (Whitten *et al.*, 2007), the new taxonomic treatment for the subtribe proposed by Blanco et al. (2007) and Pridgeon et al. (2009) recognized the division of *Maxillaria* Ruiz & Pav. into a core Maxillariinae of 17 genera, some of them segregated or resuscitated from *Maxillaria: Brasiliorchis* R.B.Singer, S.Koehler & Carnevali (*Maxillaria picta* group), *Camaridium* Lindl., *Christensonella* Szlach., Mytnik, Górniak & Smiszek, *Cryptocentrum*, *Cyrtidiorchis*, *Heterotaxis* Lindl., *Inti* M.A.Blanco, *Mapinguari* Carnevali & R.B.Singer (*Maxillaria rufences* group), *Maxillariella* M.A.Blanco & Carnevali (*Maxillaria variabilis* group), *Mormolyca*, *Nitidobulbon* Ojeda, Carnevali & G.A.Romero, *Ornithidium* Salisb. ex R.Br., *Pityphyllum*, *Rhetinantha* M.A.Blanco, *Sauvetrea* Szlach., *Trigonidium* Lindl. and *Maxillaria* Ruiz & Pav. itself. Besides the core Maxillariinae, the authors recognize as well

the genera *Anguloa* Ruiz & Pav., *Bifrenaria* Lindl., *Guanchezia* G.A.Romero & Carnevali, *Horvatia* Garay, *Lycaste* Lindl., *Neomoorea* Rolfe, *Rudolfiella* Hoehne, *Scuticaria* Lindl., *Ida* A. Ryan & Oakeley (=*Sudamerlycaste* Archila), *Teuscheria* Garay, and *Xylobium* Lindl. as belonging to the subtribe.

Since Whitten et al. (2007) and Blanco et al. (2007) studies, orchid researchers kept enhancing comprehension of Maxillariinae relationships, specially for the genera *Nitidobulbon (Ojeda et al., 2009)*, *Christensonella (Koehler et al., 2008, 2012)*, *Mormolyca* (Arévalo & Cameron, 2013; Arévalo *et al.*, 2015) and *Brasiliorchis (Novello, 2015)*, contributing to the reevaluation of generic boundaries. Subtribe relationships have also been investigated (e.g. Szlachetko *et al.*, 2012; Whitten *et al.*, 2014; Chase *et al.*, 2015; Schuiteman & Chase, 2015), and despite the new studies confirming the taxa recovered by Whitten et al. (2007), some authors disagreed with them in the taxonomic treatment adopted following their work.

For instance, attempting to summarize relationships within Maxillariinae, Szlachetko *et al.* (2012) proposed dramatic changes to the subtribe taxonomy. Besides having created new genera from *Maxillaria* s.l., as for instance *Calawaya* Szlach. & Sitko and *Pseudocymbidium* Szlach. & Sitko, the authors payed particular attention to *Camaridium* and *Ornithidium sensu* Blanco et al. (2007), which they considered to have difficult identification due to inaccurate and broadly defined generic delimitations. These two genera were then divided into 14 genera, five of which were proposed by the authors, and added to further divisions of *Maxillaria* s.l. and other recircumscriptions. The revision of the subtribe resulted in 36 genera, easily distinguishable through the dichotomous key they presented. However, this classification is not adopted by orchid researchers because most of the proposed genera are either para- or polyphyletic even according to the authors own phylogeny, or monophyletic groups embedded within larger polyphyletic clades (Chase *et al.*, 2015; Schuiteman & Chase, 2015).

The most recent Maxillariinae reassessments (Chase *et al.*, 2015; Schuiteman & Chase, 2015) suggested to bring back together the clades proposed by Blanco et al. (2007) and Pridgeon et al. (2009) into a *Maxillaria* megagenus composed of 634 species subdivided into 17 sections. According to the authors, the splitting of *Maxillaria* in 17 genera would be

just a proliferation of taxonomic names and they defend that: a) a single large *Maxillaria* genus would be more easily identifiable; b) the identification key proposed in the *Genera Orchidacearum* (Pridgeon *et al.*, 2009) for these genera contained flaws and the diagnostic characters were not good enough; and c) the reduction in the number of clades would assist in teaching botany. Maxillariinae would hence be composed by *Anguloa* Ruiz & Pav., *Bifrenaria* Lindl., *Guanchezia* G.A.Romero & Carnevali, *Horvatia* Garay, *Lycaste* Lindl., *Neomoorea* Rolfe, *Rudolfiella* Hoehne, *Scuticaria* Lindl., *Sudamerlycaste* Archila, *Teuscheria* Garay, *Xylobium* Lindl., and a large *Maxillaria* Ruiz & Pav.

Regardless all the taxonomic efforts to enlight relationships within Maxillariinae, the subtribe is far from being well resolved, mainly due to shorten in the sampling with many genera underrepresented in Maxillariinae phylogenies (e.g. *Scuticaria*, *Xylobium*, *Rudolfiella*, *Rhetinantha*). This fact represents a serious problem, because in neotropical flora, where the subtribe constitute an important component (Williams & Mark Whitten, 2003), botanical exploration still lags far behind the biodiversity (dos Santos *et al.*, 2015; Hopkins, 2019). The advance of molecular phylogenies to support orchid and general biodiversity and ecological studies in Amazon and Atlantic Forest is urgent, due to the pressures of understanding species dynamics under climate changes and the serious deforestation in these sites, specially nowadays, when anti-environmental and pro-development policies are taking place in Brazilian government (de Area Leão Pereira *et al.*, 2019, 2020; Johnson, 2020; Stewart *et al.*, 2020). Finally, given the importance of a stable nomenclature in science (Christenhusz *et al.*, 2015; Christenhusz, 2020), genera within Maxillariinae urge for unassailable delimitation and resolution so researchers can achieve consensus in the taxonomic treatment of the subtribe.

2. Species Distribution Models (SDM)

Scientists have been observing for centuries the relationships between species distributions and environmental conditions and gradients (Murray, 1866; Schimper, 1902; Grinnell, 1904), highlighting responses of species occurrence to the environment (Cain, 1931; Whittaker, 1956; Harmon *et al.*, 1984). Despite early studies in said matter being rather qualitative (Elith & Leathwick, 2009), they set the foundation for biogeography and provided

the conceptual framework for the development of numerical, explanatory methods (Malanson & Peet, 2020). Such quantitative methods started from linear multiple regressions, discriminant function analysis over presence-absence data and Generalized Linear Models (GLMs), whose key structural features are a component of the most advanced Species Distribution Model (SDM) techniques currently used, as for instance maximum entropy models (reviewed in Elith & Leathwick, 2009).

The understanding of relationships between a species or community and its environment and the drivers of species distribution is a central goal in science and rapidly moved developments to SDM techniques, including advances in Geographic Information Systems (GIS; Huang, 2018; Bareth & Waldhoff, 2018) and in physical geography, which allowed fine resolution digital models of Earth's surfaces, climate and vegetation (*e.g.* Olson & Dinerstein, 1998; Olson *et al.*, 2001; Hijmans *et al.*, 2005; Danielson & Gesch, 2011; Karger *et al.*, 2017). With such enhancements, SDMs became a key tool for describing patterns and making predictions and is widely used in an extensive range of applications as for instance forecasting species occurrence dynamics under impending climate change scenarios (Sinclair *et al.*, 2010; Park *et al.*, 2015), informing management plans (Van Echelpoel *et al.*, 2015), conservation policies (Mateo *et al.*, 2019; Kaky *et al.*, 2020) and researching biological issues as ecology (Soberón, 2010; Guo *et al.*, 2015; Norberg *et al.*, 2019), paleobiology (Svenning *et al.*, 2011), systematics (Kharouba *et al.*, 2013), biogeography (Guisan *et al.*, 2006; Fois *et al.*, 2018), invasiveness (Srivastava *et al.*, 2019; Liu *et al.*, 2020) and many other applications (reviewed in Guisan & Thuiller, 2005; Booth *et al.*, 2014; Hao *et al.*, 2019).

Following the increasing utilization of SDMs in sciences, many algorithms were developed, including GLM and Generalised Additive Models (GAM), Maximum Entropy Modelling (MaxEnt), MaxLike, Random Forests (RF), Boosted Regression Trees (BRT), Artificial Neural Networks (ANN), Bayesian Hierarchical Modelling (BHM), Support-Vector Machine (SVM), Classification and Regression Trees (CART) and Flexible Discriminant Analysis (FDA) among others, besides Ensemble Modellig, which combines multiple SDMs from different methods into one prediction by averaging the models (Araújo & New, 2007; Marmion *et al.*, 2009). These techniques have been extensively compared and tested on their accuracy and performance (Phillips *et al.*, 2004; Elith *et al.*, 2006; Elith & Graham, 2009; Buckley *et al.*, 2010; Liu *et al.*, 2011; Li & Wang, 2013; Silva *et al.*, 2014; Merow & Silander, 2014; Duan *et*

al., 2014; Fiedler *et al.*, 2018; Hao *et al.*, 2020; Perkins-Taylor & Frey, 2020; Kaky *et al.*, 2020). The conclusion is that there is no single SDM algorithm that can outperform the others under all experiment designs (Li & Wang, 2013), leaving the choice on which technique to use in the dependance of the objectives of each study and the data available (Guillera-Arroita *et al.*, 2015). Yet, MaxEnt (Phillips *et al.*, 2006; Elith *et al.*, 2011) was shown to outperform single model based SDMs in many studies and to produce predictions of comparable accuracy to ensembling methods (Kaky *et al.*, 2020).

MaxEnt stands for maximum entropy, a machine learning algorithm which compares the environmental variables underlying species observation points against a random set of pseudo-absence points, *i.e.* the background. As a result, a distribution model is produced (Phillips & Dudík, 2008), represented by a map of probability of suitability for occurrence (Figure 1). MaxEnt is considered a stable and high performance method even under default parameters (Phillips & Dudík, 2008) or using small sample sizes (Wisz *et al.*, 2008), and estimates the distribution of a given species by finding the least biased distribution possible (*i.e.* the distribution with Maximum Entropy). Additionally, it does not require true absence data, but only presence and background (Phillips *et al.*, 2006; Elith *et al.*, 2006), is implemented through free, user-friendly and open source R packages ('ENMTools' Warren *et al.*, 2010; 'sdm' Naimi & Araújo, 2016; e.g. 'dismo' Hijmans *et al.*, 2017; Phillips *et al.*, 2017), and does not require massive computational power, all these reasons making MaxEnt the most popular choice among researchers (Kaky *et al.*, 2020).

The MaxEnt output comprises a series of statistics on the probability of suitability for occurrence in response to variables and on the importance and contribution of the imputed predictor variables to the model. Examples of such statistics are Percent contribution, Permutation importance and the Jackknifes for training gain, test gain and AUC. *Gain* is a central concept in MaxEnt and it is basically the deviance in the probability of the presence over the background. For Permutation importance and Percent contribution to the Model statistics, MaxEnt tracks how much the overall model gain is increased or decreased when a coefficient is changed in each iteration of the training algorithm. For Percent Contribution to the Model, the increase or decrease in the regularized gain is added or subtracted to the contribution of the corresponding variable. Finally, for the Permutation Importance estimate, the values of the variable in turn are randomly permuted, decreasing the possible bias

caused by the order of input of the variables in the first estimate. The Jackknife test of variable importance accounts dependencies between predictors by estimating how well a model would perform if using only one variable in contrast to the performance of a model with all variables but the given one and comparing both data with the overall model performance. The variables with higher gain when used alone are the ones that contribute the most to the model, therefore they might have the most useful information by themselves. The variables which, when omitted, cause the gain to be lower compared to the overall model would have the most information that is not present in the other variables (for further explanations, see Elith *et al.*, 2011; Phillips, 2017; Phillips *et al.*, 2017).

MaxEnt models have been criticized for having produced overfitting models (Merckx *et al.*, 2011; Halvorsen, 2013; Radosavljevic & Anderson, 2014) and for being oversimplified or incorrectly applied (Yackulic *et al.*, 2013; Morales *et al.*, 2017). A good and contemporary example of such is the recent publication stating that, according to SDM forecasts, climate would naturally be a primary regulator for the spread of the infection by the SARS-CoV-2 Coronavirus, which would make a synchronous global pandemic of the disease unlikely (Araujo & Naimi, 2020). The scientific community immediately responded to that pointing out the inadequacy of the application of SDMs to the SARS-CoV2 Coronavirus (Chipperfield *et al.*, 2020; Harbert *et al.*, 2020), but we also have seen such statement to be proven wrong given the quick spread of the virus. Nonetheless, many researches have been addressing the sources of uncertainty in predictions, as choice and adequacy of SDMs, model parameters and complexity, sample size, spatial distribution, spatial scale, variable selection and model evaluation (e.g. Wisz *et al.*, 2008; Warren & Seifert, 2011; Anderson & Gonzalez, 2011; Halvorsen *et al.*, 2016; Khosravi *et al.*, 2016; Kiedrzyński *et al.*, 2017; Shabani *et al.*, 2018; Fourcade *et al.*, 2018; Kong *et al.*, 2019; Fernández & Morales, 2019; Sanei *et al.*, 2020). Following, guidelines have been published (Merow *et al.*, 2013; Araújo *et al.*, 2019; Zurell *et al.*, 2020) in order to help researchers to fit adequate SDMs and properly report the methods and results. Such initiative should make SDM results more comparable throughout methods, space and taxa, allowing both a deeper comprehension of the performance of models and the improvement of the algorithms.

Meanwhile, well applied SDMs have already been fitting their purposes in providing valuable data to ecological studies. For instance, researchers combined data from MaxEnt

SDMs and genomic data to demonstrate that orchid bees might be susceptible to genetic disruption in the event of climatic changes, since some species presented less suitable habitat during past glaciations and currently exhibit strong population structure associated with mitochondrial genome (López-Uribe *et al.*, 2014). In another example, relationships between the richness of orchid species and ecological factors was assessed, pointing out land cover and geological substrate as strong determinants of richness patterns, besides potential occurrence in poorly known areas, thus providing data to management and conservancy policies in the Czech Republic (Štívková *et al.*, 2018).

Finally, a study conducted with orchids including two Maxillariinae species combined climatic niches from SDMs with genomic data, under a phylogenetic background, and showed diversity in genome size and GC content to have adaptive consequences, besides being associated with environmental and ecological factors (Trávníček *et al.*, 2019). However, Maxillariinae were underrepresented in their sample, which was also biased towards temperate species. Hence, relationships between genomic traits and putative responses to the environment still remain to be assessed in order to achieve a better comprehension of the association of chromosomal changes with species occurrence in the subtribe.

3. Chromosomal changes and the environment

Changes in genome structure, acknowledged as a prime stimulus for evolution in flowering plants, can impose immediate effects on the fitness and the phenotype of an individual. As such, the interdependence between the genome and the environment in which species evolve and develop have been long recognized, and the development of cutting-edge, high resolution techniques in genomic and cytogenetic studies (Levin, 2002; Madlung, 2013; Mayrose & Lysak, 2020), allied to high performance modelling algorithms applied to ecology (Guo *et al.*, 2015), have allowed a progressively deeper understanding on this matter, but also raised even more questions, making the relationships between chromosomal changes, species distribution and responses to environment a central subject in modern ecology.

Extreme environmental conditions are known to lead to failure during micro- and megasporogenesis, resulting in unreduced gametes (Ramsey & Schemske, 1998; Brownfield & Köhler, 2011), commonly involved in polyploid origin and establishment (Soltis *et al.*, 2007, 2009, 2015; Parisod *et al.*, 2010; Pelé *et al.*, 2018; Rezende *et al.*, 2020). After polyploidization, diploidization takes place: a series of genome reorganization events in order to restore the "diploid-like" state (Dodsworth *et al.*, 2016; Mandáková & Lysak, 2018; Qiao *et al.*, 2019), involving gene neo- and sub-functionalization (Rastogi & Liberles, 2005), elimination of unequal homologous and illegitimate recombination and genome downsizing, all these events being potential drivers of phenotypic novelty and key innovations which would serve as catalysts for speciation (Leitch & Bennett, 2004; Soltis *et al.*, 2015; Dodsworth *et al.*, 2016; Simonin & Roddy, 2018). That given, polyploidy drives a hearty debate among scientists: some would say it is a major force in angiosperms evolution and diversification (Soltis *et al.*, 2009; Landis *et al.*, 2018; Ren *et al.*, 2018b), owing to genetic enhanced diversity and heterogeneity (Miotto & Monacelli, 2020). Meanwhile, others would state it is an evolutionary dead end, since recent studies have shown that many angiosperm speciation events followed ploidy increases, but they were not accompanied by increased speciation rates (Wood *et al.*, 2009), and that polyploids were more likely to occur at the tips of phylogenetic trees, insinuating that they would rather be prone to extinction (Mayrose *et al.*, 2011).

Yet, the same study suggested that in those cases in which polyploids indeed succeed to establish as new species, the enhanced genetic diversity is probably the key (Mayrose *et al.*, 2011). It is also widely accepted that the duplicated gene copies after subsequent rearrangements can potentially assume new functions and allow distinct responses to environmental factors, promoting ecological niche shift (Madlung, 2013). It is suggested that, usually, the formed polyploid species tend to tolerate harsher conditions compared to diploid/parentals, and also expand to new habitats (Pandit *et al.*, 2011; Linder & Barker, 2014; Visger *et al.*, 2016; Pfennig *et al.*, 2016; Blaine Marchant *et al.*, 2016). In that sense, polyploidy, specially allopolyploidy, is believed to "*continue to be an effective speciation mechanism to sustain habitat disturbance emanating from rigors of climate change*" (Lavania, 2020).

Besides polyploidy, dysploidy (*i.e.* changes in chromosome number without significant gain or loss of genetic material) is known to be frequent across angiosperm taxa and to persist longer over evolutionary time than polyploid changes (Escudero *et al.*, 2014), owing to bring less disadvantageous changes to genome compared to polyploidy and aneuploidy (*i.e.* changes in chromosome number with gain or loss of genetic material). Dysploidy derives from chromosomal rearrangements as Robertsonian translocations, with fusions and fissions that can dramatically change the architecture of chromosomes, affecting the chromosome number, without significant loss or gain of DNA sequences, except for some sequence loss at newly telomeric distal regions and sequence gain at newly pericentromeric proximal regions (Roalson *et al.*, 2007; Ren *et al.*, 2018a). Hence, implying structural changes to the genome, dysploidy can also potentially affect the species adaptive diversification and distribution (Pandit *et al.*, 2014; Mas de Xaxars *et al.*, 2016; Mandáková & Lysak, 2018).

Regarding DNA content, when not caused by changes in chromosome number, genome size variations are caused by changes in the repetitive DNA fraction, which could represent up to 90% of the plant genome (Bennett & Leitch, 2005). The repetitive fraction of the genome is mainly composed of two categories: sequences dispersed throughout the genome - mainly retrotransposons - and sequences organized in tandem - mainly families of satellite DNA (satDNA) (Lee & Kim, 2014; Biscotti *et al.*, 2015; Garrido-Ramos, 2017; Hartley & O'Neill, 2019). The activation of retrotransposons configures the main cause of genome size gain (Bennetzen *et al.*, 2005; Michael, 2014) and was already demonstrated to cause massive increase in DNA content as a consequence of chromosome rearrangements (Winterfeld *et al.*, 2020). Additionally, transcriptional errors involving satDNA, often due to stressful environmental situations (Pezer *et al.*, 2012), constitute an alternative mechanism of genome size change (Mehrotra & Goyal, 2014). All together, these DNA content variations come with consequences to the plant.

The variation in genome size influences the plant phenotype and genotype, affecting the volume and size of chromosomes (Bennett *et al.*, 1983) and other cellular structures as the nucleus (Baetcke *et al.*, 1967), centromere (Bennett *et al.*, 1981), pollen grains (Bennett, 1972) and stomatal cells (Masterson, 1994). The genome size is also correlated with cell cycle duration (Levin, 2002), an important aspect of metabolic constraints because cell cycle duration scales up to developmental speed and generation times. Nevertheless, metabolic

rates, including demographic rates, increase with local temperature (Brown *et al.*, 2004; Savage *et al.*, 2004) and may thus compensate genome size mediated lower metabolic rates. As a consequence, the variation in GS should be associated with different abiotic preferences and life-history traits (Knight & Ackerly, 2002; Leitch & Bennett, 2007; Díez *et al.*, 2013; Pustahija *et al.*, 2013).

In fact, it has been demonstrated that species with large genome size are often perennial, slow-growing (Bennett, 1972), do not show great morphological variation and are more sensitive to radiation (Sparrow & Miksche, 1961) and to pollution, especially by heavy metals (Temsch *et al.*, 2010). The geographic distribution of species with large genome sizes is constrained to non-extreme environments (Knight & Ackerly, 2002; Knight & Beaulieu, 2008), to non-epiphytic habit (Veselý *et al.*, 2012), and the big size of their seeds hinders long-distance dispersal (LDD) (Knight *et al.*, 2005; Ogutcen & Vamosi, 2016). In contrast, polyploidy, which inherently causes the genome size to increase, facilitates the LDD, at least for seeds with good dispersal ability (Linder & Barker, 2014). A further contrast between polyploidization and genome size increase is the fact that while the first is positively associated with species diversification, the second would tend to hinder it (Vinogradov, 2003). The disadvantageous aspects of the increase in genome size are probably due to the fact that the higher volume of DNA makes cell division more expensive, causing higher demand of resources for the development and metabolism of the organism (Knight *et al.*, 2005; Gregory, 2005), without the benefits of increased recombination possibilities and heterogeneity provided by polyploidy.

There is still no consensus about the relationship between genomic changes with plant niche preferences and distributions, since researchers found contrasting results while studying this matter. For instance, there is significant positive correlation between genome size and growing elevation in wild populations of *Corchorus olitorius* L. (Malvaceae S.l.) (Benor *et al.*, 2011), while among cytotypes of *Dianthus broteri* Boiss. & Reut. (Caryophyllaceae) from diploid to dodecaploid, with great variance in genome size inclusively within some cytotypes, DNA content variations were not straightforwardly consistent with geographical distribution, regarding elevation, longitude and longitude (Balao *et al.*, 2009). A more recent study, however, conducted in the same *D. broteri* complex, analyzed ploidy instead of genome size, and using modern ecoinformatics and phylogenetic comparative

methods, found the *D. broteri* cytotypes occupying distinct niches, with distributions constrained by soil characteristics, temperature and water stress (López-Jurado *et al.*, 2019).

Another study, conducted with 14 *Eugenia* L. (Myrtaceae) species in brazilian flora, revealed different spatial distribution patterns between diploids and polyploids, with polyploid individuals associated to more adverse environments, usually at higher elevations (Silveira *et al.*, 2016). Also the increase in genome size among 23 wild *Coffea* L. (Rubiaceae) species was found to be associated with longitudinal/latitudinal gradients (Razafinarivo *et al.*, 2012). On the other hand, the authors found no correlation between genome and stomata sizes (Razafinarivo *et al.*, 2012), despite this being considered a common relationship across angiosperm trees (Hodgson *et al.*, 2010).

Overall, studies of genomic traits associations with geographical and environmental factors have been presenting both positive and negative correlations, depending on the particular taxa, environmental variables and methodologies applied (Knight *et al.*, 2005; Bennett & Leitch, 2005). Nevertheless, it has been demonstrated that factors related to species preferences and distribution are expected to be primarily associated not with the size, neither small or big, of the genome, but with size changes, either increase or decrease, and with factors underlying these genomic alterations, such as duplications, transposable elements and selective pressures (Kraaijeveld, 2010; Puttick *et al.*, 2015).

4. Tying the knots: Chromosomal changes relationships with the environmental and ecological variables associated to species distribution in Maxillariinae

Orchidaceae Juss. is the largest family among plants (Christenhusz & Byng, 2016), comprising, besides morphological and ecological, a huge karyotypic diversity: the second largest variation in genome size among plants, about 168x (Leitch *et al.*, 2009). Studies in Orchidaceae have already revealed an association between smaller genome sizes and epiphytic habit (Leitch *et al.*, 2009), which could be due to the association between smaller genome and smaller guard cells allowing to cope more efficiently with the water stress (Aasamaa *et al.*, 2001; Hetherington & Woodward, 2003). A recent study has also associated species with bigger genomes in temperate climates with the geophytic habit (Trávníček *et al.*, 2019), which the author attributed to rapid growth by expansion of preformed cells,

specifically in the early season, as an evolutive advantage in cold environments (Veselý *et al.*, 2012). As for general growing speed, analyzes for several genotypes of the subtribe Oncidiinae revealed that between sibling clades, both fast- and slow-growing plants can have small genomes, but fast-growing plants do never have large genome sizes, even within the moderate variation observed inside subtribe Oncidiinae (from 1.10 to 4.60pg, except by Rossioglossum with 7.70pg; Chase *et al.*, 2005).

Regarding chromosome number, niche preference changes are often associated to numeric changes, specially considering species-rich plant groups (Hijmans *et al.* 2007; Manzaneda *et al.* 2012; Thompson *et al.* 2014; Godfree *et al.* 2017), as Orchidaceae (Félix & Guerra, 2000; Yamagishi-Costa & Forni-Martins, 2009; Felix & Guerra, 2010). However, a first assessment comparing diploid vs. polyploid niche variation across 46 plant families showed no association of ecological and geographical gradients with chromosome number, but showed association of these gradients with shared evolutionary history (Martin & Husband, 2009). In another example, independently conducted ordinary least-squares models associated the genome size variation across Orchidaceae with nine out of 10 environmental, ecological and biological predictors, while the phylogeny-corrected PGLS model held only five predictors (Trávníček *et al.*, 2019). Whereas further studies are needed to address the ecological and karyotypic associations, the mentioned studies reinforced the importance of ecological analysis with a phylogenetic background.

Within Orchidaceae, the subtribe Maxillariinae have been the focus of discussion among researchers, regarding the best taxonomic treatment to properly represent the evolution of this clade (Blanco *et al.*, 2007; Whitten *et al.*, 2007; Szlachetko *et al.*, 2012; Schuiteman & Chase, 2015). Difficulties pose mainly in the fact that phylogenetic relationships are not yet resolved enough to achieve a consensus in that matter and morphological traits are also too diverse to allow unambiguous diagnoses (see Literature Review topic 1, “A brief history of Maxillariinae”). Nonetheless, genomic traits are as well rather diverse among Maxillariinae orchids, with chromosome numbers ranging from 2n=36 in *Christensonella uncata* (Lindl.) Szlach. to 2n=76 in *Bifrenaria tyrianthina* (Lodd. ex Loudon) Rchb.f. and genome sizes ranging from 1C=1.70pg in *Trigonidium egertonianum* Bateman ex Lindl. to 1C=5.69pg in *Scuticaria hadwenii* (Lindl.) Planch. (Koehler *et al.*, 2008; Moraes *et al.*, 2012, 2017). The karyotypic divergences in Maxillariinae are attributed to polyploidy,

dysploid numeric changes and genome content accumulation through acquisition of tandem repeats (Moraes *et al.*, 2012, 2017), but other chromosomal changes were observed in the subtribe, such as chromosome inversion, as evidenced by patterns of 5S rDNA sites in chromosomes of *Heterotaxis* species (Moraes *et al.*, 2016).

Regarding niche associations, there are indicatives that genome size increase might be related to plant size increase and desertion of epiphytism as obligatory habit in *Bifrenaria* species (Koehler & do Amaral, 2004; Moraes *et al.*, 2017), as expected in accordance to the theories of the Nucleotypic Effect (Doyle & Coate, 2019) and the Large Genome Constraints (Knight *et al.*, 2005) respectively. However, no comprehensive study has further assessed genomic and environmental relationships in Maxillariinae species.

STUDY SCOPE AND OBJECTIVES

Aiming to contribute to the knowledge of neotropical biodiversity, a team of Brazilian researchers and international collaborators in a multidisciplinary project has been brought together. This thesis, to be submitted as a fulfillment of a requirement for the degree of Doctor, directly results from such collaborations and consists in two chapters.

Chapter 1 presents a comprehensive phylogeny for Maxillariinae, including more species from the Brazillian biodiversity for some underrepresented genera, contributing to the comprehension of relationships within the clade and to discussions to the current taxonomic treatment proposals for the subtribe.

Chapter 2 presents distribution models and macroevolutionary studies for Maxillariinae species, appraising the relationships of genomic traits with the environmental and ecological variables associated with species distribution.

Our general goal is to improve the comprehension of genomic traits evolution as a putative driver of distribution patterns and species diversity in the Neotropics, and we do so

with a phylogenetic background, using the subtribe Maxillariinae, a diverse, widespread, representative and important component of our flora, as a study model. Specific objectives include:

a) Providing DNA sequences of representatives of neotropical orchids to support further phylogenetic studies;
b) Assessing relationships within Maxillaiinae to achieve a better comprehension of the evolution of the subtribe;
c) Contributing to the discussion on the best taxonomic treatment for Maxillariinae;
d) Assessing putative patterns of species distribution in response to environmental variables;
e) Determining the main environmental variables affecting species distribution among Maxillariinae species;
f) Summarizing the available data on Maxillariinae chromosome number and genome size to support and encourage the use of genomic data in further analyzes, as well as adding new information to these data;
g) Assessing putative correlations between genomic traits in Maxillariinae and environmental and ecological variables.

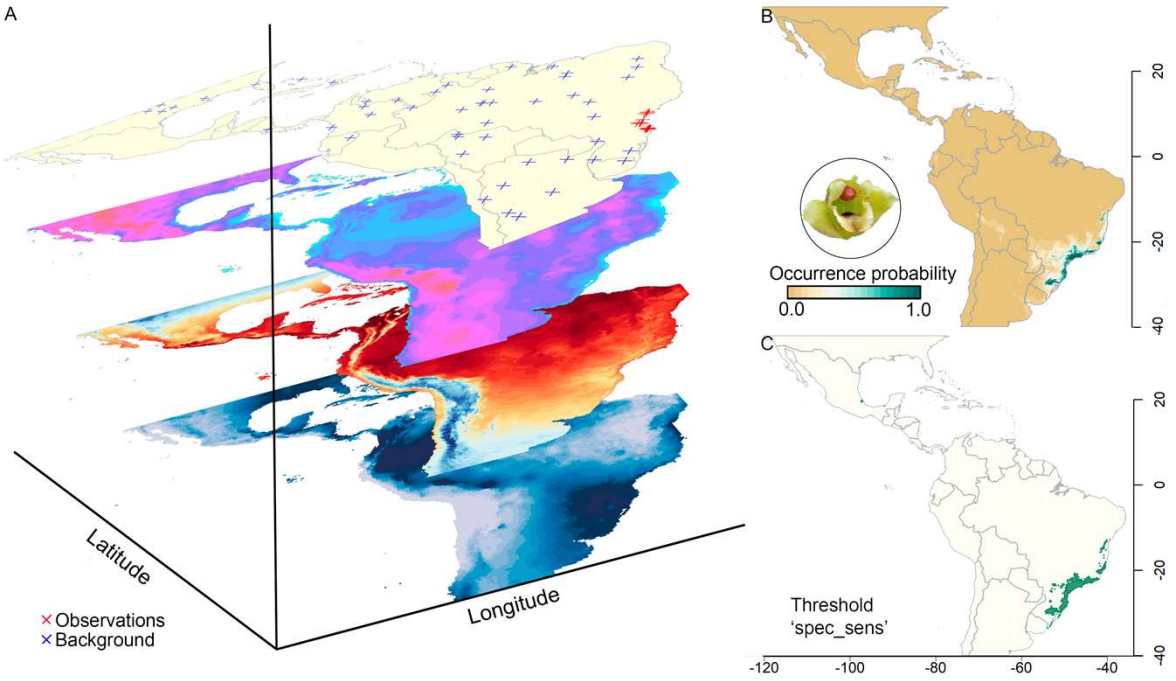

Figure 1 – SDM input and outputs. A - true presence observations (red points) and background (blue points) using *Christensonella ferdinandiana* (in detail in panel B) data as example. Observation and background points are overlaid over predictor variables (in the figure, examples of predictor variables are the climate variables: seasonality, temperature and precipitation). Data on the values of the variables in the locations of observation and background points are extracted from the variable layers. B - logistic output of SDM showing probability of species occurrence ranging from 0 (beige) to 1 (green). C - Binary representation (0 or 1) of species environmental suitability using the value at which the sum of the sensitivity (true positive rate) and specificity (true negative rate) was highest as threshold ('spec_sens'). Map areas in which the probability of environmental suitability is greater than the threshold receive the number 1, the remaining areas receive 0, and all areas with the assigned value 1 are converted to a polygon (green).

CHAPTER 1 - A NOTE ON MAXILLARIINAE (ORCHIDACEAE) RECIRCUMSCRIPTIONS: TO LUMP OR TO SPLIT?

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Abstract

Introduction: The morphological diversity of Maxillariinae, a species rich and widely distributed neotropical subtribe, imposes challenges for the achievement of a consensus about the best taxonomic treatment to reflect species evolution in the clade, driving discussions on whether to lump or split taxa. In the present study we aim to contribute to this matter by providing new DNA sequences and a new phylogeny to appraise relationships among and within Maxillariinae genera.

Methodology: Phylogenetic relationships were inferred using Bayesian Inference analysis of combined nrITS, the plastid matK gene and flanking trnK intron, and the plastid atpB-rbcL intergenic spacer for 223 species.

Results: A mostly well resolved and highly supported mojority rule tree was presented reinforcing the monophyly of most of the taxa segregated from *Maxillaria s.l.* as well as for basal genera (exceptions are *Nitidobulbon* and *Scuticaria* respectively).

Discussion: While both lumping and splitting are suggested for taxa recircumscriptions in the subtribe, given our results and the available literature we support a taxonomic treatment in which *Maxillaria* s.l. was splitted in 17 genera as the best representation of the evolution of Maxillariinae. We encourage further molecular and morphological monographic studies in order to appraise uncertain relationships and hopefully achieve consensus in generic delimitations.

Keywords: Maxillaria, Bifrenariinae, Lycastinae, molecular phylogeny, Bayesian Inference, orchids.

1. Introduction

Maxillariinae Bentham, circumscribed in the Cymbideae tribe, subfamily Epidendroideae (Orchidaceae), is an important subtribe in the composition of the epiphytic floras of Neotropical rainforests. It comprises about 720 species distributed from the South of the United States to the north of Argentina (Blanco *et al.*, 2007; Whitten *et al.*, 2007; Koehler *et al.*, 2008). The taxonomic treatment of Maxillariinae is rather challenging due to the difficulties in determining morphological synapomorphies in its largest genus *Maxillaria* Ruiz & Pav. (sensu Pavon & Ruiz, 1794). As originally circumscribed, *Maxillaria* had about 570 species (Christenson, 2013), which makes *Maxillaria sensu lato* one of the largest genera of Orchidaceae and Angiosperms (Cribb, 2005; Schuiteman & Chase, 2015). In addition, due to its wide distribution, adaptations to different environments contribute to a great diversity of vegetative morphologies, although its flowers have a relatively uniform structure (Szlachetko *et al.*, 2006).

In the early 2000s, following the increasing availability of DNA analyses with fine resolution, a few molecular phylogenies suggested *Maxillaria s.l.* was not monophyletic (Whitten *et al.*, 2000; Koehler *et al.*, 2002; Williams & Mark Whitten, 2003; Dathe & Dietrich, 2006; Singer *et al.*, 2007), and the first comprehensive phylogeny for the megagenus, comprising 354 species, revealed that clades often recognized as genera (i.e. *Anthosiphon* Schltr. and *Chrysocycnis* Linden & Rchb.f.), and even clades easily diagnosed by their distinct morphologies (i.e. *Cryptocentrum* and *Trigonidium)*, were embedded in *Maxillaria s.l.* Since lumping all genera to a monophyletic *Maxillaria* would make it too morphologically diverse, and hence undiagnosable (Whitten *et al.*, 2007), a new taxonomic treatment for the subtribe was proposed (Blanco *et al.*, 2007; Pridgeon *et al.*, 2009), recognizing the division of *Maxillaria* in 17 genera, some of them segregated or resuscitated from *Maxillaria: Brasiliorchis* R.B.Singer, S.Koehler & Carnevali (*Maxillaria picta* group), *Camaridium* Lindl., *Christensonella* Szlach., Mytnik, Górniak & Smiszek, *Cryptocentrum*, *Cyrtidiorchis*, *Heterotaxis* Lindl., *Inti* M.A.Blanco, *Mapinguari* Carnevali & R.B.Singer (*Maxillaria rufences* group), *Maxillaria* Ruiz & Pav., *Maxillariella* M.A.Blanco & Carnevali (*Maxillaria variabilis* group), *Mormolyca*, *Nitidobulbon* Ojeda, Carnevali & G.A.Romero, *Ornithidium* Salisb. ex R.Br., *Pityphyllum*, *Rhetinantha* M.A.Blanco, *Sauvetrea* Szlach. and *Trigonidium* Lindl. Besides the core Maxillariinae, the authors recognize as well the genera *Anguloa* Ruiz & Pav., *Bifrenaria*

Lindl., *Guanchezia* G.A.Romero & Carnevali, *Horvatia* Garay, *Lycaste* Lindl., *Neomoorea* Rolfe, *Rudolfiella* Hoehne, *Scuticaria* Lindl., *Ida* A. Ryan & Oakeley (=*Sudamerlycaste* Archila), *Teuscheria* Garay, and *Xylobium* Lindl. as belonging to the subtribe.

A second taxonomic effort resulted in the division of *Maxillaria s.l.* into 32 genera (Szlachetko *et al.*, 2012), but it was not adopted by orchid researchers because it failed to meet basic recommendations for taxa recircumpscription, such as to designate as taxa only monophyletic and well supported groups, and to minimize as possible the nomenclatural disruption (Backlund & Bremer, 1998; Entwisle & Weston, 2005).

However, recent works are defending the lumping of the core Maxillariinae genera back together to a broad *Maxillaria s.l. (Whitten et al., 2014; Molinari-Novoa, 2015; Schuiteman & Chase, 2015)*, which would even include *Hylaeorchis (Schuiteman & Chase, 2015)*, a genus that was never considered to be *Maxillaria* before. The authors recognize that their results, as well as other molecular phylogenies for genera (Koehler *et al.*, 2008, 2012; Ojeda *et al.*, 2009; Arévalo & Cameron, 2013; Arévalo *et al.*, 2015; Novello, 2015), provide statistical support for these taxa, but in disagreement with the orchid splitters (Blanco *et al.*, 2007; Whitten *et al.*, 2007; Pridgeon *et al.*, 2009), they advocate that: a) *Maxillaria s.l.* can be easily recognized by three morphological traits: column foot with hinged lip, unifloral inflorescences and conduplicated lips; b) Some genera derived from *Maxillaria s.l.* cannot be characterised by autapomorphies, but by sets of traits, and would be difficult to identify; c) The key for identification fails to properly assign some species to the right genera; d) This classification would be disruptive nomenclature wise, much more difficult to use for non-specialists and could hinder taxonomic learning.

Lumping has also participated in the recircumscription of the basal Maxillariinae genera. A molecular phylogeny placed *Xylobium* as basal to Maxillariinae (sensu Ruiz & Pavón), and sister to the former Lycastinae genera (*Anguloa* and *Lycaste*), which in its turn was derivate to the former Bifrenariinae genera (Whitten *et al.*, 2000). Recognising Bifrenariinae, Lycastinae and Maxillariinae as separate subtribes would imply eather the creation of a subtribe for *Xylobium* alone, or keeping the genus as incertae sedis. Orchid taxonomists favoured a broader Maxillariinae to encompass the four groups. However, the

position of *Xylobium* in the subtribe remains controversial (see Whitten *et al.*, 2000, 2007, 2014; Freudenstein & Chase, 2015; Schuiteman & Chase, 2015)

The discussion on whether to lump or to split clades should be supported by objective characters such as DNA sequences, chemistry and morphological traits selected to account for trait convergence and phenotypic plasticity (Christenhusz, 2020). In this sense, since Whitten (2007) many studies were published, enlightening inter and infrageneric boundaries within Maxillariinae (Koehler *et al.*, 2008, 2012; Ojeda *et al.*, 2009; Szlachetko *et al.*, 2012; Arévalo & Cameron, 2013; Whitten *et al.*, 2014; Arévalo *et al.*, 2015; Novello, 2015; Schuiteman & Chase, 2015). Yet, regardless of all the taxonomic efforts, it is clear that Maxillariinae is far from being well resolved and lacks data for achieving consensus on a conclusive, unassailable classification. In this study we present a new phylogeny, including species of genera that are underrepresented in Maxillariinae phylogenies (e.g. *Scuticaria*, *Xylobium*, *Rudolfiella*, *Rhetinantha*). We aim to contribute to the comprehension of relationships within the core Maxillariinae, but also among the basal genera of the subtribe, which are generally overlooked in the taxonomic reviews.

2. Material and methods

2.1. Plant material

All plant material was provenient from the living collection of the Frederico Carlos Hoehne Orchidarium of the São Paulo Institute of Botany or collected during field expeditions and all vouchers were deposited in HUFABC herbarium (acronym following Thiers, 2020).

2.2. Extraction, amplification and sequencing.

Genomic DNA extraction followed the CTAB 2x protocol (Doyle & Doyle, 1987) excluding β-mercaptoethanol. We used primers 17SE and 26SE (Sun *et al.*, 1994) to amplify

the internal transcribed spacers from the nuclear ribosomal DNA (*nr*ITS) and the primers 19F (Goldman *et al.*, 2001) and trnK2R (Johnson & Soltis, 1994) to amplify the *matK-trnK* region from the plastidial genome, with a third internal primer for sequencing, the 308F. The *atpB-rbcL* intergenic spacer was amplified with the primers MaxF and MaxR , designed by Mark Whitten (Whitten *et al.*, 2007). The PCRs parameters and reagent volumes are described in Whitten et al. (2007). Purification and sequencing were performed by Macrogen (Seoul, South Korea - http://dna.macrogen.com/eng/), using the same primers mentioned above. The obtained sequences will be submitted to GenBank.

2.3. Species matrix

A total of 31 *nr*ITS, 32 *atpB-rbcL* and 35 *matK-trnK* sequences were produced for the present work (Table 1), from which 15 species are new to comprehensive Maxillariinae phylogenies (see "*" in Table 1). In addition to the specimens we have sequenced, we compiled from the GenBank database sequences for *nr*ITS, *matK-trnK* and *atpB-rbcL* of Maxillariinae (Supplementary Table1). Among the sequences compiled, we select whenever possible those that: a) were published by Whitten et al. (2007); b) had information about the species voucher; c) had sequences for the three DNA regions from the same individual; d) had greater length and better quality, and; e) have genomic traits information published in literature, to support parallel works being carried out by our group. The final matrix comprised 223 species, being 212 *nr*ITS, 221 *matK-trnK and* 204 *atpB-rbcL.*

2.4. Alignments

The homologous sequences were aligned using the MAFFT v1.3.5 plug-in (Katoh & Standley, 2013; Katoh *et al.*, 2019) and MUSCLE v3.5 (Edgar, 2004) in the Geneious software v9.1.4 (Kearse *et al.*, 2012). All alignments were verified, compared and edited manually for minor inconsistencies and ambiguities. MUSCLE showed better results for the *atpB-rbcL* spacer region, so we used the MUSCLE alignments for that region and MAFFT for the others. The matrices were concatenated into a single matrix using the Geneious 9.1.4 software (Kearse *et al.*, 2012).

2.5. Bayesian inference

For Bayesian Inference (IB), the best evolutionary models for nucleotide substitution for each marker were chosen using the Corrected Akaike Information Criterion (AICc) test (Hurvich & Tsai, 1993), implemented at jModelTest2 (Posada, 2008; Darriba *et al.*, 2012), through the online platform CIPRES Science Gateway (http://www.phylo.org/; Miller *et al.*, 2010, 2011). BI analyzes were performed using MrBayes 3.2.2 on XSED at CIPRES (Huelsenbeck & Ronquist, 2001; Ronquist *et al.*, 2012). Due to the different evolutionary models of each DNA region, data partitions were created, which were treated independently in terms of their parameters. The BI consisted of two simultaneous runs of four MCMC chains (Markov chain Monte Carlo - Monte Carlo via Markov chains) for 10,000,000 generations. We discarded the initial 2,500,000 generations, setting a 25% burn-in. The remaining trees were used for the inference of a tree by majority consensus (50% majority-rule consensus tree), with the frequency with which each clade is observed between the trees representing the posterior probability of that clade. Because PP in Bayesian analyses generally holds higher values than other estimates (e.g. bootstrap percentages in maximum parsimony; Erixon *et al.*, 2003), we use the criteria of standard statistics, considering well supported those clades with PP≥95.

3. Results

All DNA regions yielded good sequences, being *atpB-rbcl* the most difficult to amplify and align. Amplification and sequencing with the primers 19F, 308R and trnK2R yielded a *matK-trnK* intron region and a nearly complete portion of matK, and the alignment also revealed indels. The phylogenetic hypothesis from our data added to geneBank sequences can be seen in Figure 1 (species sequenced for this study are marked by filled circles in the tips) and presented well resolved clades with strong support for almost all genera (PP>95). The exceptions were a) *Lycaste* (PP=84); b) a paraphyletic *Scuticaria*, divided in two well supported clades, one formed by three specimens of *S. hadwenii* (PP=100), and the other

formed by *S. salesiana* and *S. steelei* (PP=100); c) the polyphyletic *Nitidobulbon*, with *N. cymbidioides* and *N. nasutum* as sisters to *Heterotaxis* and *N. proboscideum* sister to *Ornithidium*, despite the three genera formed a well supported clade (PP=100). The core Maxillariinae was strongly supported (PP=100), as well as some clades within. *Maxillaria s.s.* and *Trigonidium* (PP=98) were placed as sisters to *Camaridium* (PP=98), *Sauvetrea* to *Cyrtidiorchis* (PP=100), and the clade these genera formed with *Christensonella, Mapinguari*, *Maxillariella*, *Rhetinantha* and *Mormolyca* was also well supported (PP=99), despite moderate resolution within. These taxa plus *Brasiliorchis* were placed as sister to *Inti* and *Cryptocentrum* (PP=98), *Inti* and *Cryptocentrum* to *Pityphyllum* (PP=96), and *Pityphyllum* to the *Heterotaxis*, *Nitidobulbon* and *Ornithidium* clade (PP=100). The core Maxillariinae was presented with moderate support (PP=91) as sister to a clade of *Anguloa* and *Lycaste* (PP=100) plus *Xylobium* (PP=97), and all these genera were sisters to a clade formed by *S. hadwenii* as sister to the extant *Scuticaria* (PP=100), which were sisters to an unresolved clade of *Hylaeorchis*, *Rudolfiella* and *Bifrenaria*.

4. Discussion

The presence of reading frameshifts in *matk*, an evidence that it is a pseudogene (Sheetlin *et al.*, 2014), was already observed in Maxillariinae (Whitten *et al.*, 2007) and other orchid and angiosperm clades (Kores *et al.*, 2000; Whitten *et al.*, 2000; Goldman *et al.*, 2001; Cameron *et al.*, 2001; Freudenstein & Senyo, 2008), but there are indicatives that it is very common in plants and other organisms and does not necessarily imply in function loss (Sheetlin *et al.*, 2014; Goodhead & Darby, 2015; Xie *et al.*, 2019). Despite new works have been suggesting the chloroplast open reading frame 1 (*ycf1*), another plastid gene, to be more variable than *matk-trnk* in orchids (Neubig *et al.*, 2009) and to perform better in plant phylogenies (Dong *et al.*, 2015), the phylogenetic hypothesis resulting from *matK-trnK* combined with *nr*ITS and *atpB-rbcL* delivered a topology comparable to that of *nr*ITS, *nr*ITS/*matK-trnK*/*ycf1* and *matk-trnk*/*ycf1* (Engel *et al.* in prep. Whitten *et al.*, 2014; Moraes *et al.*, 2017), proving it to be useful to the comprehension of orchids relationships. The overall topology of the phylogeny we present (Figure 1) confirms the monophyly of most the

genera, and suggests some clade relationships, with poor to high support, that were unresolved in polytomies in previous works, representing a step further in the comprehension of the subtribe. Clade and genera diagnoses and morphological descriptions are not the scope of the present note, since they were already depicted (Blanco *et al.*, 2007; Whitten *et al.*, 2007; Pridgeon *et al.*, 2009), but in the next paragraphs we delve into some particularities of the main clades supported by our results.

4.1. Basal Maxillariinae

Relationships among the basal genera of Maxillariinae were never deeply accessed, and despite they still need clarification, our results provided good resolution for this group. For instance, despite being easily diagnosable by terete leaves, *Scuticaria* was actually found to be polyphyletic, in accordance with previous suggestions (Whitten *et al.*, 2014; Moraes *et al.*, 2017). That could tentatively reflect geographic structure since the clades are differentially occurying in Atlantic (*S. hadwenii* (Lindl.) Planch.) and Amazon (*S. steelei* (Hook.) Lindl.) rainforests. Additionally, relationships among *Rudolfiella*, *Hylaeorchis* and *Bifrenaria* remains uncertain here as in previous works (Koehler *et al.*, 2002; Szlachetko *et al.*, 2012; Whitten *et al.*, 2014; Moraes *et al.*, 2017), but our results confirmed these genera to be sister to the amazonian *Scuticaria,* and this group in its turn is sister to the atlantic *Scuticaria*. These four genera once composed the subtribe Bifrenariinae, known by plicate leaves and strap-like viscidia, and despite *Scuticaria* presenting terete whip-like leaves, its flowers are often similar to those of *Rudolfiella*, and the few-flowered inflorescence is a synapomorphy shared with *Bifrenaria (Whitten et al., 2000)*.

Also strongly supported, a clade of *Anguloa* and *Lycaste* (former Lycastinae) as sister to *Xylobium* was recovered here as sister to the core Maxillariinae with moderate support. These relationships were already presented in literature (Whitten *et al.*, 2014; Freudenstein & Chase, 2015), but different results suggest that *Anguloa* and *Lycaste* might be basal to a clade in which *Xylobium* is sister to the core Maxillariinae (Engel *et al.* in prep. Whitten *et al.*, 2000; Ryan, 2001; Moraes *et al.*, 2017). This topology once supported the lumping of the former subtribes Lycastinae and Bifrenariinae with Xylobium and Maxillariinae into one

broad Maxillariinae, but discrepancies among the mentioned studies in the position of the genus suggest it should be further investigated.

4.2. Core Maxillariinae

Almost all the core Maxillariinae genera were recovered as monophyletic and with strong support, with exception to *Nitidobulbon* which was found here to be polyphyletic, possibly owing to the influence of *matK-trnK* and *atpB-rbcL,* since in phylogenies with separate markers, *nr*ITS helds this genus monophyletic (Ojeda *et al.*, 2003). *Nitidobulbon* shares with *Heterotaxis* and *Ornithidium* a greatly reduced column foot, and they compose a clade which is consistently held with strong support across molecular phylogenies (Engel *et al.* in prep. Whitten *et al.*, 2000, 2007). The three genera are also consistently held as basal to *Pityphyllum,* which is basal to monophyletic *Inti* and *Cryptocentrum*, remaining to understand if they are basal to *Brasiliorchis* as or data suggest, or rather sisters to this genus (Whitten *et al.*, 2007).

A further clade consistently held is that of *Sauvetrea* and *Cyrtidiorchis*, both monophyletic and strongly supported across phylogenies, despite its position within the core Maxillariinae is not well resolved. The clade formed by *Trigonidium* and *Maxillaria* was already observed (Engel *et al.* in prep. Whitten *et al.*, 2000), but while we found this clade to be sister to *Camaridium*, in Whitten *et al.* (2007) *Trigonidium* was placed as basal to a *Camaridium* and *Maxillaria* clade when using the same DNA regions we used, and the three genera formed a polytomy with *Maxillariella* and *Rhetinantha* after the addition of the RNA polymerase beta subunit 1 (*rpoC1*) to the matrix (Whitten *et al.*, 2007). Relationships of this clade with other clades formed by *Mormolyca, Rhetinantha, Maxillariella, Sauvetrea and Cyrtidiorchis, Christensonella* and *Mapinguari* are partially resolved with poor to high support in our results and presented mostly polytomies in those of Whitten et al. (2007), meaning much more research is necessary in these taxa. Another study with comprehensive sampling of Maxillariinae is available (Whitten *et al.*, 2014), but the authors have, probably by mistake, replaced the Figure 5, where relationships within *Maxillaria s.l.* were depicted, with a

repeated Figure 3, and they did not describe these results, hence it is not possible to know the position of the above mentioned genera.

4.3. To lump or to split?

Name conservatism in taxonomy is important because name changes cause the knowledge about an organism to be associated with it under different nomenclatures, imposing challenges and losses in the use of information (Christenhusz, 2020). However, name changes are necessary when the data shows a treatment to reflect poorly the evolution of taxa. In that context, discussions on how to recircumscribe and whether to lump or to split clades were raised since the beginnings of taxonomy, having Darwin himself pondered about it (Christenhusz *et al.*, 2015; Christenhusz, 2020). A recent research conducted with plant taxonomists revealed that between lumping or splitting clades, lumping would be slightly prefered, but most taxonomists would rather decide depending on the taxa (Christenhusz *et al.*, 2015). We agree that circumscriptions should be analyzed clade by clade to better reflect the natural history and the traits (morphological, chemical, genomic) of coherent and well supported groups of species.

Regarding Maxillariinae taxa, despite the available information allowing interesting conclusions, further studies are clearly necessary. For instance, given our results, we suggest *Scuticaria* should be reviewed in order to restore monophyly, but the genus comprises 10 species and there is no phylogeny to enlighten relationships within it, thus requiring deeper investigations for putative new delimitations

About the division of the core Maxillariinae into 17 genera, splitters would say that a wide *Maxillaria s.l.* is too morphologically diverse to be diagnosable, while the smaller taxa are highly supported by molecular data (Blanco *et al.*, 2007; Whitten *et al.*, 2007; Pridgeon *et al.*, 2009; Szlachetko *et al.*, 2012). Lumpers would say that the new nomenclature is too disruptive and some of the new genera are poorly characterized (Molinari-Novoa, 2015; Chase *et al.*, 2015; Schuiteman & Chase, 2015). We believe they might both be right and we recognize that achieving consensus in such delimitations is rather challenging, especially when there is not enough data to draw unarguable boundaries. Yet, we favour the splitting of

Maxillaria, because the recognition of well supported and too morphologically diverse taxa under a single genus creates a heterogeneous assembly of species while the division better reflects the natural history, the geographic structure and the morphological cohesion of the taxa.

For example, lumping the core Maxillariinae would group genera that are considered unequivocally distinguishable, as *Trigonidium,* very distinctive florally wise, lacking a prominent column foot while a column foot with hinged lip is considered one of the diagnostic characters of a broad *Maxillaria s.l.* The genus *Cryptocentrum* is another example of unambiguous morphological diagnosis, with monopodial shoots, wiry inflorescences and star shaped flowers with a nectariferous spur (Carnevali, 1997, 2001). On the other hand, some species of *Camaridium* and *Ornithidium* were repeatedly transferred between these two genera because they share morphological similarities (i.e. abundant nectar, orange or yellow flowers), possibly reflecting homoplasious traits associated with pollination by bees or hummingbirds (Whitten *et al.*, 2007). Yet, these two genera are not closely related, and despite being morphologically diverse, they are both monophyletic, strongly supported by molecular data.

In this sense, we disagree with the lumping proposed by Chase *et al.* (2015), especially when they propose to include *Hylaeorchis* in *Maxillaria s.l.* (Chase *et al.*, 2015, p. 159). Given the position of this genus in the phylogeny, it would be necessary to lump all basal Maxillariinae into *Maxillaria* to restore monophyly, making it even more challenging to diagnose morphology wise. But we also strongly disagree with further splitting for genera within Maxillariinae as proposed by Szlachetko *et al.* (2012), since this treatment would be unnecessarily too disruptive and, mainly, because many of the new taxa are poly or paraphyletic.

5. Conclusion

Our data reinforced the monophyly of most the core Maxillariinae genera, supporting the taxonomic treatment proposed by Blanco *et al.* (2007) and Pridgeon (2009) following Whitten *et al.* (2007). We consider the splitting of *Maxillaria s.l.* in 17 cohesive, well

supported and mostly morphologically diagnosable genera the best representation of the evolution of the subtribe, while we acknowledge the need of further molecular and morphological studies to improve the circumscription of some clades. Minor inconsistencies between the phylogeny we presented here and those previously published, as well as the non monophyletic taxa we recovered, for both basal and core Maxillariinae genera, are outwardly troublesome, as they point towards the clades for which deeper investigations are critical to appraise relationships within the subtribe. In that sense, besides our contributions to the understanding of Maxillariinae and the achievement of a consensual taxonomic treatment, the new DNA sequences we provided here will be of use not only for future phylogenies, but also for any researchers addressing important representatives of our neotropical flora.

6. References

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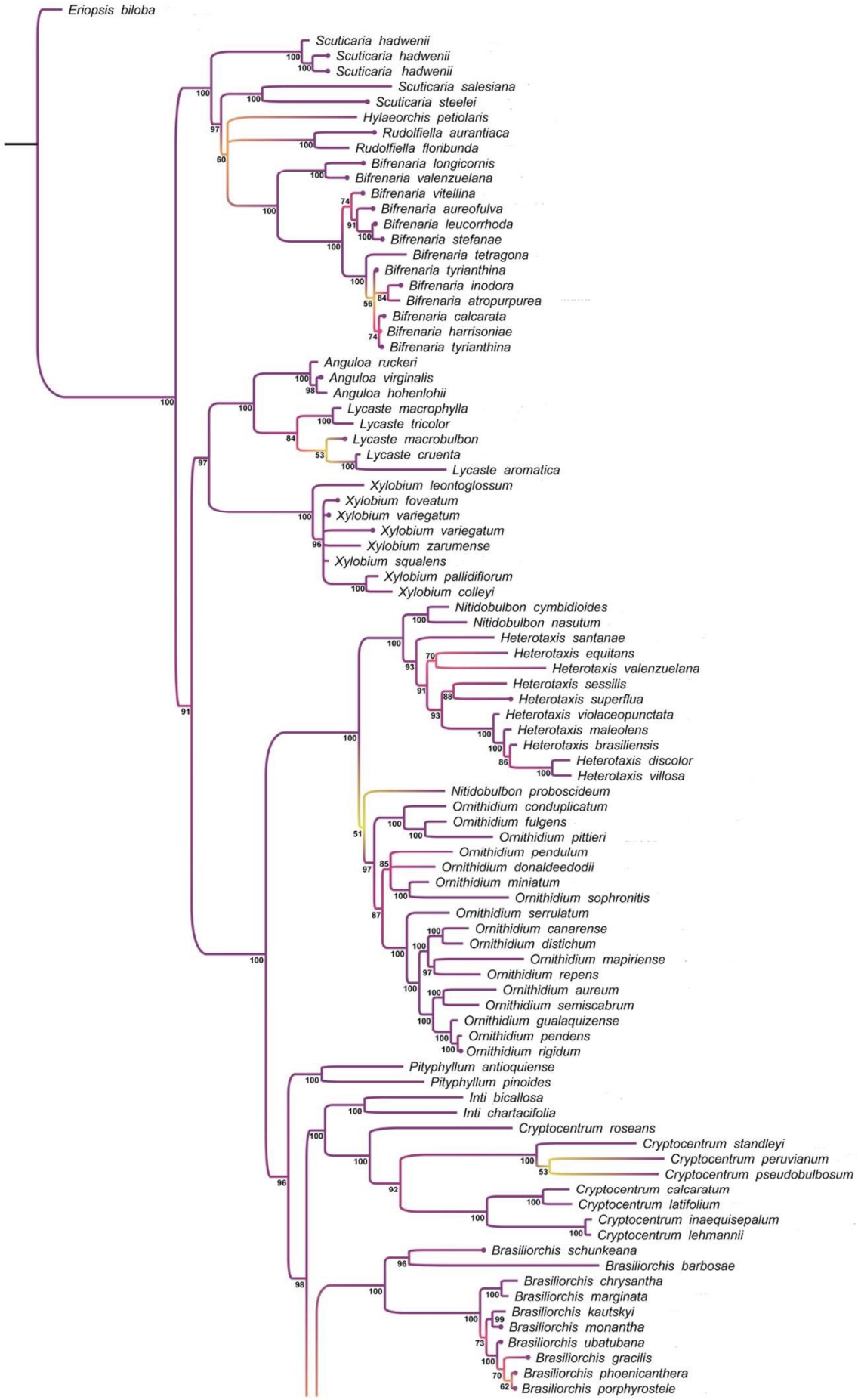
Table 1. Maxillariinae DNA sequences used in the phylogenetic analysis. For each species, one sequence from nuclear genome, *nr*ITS, and two sequences from the chloroplast genome, *matK-trnK* and *atpB-rbcl*, were used allways that available. For each species and each sequence, the species voucher is provided.

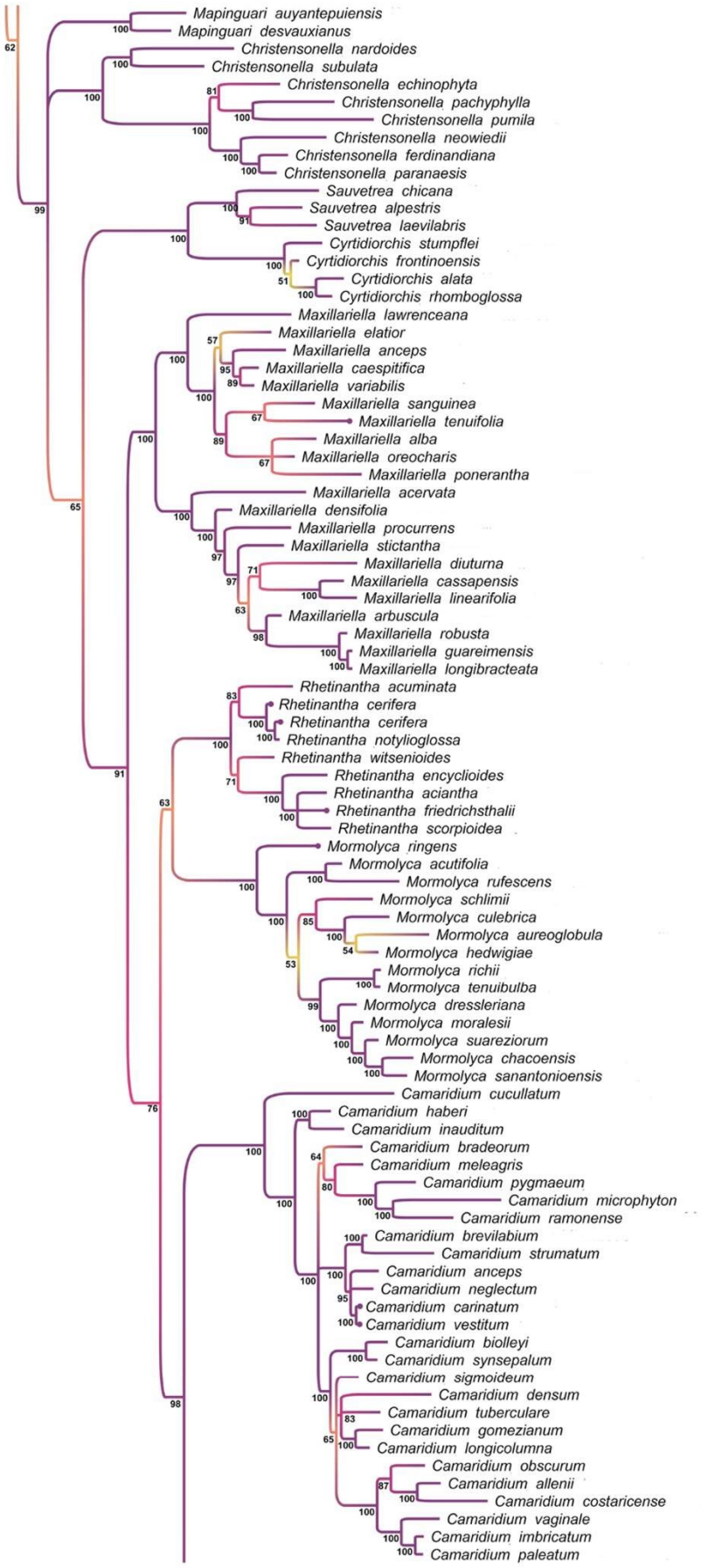
Species	**Authority**	***nr*ITS**	***matK-trnK***	***atpB-rbcl***
Anguloa virginalis	Linden ex B.S.Williams	A.P.Moraes 121	A.P.Moraes 121	A.P.Moraes 121
*Bifrenaria aureofulva**	Lindl.	IB10468	IB10468	IB10468
*Bifrenaria calcarata**	Barb.Rodr.		A.P.Moraes 31	A.P.Moraes 31
*Bifrenaria harrisoniae**	(Hook.) Rchb.f.	A.P.Moraes 45	A.P.Moraes 45	A.P.Moraes 45
Bifrenaria inodora	Lindl.	IB16946	IB16946	IB16946
*Bifrenaria leucorrhoda**	Rchb. f.	A.P.Moraes 17	A.P.Moraes 17	A.P.Moraes 17
Bifrenaria longicornis	Lindl.	IB P6285	IB P6285	IB P6285
*Bifrenaria stefanae**	V.P.Castro	A.P.Moraes 16	A.P.Moraes 16	A.P.Moraes 16
Bifrenaria tyrianthina	(Lodd. ex Loudon) Rchb.f.	IB11261	IB11261	IB11261
Bifrenaria tyrianthina	(Lodd. ex Loudon) Rchb.f.	IB5298	IB5298	IB5298
Bifrenaria venezuelana	C.Schweinf.	IB P6289	IB P6289	IB P6289
*Bifrenaria vitelina**	Lindl.	to confirm	to confirm	
Brasiliorchis barbosae	(Loefgr.) R.B.Singer, S.Koehler & Carnevali	IB11717		
Brasiliorchis gracilis	(Lodd., G.Lodd. & W.Lodd.) R.B.Singer, S.Koehler & Carnevali		IB654	IB654
Brasiliorchis phoenicanthera	(Barb.Rodr.) R.B.Singer, S.Koehler & Carnevali	IB2732	IB2732	IB2732
*Brasiliorchis monantha**	(Barb.Rodr.) Campacci	IB5442	IB5442	IB5442
Brasiliorchis porphyrostele	(Rchb.f.) R.B.Singer, S.Koehler & Carnevali	IB4735	IBt SP 4735	IB4735
Brasiliorchis schunkeana	(Campacci & Kautsky) R.B.Singer, S.Koehler & Carnevali	A.P.Moraes 131	A.P.Moraes 131	A.P.Moraes 131
Brasiliorchis ubatubana	(Hoehne) R.B.Singer, S.Koehler & Carnevali	IB618	IB618	IB618
Camaridium carinatum	(Barb.Rodr.) Hoehne	IB16781	IB16781	IB16781
Camaridium vestitum	(Sw.) Lindl.		to confirm	to confirm
Heterotaxis superflua	(Rchb.f.) F.Barros	IB P3935	IB P3935	IB P3935
*Lycaste macrobulbon**	(Hook.) Lindl.	IB11785	IB11785	
*Maxillaria kegelii**	Rchb.f.	A.P.Moraes 77	A.P.Moraes 77	A.P.Moraes 77
Maxillaria leucaimata	Barb.Rodr.		IB15044	IB15044
Maxillariella robusta	(Barb.Rodr.) M.A.Blanco & Carnevali	IB8510	IB8510	IB8510

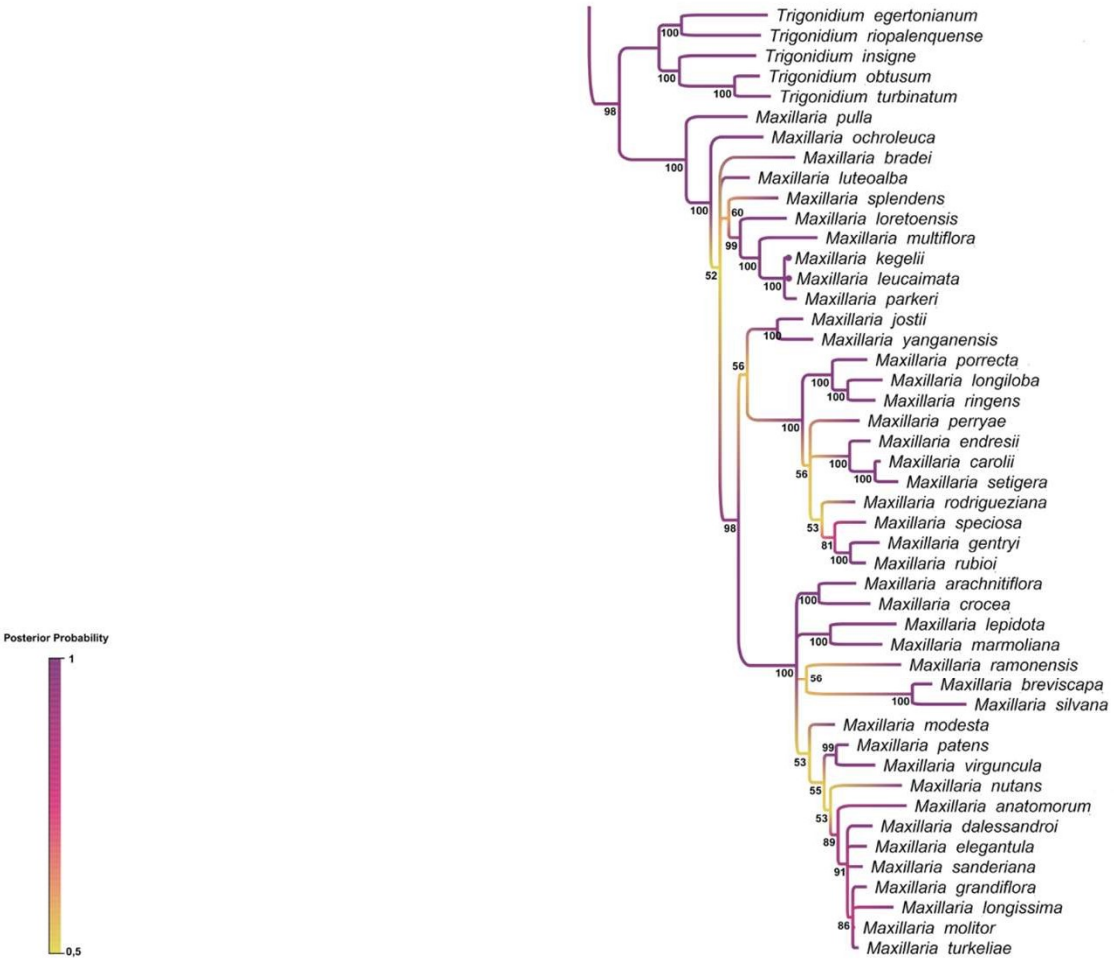
Maxillariella tenuifolia	(Lindl.) M.A.Blanco & Carnevali		A.P.Moraes 09	A.P.Moraes 09
Mormolyca ringens	(Lindl.) Gentil	IB16981	IB16981	IB16981
Ornithidium rigidum	(Barb.Rodr.) M.A.Blanco & Ojeda	IB7103	IB7103	IB7103
*Rhetinantha cerifera**	(Barb.Rodr.) M.A.Blanco	IB6103	IB6103	IB6103
*Rhetinantha cerifera**	(Barb.Rodr.) M.A.Blanco	IB7078	IB7078	IB7078
*Rhetinantha friedrichsthalii**	(Rchb.f.) M.A.Blanco		IB15196	IB15196
*Rudolfiella aurantiaca**	(Lindl.) Hoehne	IB10110	IB10110	IB10110
Scuticaria hadwenii	(Lindl.) Planch.	IB18323	IB18323	IB18323
Scuticaria hadwenii	(Lindl.) Planch.	IB11996	IB 11996	IB11996
Scuticaria steelei	(Hook.) Lindl.	A.P.Moraes 75	A.P.Moraes 75	A.P.Moraes 75
*Xylobium foveatum**	(Lindl.) G.Nicholson		IB P3929	IB P3929
*Xylobium variegatum**	(Ruiz & Pav.) Garay & Dunst.	A.P.Moraes 03	A.P.Moraes 03	A.P.Moraes 03

* Species that are not present in comprehensive Maxillariinae phylogenies (Whitten et al. 2007, 2014)

Figure 1: Maxillariinae phylogenetic tree from Bayesian Inference (BI), based on *nr*ITS, *matK-trnK* and *atpB-rbcl* markers. Branches support, Posterior Probability, is indicated by numbers in the phylogeny nodes and by the branch color according to a color scale ranging from yellow (low support) to purple (high support). Circles in the branch tips indicate species sequenced in the present study, while the extant species sequences were obtained in the GeneBank database.


Eriopsis biloba
Scuticaria hadwenii
Scuticaria hadwenii
Scuticaria hadwenii
Scuticaria salesiana
Scuticaria steelei
Hylaeorchis petiolaris
Rudolfiella aurantiaca
Rudolfiella floribunda
Bifrenaria longicornis
Bifrenaria valenzuelana
Bifrenaria vitellina
Bifrenaria aureofulva
Bifrenaria leucorrhoda
Bifrenaria stefanae
Bifrenaria tetragona
Bifrenaria tyrianthina
Bifrenaria inodora
Bifrenaria atropurpurea
Bifrenaria calcarata
Bifrenaria harrisoniae
Bifrenaria tyrianthina
Anguloa ruckeri
Anguloa virginalis
Anguloa hohenlohii
Lycaste macrophylla
Lycaste tricolor
Lycaste macrobulbon
Lycaste cruenta
Lycaste aromatica
Xylobium leontoglossum
Xylobium foveatum
Xylobium variegatum
Xylobium variegatum
Xylobium zarumense
Xylobium squalens
Xylobium pallidiflorum
Xylobium colleyi
Nitidobulbon cymbidioides
Nitidobulbon nasutum
Heterotaxis santanae
Heterotaxis equitans
Heterotaxis valenzuelana
Heterotaxis sessilis
Heterotaxis superflua
Heterotaxis violaceopunctata
Heterotaxis maleolens
Heterotaxis brasiliensis
Heterotaxis discolor
Heterotaxis villosa
Nitidobulbon proboscideum
Ornithidium conduplicatum
Ornithidium fulgens
Ornithidium pittieri
Ornithidium pendulum
Ornithidium donaldeedodii
Ornithidium miniatum
Ornithidium sophronitis
Ornithidium serrulatum
Ornithidium canarense
Ornithidium distichum
Ornithidium mapiriense
Ornithidium repens
Ornithidium aureum
Ornithidium semiscabrum
Ornithidium gualaquizense
Ornithidium pendens
Ornithidium rigidum
Pityphyllum antioquiense
Pityphyllum pinoides
Inti bicallosa
Inti chartacifolia
Cryptocentrum roseans
Cryptocentrum standleyi
Cryptocentrum peruvianum
Cryptocentrum pseudobulbosum
Cryptocentrum calcaratum
Cryptocentrum latifolium
Cryptocentrum inaequisepalum
Cryptocentrum lehmannii
Brasiliorchis schunkeana
Brasiliorchis barbosae
Brasiliorchis chrysantha
Brasiliorchis marginata
Brasiliorchis kautskyi
Brasiliorchis monantha
Brasiliorchis ubatubana
Brasiliorchis gracilis
Brasiliorchis phoenicanthera
Brasiliorchis porphyrostele


Mapinguari auyantepuiensis
Mapinguari desvauxianus
Christensonella nardoides
Christensonella subulata
Christensonella echinophyta
Christensonella pachyphylla
Christensonella pumila
Christensonella neowiedii
Christensonella ferdinandiana
Christensonella paranaesis
Sauvetrea chicana
Sauvetrea alpestris
Sauvetrea laevilabris
Cyrtidiorchis stumpflei
Cyrtidiorchis frontinoensis
Cyrtidiorchis alata
Cyrtidiorchis rhomboglossa
Maxillariella lawrenceana
Maxillariella elatior
Maxillariella anceps
Maxillariella caespitifica
Maxillariella variabilis
Maxillariella sanguinea
Maxillariella tenuifolia
Maxillariella alba
Maxillariella oreocharis
Maxillariella ponerantha
Maxillariella acervata
Maxillariella densifolia
Maxillariella procurrens
Maxillariella stictantha
Maxillariella diuturna
Maxillariella cassapensis
Maxillariella linearifolia
Maxillariella arbuscula
Maxillariella robusta
Maxillariella guareimensis
Maxillariella longibracteata
Rhetinantha acuminata
Rhetinantha cerifera
Rhetinantha cerifera
Rhetinantha notylioglossa
Rhetinantha witsenioides
Rhetinantha encyclioides
Rhetinantha aciantha
Rhetinantha friedrichsthalii
Rhetinantha scorpioidea
Mormolyca ringens
Mormolyca acutifolia
Mormolyca rufescens
Mormolyca schlimii
Mormolyca culebrica
Mormolyca aureoglobula
Mormolyca hedwigiae
Mormolyca richii
Mormolyca tenuibulba
Mormolyca dressleriana
Mormolyca moralesii
Mormolyca suareziorum
Mormolyca chacoensis
Mormolyca sanantonioensis
Camaridium cucullatum
Camaridium haberi
Camaridium inauditum
Camaridium bradeorum
Camaridium meleagris
Camaridium pygmaeum
Camaridium microphyton
Camaridium ramonense
Camaridium brevilabium
Camaridium strumatum
Camaridium anceps
Camaridium neglectum
Camaridium carinatum
Camaridium vestitum
Camaridium biolleyi
Camaridium synsepalum
Camaridium sigmoideum
Camaridium densum
Camaridium tuberculare
Camaridium gomezianum
Camaridium longicolumna
Camaridium obscurum
Camaridium allenii
Camaridium costaricense
Camaridium vaginale
Camaridium imbricatum
Camaridium paleatum


Trigonidium egertonianum
Trigonidium riopalenquense
Trigonidium insigne
Trigonidium obtusum
Trigonidium turbinatum
Maxillaria pulla
Maxillaria ochroleuca
Maxillaria bradei
Maxillaria luteoalba
Maxillaria splendens
Maxillaria loretoensis
Maxillaria multiflora
Maxillaria kegelii
Maxillaria leucaimata
Maxillaria parkeri
Maxillaria jostii
Maxillaria yanganensis
Maxillaria porrecta
Maxillaria longiloba
Maxillaria ringens
Maxillaria perryae
Maxillaria endresii
Maxillaria carolii
Maxillaria setigera
Maxillaria rodrigueziana
Maxillaria speciosa
Maxillaria gentryi
Maxillaria rubioi
Maxillaria arachnitiflora
Maxillaria crocea
Maxillaria lepidota
Maxillaria marmoliana
Maxillaria ramonensis
Maxillaria breviscapa
Maxillaria silvana
Maxillaria modesta
Maxillaria patens
Maxillaria virguncula
Maxillaria nutans
Maxillaria anatomorum
Maxillaria dalessandroi
Maxillaria elegantula
Maxillaria sanderiana
Maxillaria grandiflora
Maxillaria longissima
Maxillaria molitor
Maxillaria turkeliae
Posterior Probability
1
0,5

Suplementar Information 1. Maxillariinae DNA sequences compiled from geneBank for the phylogenetic analysis. For each species, one sequence from nuclear genome, *nr*ITS, and two sequences from the chloroplast genome, *matK-trnK* and *atpB-rbcl*, were used allways that available. For each species and each sequence, the GenBank assession number is provided.

Species	**Authorship**	**ITS**	**matK**	**atpB**
Anguloa hohenlohii	C.Morren	AF239333	AF239429	-
Anguloa ruckeri	Lindl.	EF079426	EF065565	-
Bifrenaria atropurpurea	Lindl.	AF239336	AF239432	-
Bifrenaria tetragona	(Lindl.) Schltr.	AF239335	DQ210751	-
Brasiliorchis chrysantha	(Barb.Rodr.) R.B.Singer, S.Koehler & Carnevali	DQ210113	DQ210644	DQ209424
Brasiliorchis kautskyi	(Pabst) R.B.Singer, S.Koehler & Carnevali	DQ210115	DQ210646	DQ209426
Brasiliorchis marginata	(Lindl.) R.B.Singer, S.Koehler & Carnevali	-	DQ210718	DQ209495
Camaridium allenii	(L.O.Williams) M.A.Blanco	DQ210068	DQ210611	DQ209382
Camaridium anceps	(Rchb.f.) M.A.Blanco	DQ210007	DQ209874	DQ209322
Camaridium biolleyi	(Schltr.) Schltr.	DQ210050	DQ209897	DQ209365
Camaridium bradeorum	Schltr.	DQ210481	DQ210963	DQ209764
Camaridium brevilabium	(Ames & Correll) M.A.Blanco	DQ210456	DQ210940	DQ209740
Camaridium costaricense	Schltr.	DQ210028	DQ210600	DQ209342
Camaridium cucullatum	(Lindl.) M.A.Blanco	DQ210178	DQ210708	DQ209485
Camaridium densum	(Lindl.) M.A.Blanco	DQ210091	DQ210629	DQ209403
Camaridium gomezianum	(J.T.Atwood) M.A.Blanco	DQ210297	DQ210796	DQ209586
Camaridium haberi	(J.T.Atwood) M.A.Blanco	DQ210032	DQ209881	DQ209346
Camaridium imbricatum	Schltr.	DQ210043	DQ209890	DQ209358
Camaridium inauditum	(Rchb.f.) M.A.Blanco	DQ210524	DQ211004	DQ209806
Camaridium longicolumna	(J.T.Atwood) M.A.Blanco	DQ210495	DQ210978	DQ209779
Camaridium meleagris	(Lindl.) M.A.Blanco	DQ210539	DQ211014	DQ209821
Camaridium microphyton	(Schltr.) M.A.Blanco	DQ210539	DQ210976	DQ209777
Camaridium neglectum	(Schltr.) M.A.Blanco	DQ210383	DQ210874	DQ209669
Camaridium obscurum	(Linden & Rchb.f.) M.A.Blanco	DQ210048	DQ209895	DQ209363
Camaridium paleatum	(Rchb.f.) M.A.Blanco	DQ210420	DQ210907	DQ209705

Camaridium pygmaeum	M.A.Blanco	DQ210477	DQ210959	DQ209760
Camaridium ramonense	(Schltr.) M.A.Blanco	DQ210460	DQ210944	DQ209744
Camaridium sigmoideum	(C.Schweinf.) M.A.Blanco	DQ210018	DQ210590	DQ209332
Camaridium strumatum	(Endres & Rchb.f.) M.A.Blanco	DQ210179	DQ210709	DQ209486
Camaridium synsepalum	(J.T.Atwood) M.A.Blanco	DQ210076	DQ210619	DQ209389
Camaridium tuberculare	(J.T.Atwood) M.A.Blanco	DQ210446	DQ210931	DQ209730
Camaridium vaginale	(Rchb.f.) M.A.Blanco	DQ210306	DQ210805	DQ209594
Christensonella echinophyta	(Barb.Rodr.) Szlach., Mytnik, GÃ³rniak & Smiszek	DQ210197	DQ210727	DQ209504
Christensonella ferdinandiana	(Barb.Rodr.) Szlach., Mytnik, GÃ³rniak & Smiszek	DQ210129	DQ210660	DQ209440
Christensonella nardoides	(Kraenzl.) Szlach., Mytnik, GÃ³rniak & Smiszek	DQ210403	DQ210890	DQ209688
Christensonella neowiedii	(Rchb.f.) S.Koehler	DQ210130	DQ210661	DQ209441
Christensonella pachyphylla	(Schltr. ex Hoehne) Szlach., Mytnik, GÃ³rniak & Smiszek	DQ210203	DQ210733	DQ209510
Christensonella paranaesis	NA	DQ210120	DQ210651	DQ209431
Christensonella pumila	(Hook.) Szlach., Mytnik, GÃ³rniak & Smiszek	DQ210166	DQ210696	DQ209474
Christensonella subulata	(Lindl.) Szlach., Mytnik, GÃ³rniak & Smiszek	DQ210161	DQ210693	DQ209469
Cryptocentrum calcaratum	(Schltr.) Schltr.	DQ210487	DQ210970	DQ209771
Cryptocentrum inaequisepalum	C.Schweinf.	DQ210501	DQ210982	DQ209784
Cryptocentrum latifolium	Schltr.	DQ209999	DQ210578	DQ209315
Cryptocentrum lehmannii	(Rchb.f.) Garay	DQ210365	DQ210859	DQ209652
Cryptocentrum peruvianum	(Cogn.) C.Schweinf.	DQ210279	DQ210786	DQ209572
Cryptocentrum pseudobulbosum	C.Schweinf.	DQ210280	DQ210861	DQ209654
Cryptocentrum roseans	(Schltr.) A.D.Hawkes	DQ210416	DQ210903	DQ209701
Cryptocentrum standleyi	Ames	DQ210309	DQ210808	DQ209597
Cyrtidiorchis alata	(Ruiz & Pav.) Rauschert	DQ210569	DQ211044	DQ209849
Cyrtidiorchis frontinoensis	(Garay) Rauschert	DQ210248	-	DQ209542
Cyrtidiorchis rhomboglossa	(F.Lehm. & Kraenzl.) Rauschert	KP323286	KP278316	KM879258
Cyrtidiorchis stumpflei	(Garay) Rauschert	FJ565229	FJ564741	-
Eriopsis biloba	Lindl.	DQ461788	DQ461806	DQ461770
Eriopsis biloba	Lindl.	DQ210374	DQ210866	DQ209661

Heterotaxis brasiliensis	(Brieger & Illg) F.Barros	DQ210155	DQ210687	DQ209465
Heterotaxis discolor	(Lodd. ex Lindl.) Ojeda & Carnevali	DQ210181	DQ210711	DQ209488
Heterotaxis equitans	(Schltr.) Ojeda & Carnevali	DQ210151	DQ210683	DQ209461
Heterotaxis maleolens	(Schltr.) Ojeda & Carnevali	DQ210525	DQ209972	DQ209807
Heterotaxis santanae	(Carnevali & I.RamÃrez) Ojeda & Carnevali	DQ210526	DQ209973	DQ209808
Heterotaxis sessilis	(Sw.) F.Barros	DQ210897	DQ210897	DQ209695
Heterotaxis valenzuelana	(A.Rich.) Ojeda & Carnevali	DQ210170	DQ210700	DQ209477
Heterotaxis villosa	(Barb.Rodr.) F.Barros	DQ210202	DQ210732	DQ209509
Heterotaxis violaceopunctata	(Rchb.f.) F.Barros	DQ210202	DQ210678	DQ209457
Hylaeorchis petiolaris	(Schltr.) Carnevali & G.A.Romero	DQ210545	DQ211020	DQ209827
Inti bicallosa	(Rchb.f.) M.A.Blanco	DQ210202	DQ210998	DQ209800
Inti chartacifolia	(Ames & C.Schweinf.) M.A.Blanco	DQ210265	DQ209942	DQ209559
Lycaste aromatica	(Graham) Lindl.	-	-	-
Lycaste cruenta	(Lindl.) Lindl.	AF239342	AF239438	-
Lycaste macrophylla	(Poepp. & Endl.) Lindl.	-	EU214178	-
Lycaste tricolor	Rchb.f.	-	EU214513	-
Mapinguari auyantepuiensis	(Foldats) Carnevali & R.B.Singer	DQ210336	DQ210834	DQ209622
Mapinguari desvauxianus	(Rchb.f.) Carnevali & R.B.Singer	DQ210736	DQ210736	DQ209513
Maxillaria anatomorum	Rchb.f.	DQ210202	DQ210966	DQ209767
Maxillaria arachnitiflora	Ames & C.Schweinf.	DQ210202	DQ209909	DQ209378
Maxillaria bradei	Schltr. ex Hoehne	DQ210202	DQ210681	DQ209459
Maxillaria breviscapa	Poepp. & Endl.	DQ210544	DQ211019	DQ209826
Maxillaria carolii	Christenson	DQ210573	DQ211048	DQ209853
Maxillaria crocea	Lindl.	DQ210573	DQ210634	DQ209415
Maxillaria dalessandroi	Dodson	DQ210366	DQ210860	DQ209653
Maxillaria elegantula	Rolfe	DQ210024	DQ210596	DQ209338
Maxillaria endresii	Rchb.f.	DQ210024	DQ210586	DQ209325
Maxillaria gentryi	Dodson	DQ210492	DQ210975	DQ209776
Maxillaria grandiflora	(Kunth) Lindl.	DQ210454	DQ210938	DQ209738
Maxillaria jostii	Dodson	DQ210092	DQ210630	DQ209404

Maxillaria lepidota	Lindl.	DQ210454	DQ210857	DQ209650
Maxillaria longiloba	(Ames & C.Schweinf.) J.T.Atwood	DQ210454	DQ210919	DQ209716
Maxillaria longissima	Lindl.	DQ210515	DQ210996	DQ210996
Maxillaria loretoensis	C.Schweinf.	DQ210361	DQ210856	DQ209648
Maxillaria luteoalba	Lindl.	DQ210240	-	DQ209538
Maxillaria marmoliana	Dodson	DQ210351	DQ210848	DQ209638
Maxillaria modesta	Schltr.	DQ210195	DQ210726	DQ209503
Maxillaria molitor	Rchb.f.	DQ210369	DQ210863	DQ209656
Maxillaria multiflora	Barb.Rodr.	DQ210186	DQ210716	DQ209493
Maxillaria nutans	Lindl.	DQ210561	DQ211036	DQ209841
Maxillaria ochroleuca	Lodd. ex Lindl.	DQ210105	DQ210636	DQ209417
Maxillaria parkeri	Hook.	DQ210144	DQ210675	DQ209454
Maxillaria patens	Schltr.	DQ210528	DQ211006	DQ209810
Maxillaria perryae	Dodson	DQ461801	DQ461819	DQ461783
Maxillaria porrecta	Lindl.	DQ209985	DQ210576	DQ209302
Maxillaria pulla	Linden & Rchb.f.	DQ210381	DQ210872	DQ209667
Maxillaria ramonensis	Schltr.	DQ210099	DQ209918	DQ209411
Maxillaria ringens	Rchb.f.	DQ210005	DQ210583	DQ209321
Maxillaria rodrigueziana	J.T.Atwood & Mora-Ret.	DQ210061	DQ210606	DQ209376
Maxillaria rubioi	Dodson	DQ210327	DQ210826	DQ209615
Maxillaria sanderiana	Rchb.f. ex Sander	DQ210271	DQ210781	DQ209564
Maxillaria setigera	Lindl.	DQ210143	DQ210674	DQ209453
Maxillaria silvana	Campacci	DQ210391	DQ210878	DQ209676
Maxillaria speciosa	Rchb.f.	DQ210075	DQ210618	DQ209388
Maxillaria splendens	Poepp. & Endl.	DQ210152	DQ210684	DQ209462
Maxillaria turkeliae	Christenson	DQ210276	DQ209945	DQ209569
Maxillaria virguncula	Rchb.f.	DQ210504	DQ210985	DQ209787
Maxillaria yanganensis	Dodson	DQ461790	DQ461808	DQ461772
Maxillariella acervata	(Rchb.f.) M.A.Blanco & Carnevali	DQ210064	DQ210607	DQ209379
Maxillariella alba	(Hook.) M.A.Blanco & Carnevali	DQ210315	DQ210814	DQ209603

Maxillariella anceps	(Ames & C.Schweinf.) M.A.Blanco & Carnevali	DQ210518	DQ209971	DQ209801
Maxillariella arbuscula	(Lindl.) M.A.Blanco & Carnevali	DQ210555	DQ211030	DQ209836
Maxillariella caespitifica	(Rchb.f.) M.A.Blanco & Carnevali	DQ210035	DQ209883	DQ209349
Maxillariella cassapensis	(Rchb.f.) M.A.Blanco & Carnevali	DQ210256	DQ210768	DQ209550
Maxillariella densifolia	(Poepp. & Endl.) M.A.Blanco & Carnevali	DQ210253	DQ210767	DQ209547
Maxillariella diuturna	(Ames & C.Schweinf.) M.A.Blanco & Carnevali	DQ210022	DQ210594	DQ209336
Maxillariella elatior	(Rchb.f.) M.A.Blanco & Carnevali	DQ210298	DQ210797	DQ209587
Maxillariella guareimensis	(Rchb.f.) M.A.Blanco & Carnevali	DQ210565	DQ211040	DQ209845
Maxillariella lawrenceana	(Rolfe) M.A.Blanco & Carnevali	DQ210451	DQ210936	DQ210451
Maxillariella linearifolia	(Ames & C.Schweinf.) M.A.Blanco & Carnevali	DQ210096	DQ209915	DQ209408
Maxillariella longibracteata	(Lindl.) M.A.Blanco & Carnevali	DQ210353	DQ210850	DQ209640
Maxillariella oreocharis	(Schltr.) M.A.Blanco & Carnevali	DQ210488	DQ210971	DQ209772
Maxillariella ponerantha	(Rchb.f.) M.A.Blanco & Carnevali	DQ210418	DQ210905	DQ209703
Maxillariella procurrens	(Lindl.) M.A.Blanco & Carnevali	DQ210380	DQ210871	DQ210380
Maxillariella sanguinea	(Rolfe) M.A.Blanco & Carnevali	DQ210081	DQ209910	DQ209394
Maxillariella stictantha	(Schltr.) M.A.Blanco & Carnevali	DQ210538	DQ211013	DQ210538
Maxillariella variabilis	(Bateman ex Lindl.) M.A.Blanco & Carnevali	DQ210187	DQ210717	DQ210717
Mormolyca acutifolia	(Lindl.) M.A.Blanco	DQ210168	DQ210698	DQ209475
Mormolyca aureoglobula	(Christenson) M.A.Blanco	KP323358	KP278241	KM879311
Mormolyca chacoensis	(Dodson) M.A.Blanco	DQ210278	DQ210785	DQ209571
Mormolyca culebrica	BogarÃn & Pupulin	KP323354	KP278281	KM879255
Mormolyca dressleriana	(Carnevali & J.T.Atwood) M.A.Blanco	DQ209980	DQ209858	DQ209297
Mormolyca hedwigiae	(Hamer & Dodson) M.A.Blanco	DQ210182	DQ210712	DQ209489
Mormolyca moralesii	(Carnevali & J.T.Atwood) M.A.Blanco	DQ210295	DQ210794	-
Mormolyca richii	(Dodson) M.A.Blanco	DQ461784	DQ461802	DQ461766
Mormolyca rufescens	(Lindl.) M.A.Blanco	DQ210191	DQ210721	DQ209498
Mormolyca sanantonioensis	(Christenson) M.A.Blanco	DQ210415	DQ210902	-
Mormolyca schlimii	(Linden & Rchb.f.) M.A.Blanco	DQ210350	DQ210847	DQ209637
Mormolyca suareziorum	(Dodson) M.A.Blanco	DQ210523	DQ211003	DQ209805
Mormolyca tenuibulba	(Christenson) M.A.Blanco	DQ210552	DQ211027	DQ209833

Nitidobulbon cymbidioides	(Dodson, J.T.Atwood & Carnevali) Ojeda & G.A.Romero	DQ209987	DQ209863	DQ209304
Nitidobulbon nasutum	(Rchb.f.) Ojeda & Carnevali	DQ210169	DQ210699	DQ209476
Nitidobulbon proboscideum	(Rchb.f.) Ojeda & Carnevali	DQ209979	DQ209857	DQ209296
Ornithidium aureum	Poepp. & Endl.	DQ210318	DQ210817	DQ209606
Ornithidium canarense	(J.T.Atwood) M.A.Blanco & Ojeda	DQ210372	DQ209959	DQ209659
Ornithidium conduplicatum	Ames & C.Schweinf.	DQ210041	DQ209889	DQ209356
Ornithidium distichum	Lindl.	DQ461791	DQ461809	DQ461773
Ornithidium donaldeedodii	Ackerman & Whitten	GU177875	KF660302	-
Ornithidium fulgens	Rchb.f.	DQ210225	DQ209930	DQ209525
Ornithidium gualaquizense	(Dodson) M.A.Blanco & Ojeda	DQ461796	DQ461814	DQ461778
Ornithidium mapiriense	Kraenzl.	DQ210571	DQ211046	DQ209851
Ornithidium miniatum	Lindl.	DQ210062	DQ209908	DQ209377
Ornithidium pendens	(Pabst) Senghas	DQ210104	DQ210635	DQ209416
Ornithidium pendulum	(Poepp. & Endl.) Cogn.	DQ210194	DQ210725	DQ209502
Ornithidium pittieri	Ames	DQ210060	DQ209907	DQ209375
Ornithidium repens	(L.O.Williams) M.A.Blanco & Ojeda	DQ210070	DQ210613	DQ209384
Ornithidium semiscabrum	Lindl.	KP323339	KP278300	KM879230
Ornithidium serrulatum	Lindl.	DQ210535	DQ211010	DQ209817
Ornithidium sophronitis	Rchb.f.	DQ210310	DQ210809	DQ210310
Pityphyllum antioquiense	Schltr.	DQ210371	DQ209958	DQ210371
Pityphyllum pinoides	H.R.Sweet	DQ210089	DQ209913	DQ209401
Rhetinantha aciantha	(Rchb.f.) M.A.Blanco	DQ210296	DQ210795	DQ209585
Rhetinantha acuminata	(Lindl.) M.A.Blanco	DQ210500	DQ210981	DQ209783
Rhetinantha encyclioides	(J.T.Atwood & Dodson) M.A.Blanco	DQ209983	DQ209861	DQ209861
Rhetinantha notylioglossa	(Rchb.f.) M.A.Blanco	DQ210114	DQ210645	DQ210114
Rhetinantha scorpioidea	(Kraenzl.) M.A.Blanco	DQ210058	DQ209905	DQ209373
Rhetinantha witsenioides	(Schltr.) M.A.Blanco	DQ210247	DQ209937	DQ209541
Rudolfiella floribunda	(Schltr.) Hoehne	DQ210394	DQ210881	DQ209679
Sauvetrea alpestris	(Lindl.) Szlach.	DQ210414	DQ210901	DQ209699

Sauvetrea chicana	(Dodson) M.A.Blanco	DQ461795	DQ461813	-
Sauvetrea laevilabris	(Lindl.) M.A.Blanco	DQ210334	DQ210832	DQ209621
Scuticaria hadwenii	(Lindl.) Planch.	AF239328	AF239424	-
Scuticaria salesiana	Dressler	DQ210385	DQ210875	DQ209671
Trigonidium egertonianum	Bateman ex Lindl.	DQ210184	DQ210714	DQ209491
Trigonidium insigne	Rchb.f. ex Benth. & Hook.f.	DQ210494	DQ210977	DQ209778
Trigonidium obtusum	Lindl.	DQ210110	DQ210641	DQ209421
Trigonidium riopalenquense	Dodson	DQ210252	DQ210766	DQ209546
Trigonidium turbinatum	Rchb.f.	DQ210183	DQ210713	DQ209490
Xylobium colleyi	(Bateman ex Lindl.) Rolfe	-	DQ210745	-
Xylobium leontoglossum	(Rchb.f.) Benth. ex Rolfe	DQ210254	DQ209939	DQ209548
Xylobium pallidiflorum	(Hook.) G.Nicholson	AF239338	AF239434	-
Xylobium squalens	(Lindl.) Lindl.	-	EF079255	-
Xylobium zarumense	Dodson	AF239339	AF239435	-

CHAPTER 2 - ARE CHROMOSOME NUMBER AND GENOME SIZE ASSOCIATED WITH HABIT AND ENVIRONMENTAL NICHE VARIABLES? INSIGHTS FROM THE NEOTROPICAL MAXILLARIINAE (ORCHIDACEAE).

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Summary

- The entangled relationship between chromosome number and genome size with species distribution has been the subject of study for almost a century and remainsstill an open question. Aiming to contribute to this discussion, here we used a widely distributed and karyotypically well-known orchid subtribe as a model to infer such relationships in a robust methodological scenario.
- We extracted the most important environmental variables, habits, and tested the best evolutive model for genomic traits. We also fitted null models to evaluate the putative role of polyploidy. Finally, we perform multivariate phylogenetic models to test the associations between ecological and genomic traits.
- The small genome size was related to epiphytism and possibly was adaptive in the diversification of Neotropical orchids. Polyploidy seems to be associated with reducing the number of habits, exerting epiphytism, and colonising new habitats outside humid Forests.
- The genomic traits explored here seem to be shaped by both neutral and adaptive evolution. Despite the important role of polyploidy on the ecological niche, the genome size proved to be central to Maxillariinae evolution by its association with plant physiology, environmental variables, and the epiphytic habit, a fundamental trait in Neotropical orchid diversification.

Keywords: C-value, dysploidy, epiphytic, karyotype, ecological niche, orchid, polyploidy, SDM

Introduction

Species distribution is a central subject in niche theory, and the role of chromosome numbers in this subject has been the focus of debate for almost a century (e.g. see initial works of Müntzing, 1936; Clausen *et al.*, 1940, 1945; Löve & Löve, 1943, 1949; Stebbins, 1950, 1971; Ehrendorfer, 1979). The species' realised niches are primarily determined by species inherent abiotic requirements, like environmental conditions, and associated physiological constraints, as well as by biotic requirements, often associated with resource availability and biotic interactions, and by dispersal ability (Soberón, 2010). Since the abiotic and biotic niche drivers are evolvable, they may be constrained by genomic traits, as chromosome number (Leitch & Leitch, 2008; Ramsey & Ramsey, 2014; Rice *et al.*, 2019; Baniaga *et al.*, 2020) and genome size (Knight & Ackerly, 2002; Knight *et al.*, 2005; Knight & Beaulieu, 2008; Beaulieu *et al.*, 2008; Leitch *et al.*, 2010; Pellicer *et al.*, 2018; Roddy *et al.*, 2020). Such entangled relationships between plant species' niche and genomic traits is an effervescent research topic (as reviewed in Spoelhof *et al.*, 2017; Baduel *et al.*, 2018; Lavania, 2020; Van de Peer *et al.*, 2020) but more needs to be uncovered about the impact of such genomic traits, associated or not with polyploidy, in plant species niche.

Studies on polyploid ecology have suggested the association of high ploidy levels with putative differential responses to different stress (reviewed in te Beest *et al.*, 2012), favouring the occupancy of new, extreme, or adverse environments (Ramsey & Ramsey, 2014; Dar & Rehman, 2017; Doyle & Coate, 2019). Accordingly, it is accepted that polyploidy could promote shifts in community assembly and dynamics and changes in the ecological niche (McIntyre, 2012; Segraves & Anneberg, 2016; Soltis *et al.*, 2016; Blaine Marchant *et al.*, 2016; Segraves, 2017; Baduel *et al.*, 2018; Karunarathne *et al.*, 2018; Rice *et al.*, 2019; Rezende *et al.*, 2020). Nevertheless, the phylogenetic relationships may mask the genomic effects since a higher polyploid frequency could be a consequence of the differential distribution of a polyploid-rich taxonomic group (Martin & Husband, 2009; Rice *et al.*, 2019). In this sense, phylogenetic correction is mandatory in polyploid ecology studies.

By doubling the genome, the polyploidy directly affects the genome size, a second genomic trait that could affect the ecological niche. However, changes in the repetitive DNA fraction

can promote genome size variation even without any changes in the chromosome number (Moraes *et al.*, 2012; Lee & Kim, 2014; Biscotti *et al.*, 2015; Garrido-Ramos, 2017; Hartley & O'Neill, 2019). The nucleotypic effects (i.e., phenotypic traits influenced by bulk DNA amount) could be observed both in micro- and macro morphological scale (Knight & Ackerly, 2002; Knight *et al.*, 2005; Beaulieu *et al.*, 2007b, 2008; Knight & Beaulieu, 2008; Šímová & Herben, 2012; Greilhuber & Leitch, 2013; Snodgrass *et al.*, 2017; Doyle & Coate, 2019), along with constraints to functional traits, as photosynthetic rate (Beaulieu *et al.*, 2007a; Roddy *et al.*, 2020). The mechanism by which the genome size could interfere with morphology and physiology is that once the genome size increases, it tends to scale up the cell size, affecting the cell cycle speed (higher the genome size, lower the speed; Francis *et al.*, 2008) and generation time (higher the genome size, higher the minimal time generation; Bennett, 1972; Levin, 2002; Ryan Gregory, 2004). By interfering in such essential traits, the genome size is considered to potentially affect adaptive diversification (Leitch *et al.*, 2005; Kraaijeveld, 2010; Kang *et al.*, 2014; Simonin & Roddy, 2018; Pellicer *et al.*, 2018; Carta *et al.*, 2020), habit (Chase *et al.*, 2005; Leitch *et al.*, 2009; Veselý *et al.*, 2013, 2020; Carta & Peruzzi, 2016; Hidalgo *et al.*, 2017; Trávníček *et al.*, 2019) and present associations with environmental traits (Sparrow & Miksche, 1961; Knight & Ackerly, 2002; Leitch & Bennett, 2007; Díez *et al.*, 2013; Pustahija *et al.*, 2013; Carta & Peruzzi, 2016).

Considering the above-mentioned association of genomic and ecological niche traits, a dichotomy seems to apply to plant ecology. While it is traditionally assumed that polyploidy promotes a wider plant geographic distribution and ecological niches diversity than their diploid counterparts (Stebbins, 1985), the genome size increase may limit both the distribution and the niche expansion (Blaine Marchant *et al.*, 2016; Simonin & Roddy, 2018). Such a polyploid broader niche seems to be a consequence of the polyploids heterosis, favouring the niche expansion counterbalanced by the nucleotypic effects of genome size increase (Doyle & Coate, 2019). Nevertheless, such positive correlation between polyploidy and genome size increase assumed in this dichotomy is restricted to genera and families clades (Doyle & Coate, 2019), making it mandatory to evaluate the polyploidy and genome size effects in the plant ecology at the correct taxonomic level, by choosing a group that should embrace an extensive distribution and niche variability, along with significant genomic variability.

Orchidaceae, the largest plant family (Christenhusz & Byng, 2016; Ulloa Ulloa *et al.*, 2017), meets the requirements presenting wide distribution and high genomic variability. It was suggested that the epiphytic habit presents association with genome size (Leitch *et al.*, 2009; Trávníček *et al.*, 2019); however, both studies used temperate biased samples. Nevertheless, the Neotropical clades, which represent an important component of epiphytic flora, should bring more informative results on the chromosome number and genome size mode of evolution and association between genomic and ecological traits considering the epiphytic habit. Accordingly, we choose the subtribe Maxillariinae Benth. as a study system since it is a species-rich component in humid Neotropical forests, encompassing terrestrial, lithophytic and epiphytic habits and covering a wide geographical distribution (Whitten *et al.*, 2007). Moreover, Maxillariinae orchids present chromosome number and genome size variations, along with robust ecological data availability, making the subtribe the perfect candidate study group to understand the relationships between chromosome number and genome size and ecological niche.

Based on our system, we address the following questions: (1) Are genomic traits associated with environmental variables that influence the Maxillariinae distribution? (2) Are genomic traits associated with epiphytic habit in Maxillariinae? These questions are evaluated taking the polyploidy effect and phylogenetic correction into account. To answer these questions, we follow four steps: i) gathered and generated new data for genomic traits; ii) generated a phylogenetic hypothesis ensuring that all species with genomic and/or ecological data are represented and check that the phylogenetic hypothesis is congruent with previously published studies; iii) evaluated potentially relevant environmental variables and the occurrence data used to predict species ranges; and, iv) since both genomic traits are known to frequently exhibit strong phylogenetic dependence (i.e. shared evolutionary history) we tested evolution models to fit further analyses under adequate phylogenetic constraints and then performed macroecological analyses to address the study questions listed above. We perform multivariate phylogenetic models including environmental variables and habit preferences as explanatory variables and chromosome number and genome size as response variables. To evaluate the role of polyploidy, we reconduct the analyses with and without polyploids.

Material and Methods

Plant material

All plant material was obtained from the living collection of the Frederico Carlos Hoehne Orchidarium of the São Paulo Institute of Botany or collected during field expeditions, with vouchers deposited in HUFABC and SP (acronym following Thiers, 2020; Supporting Information Tables S1 and S2). We sampled species to ensure that all Maxillariinae species with available chromosome number and genome size (Table 1), habit (Table S3), and geographic distribution data are represented in the phylogenetic tree. Here we follow the taxonomic nomenclature defined by Blanco et al. (2007).

Methodology

1. **Genomic traits - Chromosome number and Genome size data**

Chromosome count. We collected young roots from 24 species (Table S1). Pre-treatments and fixation followed Moraes *et al. (2017)* and squash technique followed Guerra & Souza (2002) using enzymatic solution of 2% (w/v) cellulase (Onozuka)/20% (v/v) pectinase (Sigma)/1% macerozyme (Sigma) at 37°C for 30 min. The chromosome preparations were stained with DAPI (1 ug ml^{-1}) for 30 min and mounted with McIlvaine: glycerol pH 7.0 buffer (Guerra & Souza, 2002). The slides were examined under an Olympus BX 53 epifluorescence microscope (Olympus Life Science), photographed with a coupled XM10 camera, and analysed using Olympus CellSens software. The images were colour inverted and processed uniformly for colour balance, contrast, and brightness using Adobe Photoshop CS5 (Adobe Systems, Inc.).

Genome size estimation by Flow cytometry. Preferably, three individuals from each species were analysed in triplicate (Table S1). For each sample, one silica-dried pollinium or 50 mg of fresh young leaf tissue was macerated with approximately 25 mg of a fresh leaf of *Pisum sativum* var. Ctirad (2C=9.09pg; Doležel *et al.*, 1998) as an internal reference standard. Both materials were macerated in 0.5 ml of cold Ebihara buffer (Ebihara *et al.*, 2005) supplied with 0.025 µg mL^{-1} RNAse using a scalpel blade. Nuclei suspensions were stained by adding 12.5 µL of a 1 mg mL^{-1} solution of propidium iodide (PI, Sigma). The analysis was performed using

the FACSCanto II cytometer (Becton Dickinson, San Jose, CA, USA) kindly made available by the Microbiology and Immunology Department of IBB-UNESP (Botucatu, Brazil). The histograms were obtained with FACSDiva software based on 5,000 events, and the statistical evaluation was performed using Flowing Software 2.5.1 (http://www.flowingsoftware.com/). The quality control of the samples was based on the coefficient of variation (CV) of each measurement, which should be below 5%, and the standard deviation (SD) among genome size measurements, which should be below 3%. Such limits ensure that the variations observed inside and among measurements are due to technical factors and should not represent intraspecific variation among individuals (Pellicer & Leitch, 2014).

Literature survey. Besides the data obtained here, we also survey chromosome number and genome size data from the scientific literature (Table 1) and, for the outgroup species, from the Chromosome counts database (http://ccdb.tau.ac.il; Rice *et al.*, 2015) and Plant C-value (https://cvalues.science.kew.org; Pellicer & Leitch, 2020).

2. Phylogenetic hypotheses

We sampled 100 individuals representing 97 species from 26 genera for phylogenetic reconstruction: 87 species distributed in 19 genera from the subtribe Maxillariinae and ten species (seven genera) as outgroup (Table S2). Besides sequences obtained from Genbank, we produced new matK-trnK sequences for 19 species and ITS sequences for 16 species (Table S2).

Extraction, amplification and sequencing. Genomic DNA extraction followed the CTAB protocol (Doyle & Doyle, 1987), excluding β-mercaptoethanol. We used primers 17SE and 26SE (Sun *et al.*, 1994) to amplify the internal transcribed spacers from the nuclear ribosomal DNA (nrITS) from 19 species and the primers 19F (Goldman *et al.*, 2001) and trnK2R (Johnson & Soltis, 1994) to amplify the matK-trnK flanking region from the plastidial genome, with a third internal primer for sequencing, the 308F (TATCAGAAGGTTTTGSA), from 12 species. The PCRs parameters and reagent volumes are described in Whitten *et al.* (2007). Purification and sequencing were performed by Macrogen (Seoul, South Korea - http://dna.macrogen.com/eng/), using the same primers mentioned above. The obtained sequences were submitted to GenBank (Table S2).

Phylogenetic reconstruction. To build the phylogenetic framework for this study, we assembled a data matrix with three markers: two plastid genome regions (*mat*K-*trn*K and ycf1) and one nuclear region (ITS) (Table S2). The multiple sequence alignments were conducted by MAFFT v. 7.453 software using its progressive alignment and iterative refinement methods (Katoh & Standley, 2014; Katoh *et al.*, 2019). The matrix was analysed under Maximum Likelihood (ML) and Bayesian Inference (BI).

The ML analysis was performed using IQ-TREE2 (Minh *et al.*, 2020) using the following nucleotide substitution models: TIM3e+G4 to nrITS, TVM+F+R3 to matK-trnK, and TVM+F+R2 to ycf1 partition, all chosen by ModelFinder applying the Bayesian Information Criterion (BIC; Kalyaanamoorthy *et al.*, 2017). We used 100 bootstrapping to assess branch supports. The BI was performed using MrBayes v. 3.2.7a (Ronquist *et al.*, 2012) with GTR+I+G as the selected evolutive model for the three partitions. Four independent runs, one hot and three cold chains, were started from different random trees to ensure that individual runs had converged to the same result. We used 20 million generations per chain with a sampling frequency of 2,000. Split frequencies below 0.01 were used to check for convergence, and the effective sample size (ESS) for each run was checked in Tracer v. 1.7.1 (Rambaut *et al.*, 2018). Twenty-five per cent of trees were excluded as burn-in. Saved trees were summarised in a majority rule consensus tree created with nodal confidence assessed by posterior probabilities (PP), which were considered strongly supported when equal to or higher than 0.95. The ML and BI trees were edited with FigTree v.1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) and Adobe Photoshop CS5 (Adobe Systems, Inc.). Alignments and trees are available from TreeBase (http://treebase.org, submission number 28311).

3. The ecological data

Habit data. For all 97 species, we compiled information on habit (terrestrial, epiphytic, or lithophytic) from two datasets: World Flora Online (http://www.worldfloraonline.org/) and Flora do Brasil 2020 (http://floradobrasil.jbrj.gov.br/) (Table S3). We defined two ecological variables based on habit information: “number of possible alternative habits” and “exclusively or not exclusively epiphytic”.

Environmental data - elevation and bioclimatic variables. We compiled occurrence information from three online databases: Species link (http://splink.cria.org.br), GBIF

(Holstein, 2001; https://www.gbif.org) and BIEN (Enquist *et al.*, 2016; Maitner *et al.*, 2018; http://bien.nceas.ucsb.edu), besides specific literature sources (Koehler *et al.*, 2008, 2012; Pridgeon *et al.*, 2009; Gomes *et al.*, 2018), expert knowledge and our collection records. We excluded duplicate occurrence entries, flawed geographic coordinates, and dubious entries, retaining a total of 5,839 occurrence points (Table S4).

From each occurrence point, we extracted information on a) elevation, obtained from the Global Multi-resolution Terrain Elevation Data 2010 (Danielson & Gesch, 2011) (Table S3), and b) 19 bioclimatic variables provided by WorldClim 2.0 (https://www.worldclim.org; Fick & Hijmans, 2017) with a spatial resolution of 30 arc seconds (~1 km^2). As elevation and bioclimatic variables are often correlated, we used the package 'virtualspecies' (Leroy *et al.*, 2016) in R (R Core Team, 2020) and the Variance Inflation Factor (Fox & Monette, 1992; Mundry, 2014) to identify and reduce multicollinearity among these variables (Methods S1). As a result, we kept elevation and six bioclimatic variables: (1) Temperature Seasonality, (2) Mean Temperature during the Coldest Quarter, (3) Mean Diurnal Temperature Range, Precipitation during the (4) Coldest Quarter, (5) Warmest Quarter, and (6) Driest Quarter.

4. Maxillariinae occurrence and its environmental drivers.

To further assess whether the sevensix environmental variables would be indeed useful for the macroevolutionary and macroecological analyses, we addressed whether these environmental features are drivers of the occurrence of Maxillariinae species by estimating the MaxEnt species distribution models (SDMs) (R package 'dismo'; Hijmans & Elith, 2013; Hijmans *et al.*, 2020; see Methods S2). As predictor variables, we used the six non-collinear bioclimatic variables described before (see also Methods S1), plus Elevation and Ecoregions (ecoregions2017.appspot.com; Olson *et al.*, 2001). We then evaluated their importances using jackknife tests (see Evaluation of the variables on Methods S2). We also analysed the response curves, a MaxEnt output, to understand how the estimated probability of environmental suitability for species presence changes in response to variation in the environmental variables.

5. Macroevolutionary and macroecological analyses

Since both genomic traits are known to frequently exhibit strong phylogenetic dependence, we tested macroevolutionary models to fit further analyses under adequate phylogenetic constraints and then performed macroecological analyses.

Relation between chromosome number and genome size. We assessed the relationship between the two genomic traits by regressing them against each other in a phylogenetic generalised least squares (PGLS), using the R package 'caper' (Orme *et al.*, 2013), with chromosome number as the response variable.

Phylogeny-based regressions approach. For regressions, we again apply the PGLS using the R package 'caper' (Revell, 2010). First, using the 'geiger' package (Pennell *et al.*, 2014), we tested three evolution models to confirm the best macroevolutionary model to our genomic traits: Brownian Motion (Felsenstein, 1973), Ornstein–Uhlenbeck (Butler & King, 2004; Cressler *et al.*, 2015), and Early Burst (Felsenstein, 1973; Harmon *et al.*, 2010). Comparing the models via an AICc, Brownian Motion was selected as the best model (Table S5). Given the overall effect of phylogeny on variation in chromosome number and genome size, we estimated the phylogenetic signal using Pagel's (λ; Pagel, 1999) under Maximum likelihood, while the scaling parameters delta (Δ) and kappa (K) were fixed to one (Table S6).

Correlations between genomic and ecological traits. To test whether the chromosome number and genome size (treated here as dependent variables) are correlated to habit and to the environmental variables, we regressed the dependent variables separately against the set of environmental variables previously selected: two habit-associated variables, the six environmental variables (see the topic "3. Ecological data") and elevation, to obtain the Maximum Models (MM), one for each genomic trait. We used the package 'MuMIn' (Bartón, 2020) for model selection, fitting every possible combination of variables within each MM and evaluating their performances. All models (i.e., all possible combinations of variables in each set), including the intercept-only model, were ranked according to their AICc weights to identify the Best Models (BM), considering that ΔAICc<2 are putative most parsimonious models (Burnham & Anderson, 2002a,b, 2004). Hence, the determination of which model is the BM lies in the knowledge of the biological sample and the predictors. For each model, we evaluated model diagnostic plots to check if regression assumptions are met.

Effect of polyploidy on the correlations between genomic and ecological traits. Finally, to enlighten the significance of polyploidy in the relationship between the genomic traits and

the ecological data, we performed the macroevolutionary analyses twice: first without the polyploidy cytotypes and following with the polyploid cytotypes.

Results

1. Genomic traits - Chromosome number and Genome size data

The chromosome number (Fig. 1) and genome size (Fig. 2) were obtained here for 24 and 28 species, respectively (Table S1). Along with the literature survey, we ensure that 92 and 62 species present chromosome number and genome size data for the analysis (Table 1).

Chromosome number. The 2*n*=40 was the most frequent chromosome number, observed in 62.65% of the Maxillariinae species. Alternative chromosome numbers indicate dysploidy as a common event in this group, with diploid chromosome numbers varying between 2*n*=36 to 2*n*=46: 2*n*=36 (7.23%), 2*n*=38 (20.48%), 2*n*=42 (8.43%), and 4*n*=46 (1.20%). One species presented dysploid cytotypes (*Mapinguari desvauxianus* (Rchb. f.) Carnevali & R.B.Singer with 2*n*=36 and 40), and three species presented polyploidy cytotypes (*Bifrenaria tetragona* (Lindl.) Schltr., *Bif. tyrianthina* (Loudon) Rchb. f. and *Christensonella subulata* (Lindl.) Szlach., Mytnik, Górniak & Åšmiszek, all with 2*n*=38 and 76) (Table 1).

Genome size. The genome size presented here (Table 1 and Table S1) indicated that the 1Cx value (i.e., C-value for the monoploid genome) varies from 1.72pg in *Trigonidium obtusum* Lindl. to 6.12pg in *Scuticaria steelei* (Hook.) Lindl., which is consistent with data obtained from the literature, which suggested that 1Cx varies from 1.70pg in *Trigonidium egertonianum* Bateman ex Lindl. to 5.69pg in *Scuticaria hadwenii* (Lindl.) Hoehne (Table 1, Fig. 2). Considering the data from the 62 species, the genome size varies 3.6x, with a modal value of 1Cx=3.41pg and an average of 1Cx= 3.38 +/- 0.96.

2. Phylogeny

The aligned *mat*K-*trn*K - ycf1 - ITS matrix comprises 2,986 sites (735 - 1541 - 710, respectively), with 33.70% of missing data, 30.14% variable sites and 16.71% parsimony informative sites. Both ML and BI analysis (Fig. S1) support the monophyly of the subtribe Maxillariinae and all sampled genera but *Trigonidium* Lindl. and *Scuticaria* Lindl. In both analyses, *Trigonidium* were placed as a polyphyletic genus close to *Maxillaria* Ruiz & Pav.,

while the two *Scuticaria* species formed a polytomy on the base of Maxillariinae subtribe in both analyses. Despite some punctual differences between ML and IB (e.g., see species relationships inside the genus *Maxillaria*, Fig. S1), both phylogenetic trees present congruent topologies with similar branch support for all genera. Based on this overall similarity, we choose to use the BI phylogenetic tree for macroecological analysis.

3. Ecological data

3.1. Habit and elevation data

The 97 species sampled here could be found in three alternative habits, with some species colonising more than one habitat (Table S3). Ninety-one Maxillariinae species (93.81%) have an epiphytic habit, 69 out of them being exclusively epiphytic (71.13%). We also observed lithophytic habit in 23 species (23.71%), none of them being exclusively lithophytic, and terrestrial habit in eight species (8.25%), four being exclusively terrestrial (4.12%). All lithophytic species are also epiphytic, except by *Mapinguari auyantepuiensis* (Foldats) Carnevali & R.B.Singer and *Eriopsis biloba* Lindl., which are lithophytic and terrestrial. Regarding the variable number of habits, 80 species (82.5%) occurred in just one habit, 23 species (23.7%) in two alternative habits and just one, *Maxillariella variabilis* Bateman ex Lindl. M.A.Blanco & Carnevali, was found in the three habits (Table S3). Species were recorded in a wide variety of elevations ranging from 0m to 5,240m (Table S3).

3.2. Maxillariinae distribution in response to environmental variables

Variables contribution. Analysing the SDMs (Methods S1) of the 87 Maxillariinae species, we identified four, out of the eight selected variables, as the most important ones to the model's performance: Ecoregion (see Note 1), Temperature seasonality, Mean Temperature of Coldest Quarter, and Precipitation of Coldest Quarter (see arrows in Fig. 3a - Permutation Importance - and for data per species, see heatmaps in Fig. S2). The three first variables were consistently among the top variables (see arrows in Fig. 3b - g), both when used alone, or when omitted. The variables Precipitation of Warmest Quarter and Mean Diurnal Temperature Range were also present in most of the jackknifes (see asterisks in Fig. 3b-g). Two variables, Temperature Seasonality and Mean Diurnal Temperature Range, showed Gaussian curves with optimum in medium values, whereas Temperature Seasonality and

Mean Diurnal Temperature Range showed decreasing sigmoid curves (Fig. 4a,b). The response curve to Mean Temperature in the Coldest Quarter was often gaussian with the optimum between 15°C and 20°C (Fig. 4c). For Precipitation in the Warmest Quarter and Precipitation in the Coldest Quarter, the response curves were sigmoidal increasing curves stabilising around 1,000mm (Fig 4d-e). Taken together, the response curves indicate that Maxillariinae species are better adapted to humid, mild, stable environments.

3.3. Macroevolution analyses

Relationship between chromosome number and genome size. Pagel's λ for the genomic traits without polyploids revealed a strong phylogenetic signal in the model residuals (λ=1.000; Table S6). A weak positive correlation was found between the genomic traits, with only 8.6% of the variance in chromosome number being explained by genome size variation (Adjusted R^2: 8.6%, *p*-value=0.0138; Table S6).

Genomic traits correlations with ecological data under a phylogeny-based approach. The Pagel's λ for the PGLS models of both the dependent variables, chromosome number and genome size, without the polyploids, against ecological variables (two habit variables, elevation and the six non-collinear bioclimatic variables) revealed a strong phylogenetic signal in the residuals for both variables (λ=0.92 and λ=1.000 respectively; Table 2). The AICc model selection based on the PGLS of the chromosome number against all nine variables (considered the Maximum Model - MM) resulted in 11 Best Model (BM) candidates (i.e., present ΔAICc<2; Fig. S3a). The BM held only the variable Elevation in positive correlation with chromosome number (R^2: 3.42%, *p*-value=0.043; Fig. 5), but the AICc did not prove the BM to perform better than the intercept-only model (i.e., the ΔAICc between Intercept-only Model and BM AICcs was not higher than 2; see Table 2).

Regarding genome size, the AICc model selection based on the MM also resulted in 11 BM candidates with ΔAICc<2 (Fig. S3b). The BM was composed by the Number of possible Habits, Elevation, and Precipitation of the Warmest Quarter variables (R^2: 12.43%, *p*-value=0.014; Table 2; Fig. S3b). While the two first variables present a positive correlation with the increase of the genome size, the last one shows a negative correlation with the increase of the genome size (Fig. 5). According to the AICc, the BM performed better than the intercept-only model (Table 2). Although the variable Habit exclusively epiphytic was not

held in the BM, it was present in many BM candidates, negatively associated with the increase in genome size (Fig. 5).

Effect of polyploidy on genomic traits correlations. Adding polyploids to the sample in the regression of chromosome number against genome size caused the model's residuals to carry no phylogenetic signal (λ=0.000; Table S6). The PGLS showed a positive relationship between these two variables, with genome size accounting for 68.42% of the variation in chromosome number (Adjusted R^2: 68.42%, *p*-value<$2.2e^{-16}$; Table S6).

Regarding chromosome numbers with polyploids, Pagel's λ showed a small phylogenetic signal in the residuals of the regression against ecological variables (λ=0.332; Table 2). The AICc model selection based on the MM indicated nine BM candidates (Fig. S3c). The variables Number of possible Habits, Habit Exclusively Epiphytic, and Precipitation of Coldest Quarter were present in all BM candidates (Fig. S3d), with a negative correlation with the increasing chromosome number, while the variable Temperature of Coldest Quarter held a positive correlation with the rising chromosome number (R^2: 32.66%, *p*-value=$3.717e^{-08}$; Table 2; Fig. 5). According to the AICc test, the BM performed better than the intercept-only model (Table 2).

Pagel's λ statistic for genome size in the sample with polyploids also showed a high phylogenetic signal in the residuals of the model (λ=0.740; Table 2), but weaker than the model without polyploids (λ=1.0; Table 2). The AICc model selection based on the MM PGLS indicated five BM candidates (Fig. S3d). The variables Temperature Seasonality and Precipitation of the Coldest Quarter were present in almost all BM candidates and composed the BM (Fig. S3d), with both variables negatively correlated to the increasing genome size (R^2: 14.56%, *p*-value=0.002; Table 2, Fig. 5).

Discussion

We used a phylogeny-based approach to seek associations between genomic (chromosome number and genome size) and ecological (habits and environmental) data, using a Neotropical orchid clade as a model. Our results suggested that, despite the solid phylogenetic signal of genomic traits, the ecological data partially explain the variation of

genomic traits. Thus, our analyses better comprehend the relationships among epiphytism, species environmental niches, and genomic traits variation.

1. Genomic traits and the phylogenetic hypothesis: correlation, mode of evolution, and phylogenetic signal

Our data concerning genomic traits and phylogeny are congruent to the literature. For those species with no previous published chromosome number and genome size, our counts agree with those reported for the genus. The chromosome number variation confirms the importance of dysploidy in orchid chromosome evolution (Cabral *et al.*, 2006; Koehler *et al.*, 2008; Felix & Guerra, 2010; Moraes *et al.*, 2012, 2016, 2017; De Oliveira *et al.*, 2015), a chromosomal process considered a critical mechanism especially following the polyploidy pulses (Escudero *et al.*, 2014; Mandáková & Lysak, 2018; Levin, 2020), while the genome sizes were deemed to be small or medium, following Leitch *et al. (1998)*. Concerning phylogeny, the subtribe and genera monophyletism was previously shown by Koehler *et al.* (2002), Whitten *et al.* (2007), Koehler *et al.* (2008), Moraes *et al.* (2016), Moraes *et al. (2017)*.

1.1. Correlation between the genomic traits

Following observations from the literature, the two genomic traits presented a weak positive correlation when considering diploid cytotypes. However, when including polyploids in the sample, a stronger positive correlation is observed between the genomic traits (e.g. Jordan *et al.*, 2015). Such a strong correlation was expected, given that polyploidy is always accompanied by genome size increase. However, such correlation is not entirely linear due to ambiguous DNA excesses purging that frequently occurs on the diploidisation process (Leitch & Bennett, 2004; Michael, 2014; Soltis *et al.*, 2015; Dodsworth *et al.*, 2016; Simonin & Roddy, 2018; Mandáková & Lysak, 2018; Qiao *et al.*, 2019).

1.2. Genomic traits mode of evolution and phylogenetic signal

Different evolution models were reported depending on taxa, sampling and

methodology (Simonin & Roddy, 2018; Burchardt *et al.*, 2018; Paule *et al.*, 2020). Here we found that Brownian Motion adequately describes chromosome number and genome size changes in Maxillariinae, contrasting with Trávníček et al. (2019), which suggested the Ornstein-Uhlenbeck model better describe the genome size variation in Orchidaceae. However, the authors' sampling biased towards temperate climates could explain the alternative result. Even more, Trávníček et al. (2019) also observed a stronger selection for genome size in temperate environments than in tropical and sub-tropical climates, where Maxillariinae preferentially occurs.

Nevertheless, it is important to note that despite the general tendency to assume that traits evolving as expected under Brownian motion are not under selection, such assumption is a simplistic interpretation of the Brownian Motion concept (Harmon, 2019). In fact, natural selection, mutation, and drift depend on many factors, and as long as properties are met, traits can evolve under Brownian Motion even under strong selection (Hansen & Martins, 1996; Harmon, 2019). Widespread taxa can have genetic changes, adaptive or not, fixed either through natural selection or genetic drift within isolated populations (Price *et al.*, 1988; Eckert *et al.*, 2008; Hooper & Price, 2015), but also, if there is extreme selective pressure on a given trait, a novelty can be fixed even in the absence of isolation (Coyne & Orr, 2004). Accordingly, although there are questions about whether genomic changes are adaptive or neutral, they can become fixed in populations and cause species diversification (Rieseberg, 2001; Whitney *et al.*, 2010; Faria & Navarro, 2010; Kang *et al.*, 2014).

The observed strong phylogenetic signals for chromosome number and genome size agrees with literature for different plant groups (Whitney *et al.*, 2010; Bainard *et al.*, 2012, 2020; Kang *et al.*, 2014; Marinho *et al.*, 2014; Jordan *et al.*, 2015; Alonso *et al.*, 2015; Du *et al.*, 2017) and implies a dependency on phylogeny for both traits. Therefore, despite the phylogenetic signal, Brownian Motion alone is not the best explanation for the variation in the chromosome number dataset with polyploids and in both genome size datasets, suggesting a combination of genetic drift and adaptive evolution should be present on both genomic traits evolution.

2. **Are genomic traits associated with environmental variables that influence the Maxillariinae distribution?**

The Maxillariinae SDMs largely agreed with the distribution known for the subtribe (Pridgeon *et al.*, 2009), with most species occurring along with the Tropical Moist Broadleaf Forests, mainly in Central America, Tropical Andes and Atlantic Forest (see Note S1). The preference for such stable environments reflects how Maxillariinae is affected by the environmental variables that define its distribution.

Among environmental variables, precipitation reveals to be a key variable. Maxillariinae species mainly occur in wet, evergreen or semi-deciduous, humid tropical forests, with little precipitation seasonality; hence they are not usually exposed to drought. Additionally, it is worth noting that most of Maxillariinae's predicted occurrence area has low Precipitation Seasonality and even the dry season is relatively humid, suggesting that precipitation in Warmest Quarter is not a limiting factor for the subtribe distribution. Therefore, the increase in genome size associated with a decrease in Precipitation in the Warmest Quarter could reflect a drift process since such a reduction in the precipitation does not exert selection pressure on genome size or species distribution.

When considering polyploids, the decrease in Precipitation in the Coldest Quarter is associated with an increase in both genomic traits, suggesting that polyploids are better adapted to dry winter, reflecting the capability to colonise unsuitable/adverse environments (Lavania, 2020; Moura *et al.*, 2021). For example, the genus *Bifrenaria* is distributed throughout Amazonia and Atlantic Rain Forest (Koehler *et al.*, 2002), humid Forests with elevated annual precipitation and low-Temperature Seasonality. The only polyploid cytotype presents an obligatory lithophytic habit, colonising exclusively open vegetation in the Cerrado biome, which shows low precipitation volumes in the Coldest Quarter (about 100-200mm during June, July and August; INPE, 2021). Similar results were observed for the close orchids *Gomesa* R. Br. and *Catasetum* Rich. ex Kunth, with polyploid species occurring in the dry Caatinga biome (Felix & Guerra, 2010; Cordeiro *et al.*, 2018), while diploid species occur in humid biomes. In this sense, the negative association between chromosome number and Precipitation in the Coldest Quarter is in accordance with the positive association between chromosome number and Temperature in the Coldest Quarter. Accordingly, such dry and hot biomes as Cerrado and Caatinga savannas could be characterized as extreme conditions, usually associated with high rates of polyploidy (Rieseberg & Willis, 2007).

Nevertheless, larger environmental tolerances are usually associated with decreasing the genome size, which influences cell size, especially the guard cells (Knight & Beaulieu, 2008; Beaulieu *et al.*, 2008; but see also for exceptions Jordan *et al.*, 2015; Roddy *et al.*, 2020). By changing the guard cells volume, genome size affects the stomata response speed (open/close movement when humidity increases/decreases). It is assumed that once the genome size increases, the guard cell gets larger, and the response gets too slow (Drake *et al.*, 2013; Veselý *et al.*, 2020). In this way, by increasing the genome size, polyploidy could favour the distribution of species with large genome size in stable climatic regions, like the ones with reduced Temperature seasonality and Precipitation seasonality.

The response to some environmental variables reflects the drift (as a response to Precipitation), while others suggest adaptive selection (as a response to temperature in warm and coldest quarters). In fact, the currently available evolutive methods have little power to disentangle the actual effect of the different evolutionary forces at work (e.g. Whitney *et al.*, 2010; Kang *et al.*, 2014). Yet, as we discuss below, our results suggest that the correlations of genomic traits with environmental variables in Maxillariinae might be mediated by the correlation of such genomic traits with a key life strategy: the habit.

3. Are genomic traits associated with epiphytic habit in Maxillariinae?

When considering only diploid species, the genome size increase is correlated with the gain of alternative habits other than epiphytic. Although the variable 'exclusively epiphytic' was not retained in the BM, it was present in other BM candidates, suggesting that species with bigger genomes might be prone to desert epiphytism. In fact, correlations between smaller genome sizes and epiphytism were already observed in Orchidaceae (Chase *et al.*, 2005; Leitch *et al.*, 2009; Trávníček *et al.*, 2019), as well as in other plant families (Veselý *et al.*, 2013; Carta & Peruzzi, 2016; Hidalgo *et al.*, 2017). However, when including polyploids in the sample, such correlations are no longer held. The association between genome size and habit might emerge from two main factors: stomata size and genome maintenance costs. By affecting the stomata response speed, the genome size correlates with species habit, possibly configuring a selective pressure since epiphytic species need an effective response to humidity variation. Additionally, larger genomes may become too

expensive in terms of nutrients, such as carbon, phosphorus, and nitrogen (Hessen *et al.*, 2010; Kang *et al.*, 2014; Guignard *et al.*, 2016) for organisms to remain epiphytic. When considering polyploids, the negative correlation between chromosome number and habit variables implies that polyploids are not adding life forms but shifting from epiphytism towards non-epiphytic habit, either terrestrial or lithophytic. Such a relationship of higher ploidy levels and the conquest of geophytic habits was traditionally assumed (e.g. Stebbins, 1971), but it was a poorly tested issue (Trávníček *et al.*, 2019), despite its importance in orchid evolution.

The epiphytic habit is a key innovation associated with Orchidaceae diversification (Gravendeel *et al.*, 2004; Freudenstein & Chase, 2015; Givnish *et al.*, 2015). It is suggested that two geographic shifts from Old World to New World have occurred, one of them on the base of tribe Cymbidieae. Such geographic shifts are associated with habit shift from terrestrial to epiphytic, suggesting that the rise of the epiphytic habit was associated with the colonisation of partially unoccupied habitats in the New World, favouring high rates of orchid diversification (Freudenstein & Chase, 2015; Givnish *et al.*, 2015). The reversion from epiphytic to non-epiphytic habit in orchid has also been associated with the acquisition of more extreme environments, as evidenced by the origin of aridity-adapted plant groups in Epidendreae (Freudenstein & Chase, 2015; Sosa *et al.*, 2016). Additionally, the differences in the orchid distribution along environmental gradients could be associated with habit and root type (Acharya *et al.*, 2011; Zhang *et al.*, 2015; Tsiftsis *et al.*, 2019; Djordjević & Tsiftsis, 2020), with terrestrial orchids richness peaking at a higher elevation (Zhang *et al.*, 2015). Accordingly, the facts of genome size in the diploid dataset increase with lower Precipitation of Warmest Quarter and higher elevations, and that chromosome number in the polyploid dataset increasing with lower Precipitation in the Coldest Quarter might be linked to the desertion of obligatory epiphytic habit. Epiphytic species possibly depend on higher humidity provided by the precipitation, while non-epiphytes can both access soil moisture and better cope with higher temperatures, commonly inherited from altitude.

The chromosome number and genome size variations, important but neglected traits in the ecological niche, are shaped in Maxillariinae by neutral (genetic drift) and adaptive evolution. Both genomic traits are correlated to bioclimatic variables and elevation at least to some degree. Polyploidy proved to be a valuable phenomenon when defining an

environmental niche, favouring colonisation of new, often stressful, regions. Moreover, by affecting the plant physiology, the genome size seems to influence the habit decisively as large genomes may require deserting epiphytism. Considering that epiphytism is a key innovation to Orchidaceae diversification, the genome size variation could have played an important role in Neotropical Orchidaceae diversification. However, leaving epiphytism could have promoted further secondary diversification events. Our findings demonstrate that the communion between cytogenetics and ecology studies provides exciting future research on plant evolution and diversification, exploring the relationships between genomic and niche traits.

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Author Contributions

APM and JSC designed the research. TBJE, ERFM, LPF, JSC, APM performed the research. TBJE, JSC, and APM analysed and interpreted the data. TBJE and APM generated the figures. TBJE, JSC, and APM wrote the manuscript. All authors reviewed the manuscript.

Data Availability

The phylogenetic analysis files are available at TreeBase (http://treebase.org, submission number 28311). All R scripts can be made available under request.

Short titles for Supplementary material

Figure S1. Maximum Likelihood and Bayesian Inference phylogenetic trees.

Figure S2. Heatmaps of variables importance to the model's performance.

Figure S3. Best Model candidates according to PGLS of environmental and ecological explanatory variables against the genomic traits.

Table S1. Maxillariinae's new chromosome number and genome size records with origin and voucher information.

Table S2. GenBank accessions for DNA sequences used in the phylogenetic analyses

Table S3. Habit and elevation information.

Table S4: Species Distribution Models for Maxillariinae and close related species.

Table S5: Evolution model test for chromosome number and genome size.

Table S6: Regressions between the genomic traits with and without polyploids in the sample.

Methods S1. Variables selection.

Methods S2. Methods on species distribution models (SDM) performed by MaxEnt.

Notes S1. Notes on Maxillariinae SDM.

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Figure legends

Figure 1. Chromosome number in Maxillariinae. (a) *Anguloa virginalis* (2*n*=40), (b) *Brasiliorchis barbosae* (2*n*=40), (c) *Bra. chrysantha* (2*n*=40), (d) *Bra. consanguinea* (2*n*=40), (e) *Bra. marginata* (2*n*=40), (f) *Bra. monantha* (2*n*=40), (g) *Camaridium carinatum* (2*n*=38), (h) *Christensonella pumila* (2*n*=36), (i) *Heterotaxis equitans* (2*n*=42), (j) *Het. superflua* (2*n*=42), (k) *Mapinguari desvauxianus* (2*n*=40), (l) *Maxillaria bradei* (2*n*=40), (m) *Max. kegelii* (2n=40), (n) *Max. leucaimata* (2*n*=40), (o) *Max. parkeri* (2*n*=40), (p) *Max. setigera* (2*n*=40), (q) *Maxillariella robusta* (2*n*=40), (r) *Maxillariella tenuifolia* (2*n*=40), (s) *Mormolyca rigens* (2*n*=40), (t) *Mor. rufescens* (2*n*=40), (u) *Nitidobulbon nasutum* (2*n*=46), (v) *Rhetinantha*

cerifera (2*n*=38), (w) *Rhe. friedrichsthalii* (2*n*=36), (x) *Scuticaria steelei* (2*n*=40). Bar=10 um.

Figure 2. Genome size in Maxillariinae and closely related species. The genome size of 62 species is presented here with an average of 1Cx=3.38 +/- 0.94. The large genome sizes are indicated in dark, average genome sizes in purple and the small genome sizes in orange. The polyploid cytotypes (*Bif. tyrianthina* and *Z. maculum*) are indicated by '**' and the haploid genome size (1C-value).

Figure 3. Variables importance in the SDM of Maxillariinae species. The variables importance are presented in each (a) Permutation Importance, (b, c) Training Gain, (d, e) Test Gain and (f, g) AUC, both with variable omitted (b, d, and f) and used alone (c, e, and g). The most important variables are indicated by an arrow and the secondary important variable by asterisks. Each bar corresponds to one variable following the variable's colour legend.

Figure 4. Performance of variables represented by the curve response. The curves show how the estimated probability of suitable occurrence changes in response to variation in an environmental variable. Each curve represents a model fitted using only one variable: (a) Temperature seasonality, (b) Mean Diurnal Temperature Range, (c) Isothermality, (d) Mean Temperature of Coldest Quarter, (e) Precipitation of Warmest quarter, and (f) Precipitation of Coldest Quarter.

Figure 5. Genomic traits correlations: chromosome number (2n) and genome size (GS) increase with environmental and ecological variables according to the PGLS analyses. The first column presents the seven variables in the Maximum Model, with the variables indicated in SDM as the most important to Maxillariinae occurrence indicated by asterisks. The next columns represent each PGLS model, indicating the variables retained in the Best Model. Arrows in the variable boxes in the models indicate the direction of the correlation with the variation of the genomic traits. Positive correlations are indicated by up arrows and purple boxes, while down arrows indicate negative correlations in orange boxes. The darker the shade of colour, the greater the importance of this variable in the model, being the light shades the least important variables. Inside each box, we present the significance codes according to p-value: ~0 = '***', <0.001 = '**', <0.01 = '*', <0.05 = '.' .

Figure S1: Maximum Likelihood (a) and Bayesian Inference (b) phylogenetic trees based on matK, ycf1 and ITS markers. Branches support, Bootstrap and Posterior Probability, respectively, are indicated by the branch colour according to the colour scale of each phylogenetic tree. The dashed branches in black represent topology differences among Maxillaria species.

Figure S2: Heatmaps of variables importance to the model's performance. (a) Permutation Importance, (b, c) Training Gain, (d, e) Test Gain and (f, g) AUC, both with variables omitted (b, d, and f) and used alone (c, e, and g). For each heatmap, a colour scale is provided. The darker the colour shade of a cell, the higher the contribution of a variable to the model for the species represented by that cell. The training, test and AUC gain tests aim to analyse how much a model's gain decreases or increases when a given variable is omitted. All the other variables are kept and when the variable is used alone to fit the model. In each test, when performed without a variable ('variable omitted'), lighter shades indicate that without that variable the gain decreases, meaning that variable has more information than the other variables. In each test, when performed with only one variable ('variable used'), the darker the colour, the higher the gain that variable provides alone, meaning it might have the most useful information by itself.

Figure S3: Model Selection showing Akaike Information Criterion corrected for small sampling sizes (AICc) cumulative weights of the Best Model candidates ($\Delta AICc<2$) according to PGLS regression of environmental and ecological explanatory variables. Figures present the results of Best Models (rows) for each dependent variable, chromosome number and genome size, without (a and b) and with polyploids (c and d) in the sample. Images in a and c present the result for chromosome number, and c and d, for genome size variation. Each Best Model is represented by one row; variables are shown in the columns, with colour columns alternating between grey and blue (for the sake of visual clarity). Whenever a variable is present in a model, the respective cell appears filled in grey or blue. Darker the colour shade, the higher the variable importance to the model.

Table legends

Table 1: Maxillariinae genomic traits. The somatic chromosome number (2n) and the haploid genome size (1C) are presented for all Maxillariinae species with available data. For polyploids cytotypes, the 1Cx value is presented inside square brackets.

Table 2. Summary of Phylogeny-corrected Regressions aiming to explain variation in chromosome number and genome size in Maxillariinae orchids using ecological variables (habit, elevation, and bioclimatic variables) associated with species distribution. Regressions were performed without and with polyploids in the sample. Under the model names we present estimates for intercept and the variables, followed by significance codes, according to p-value: ~0 = '***', <0.001 = '**', <0.01 = '*', <0.05 = '.'

Table 1. Maxillariinae genomic traits. The somatic chromosome number (2n) and the haploid genome size (1C) are presented for all Maxillariinae species with available data. For polyploids cytotypes, the 1Cx value is presented inside square brackets.

Species	Authority	Genomic Traits			
		Chromosome number		Genome size	
		2n	Reference	1C [1Cx]	Reference
***Anguloa* Ruiz & Pav. (11 species)**					
Anguloa uniflora	Ruiz & Pav.	NA		3.36	PW
Anguloa virginalis	Linden ex B.S.Williams	40	Ry00, PW	3.4	PW
Bifrenaria Lindl. (18 species)					
Bifrenaria aureofulva	Lindl.	38	MO17	3.035	MO17
Bifrenaria calcarata	Barb.Rodr.	38	MO17	NA	
Bifrenaria harrisoniae	(Hook.) Rchb.f.	38	BF61; TK84; MO17	3.71	MO17
Bifrenaria inodora	Lindl.	38	MO17	3.73	MO17
Bifrenaria leucorrhoda	Rchb. f.	38	MO17	3.035	MO17
Bifrenaria longicornis	Lindl.	38	MO17	3.33	MO17
Bifrenaria stefanae	V.P.Castro	38	MO17	NA	
Bifrenaria tetragona	(Lindl.) Schltr.	38	BF61	NA	
		76	BF61	NA	
Bifrenaria tyrianthina	(Lodd. ex Loudon) Rchb.f.	38	MO17	3.72	MO17
		76	MO17	7.17 [3.585]	MO17
Bifrenaria venezuelana	C.Schweinf.	38	MO17	3.67	MO17
***Brasiliorchis* Singer, Koehler & Carnevali (13 species)**					
Brasiliorchis barbosae	(Loefgr.) R.B.Singer, S.Koehler & Carnevali	40	PW	5.54	PW
Brasiliorchis chrysantha	(Barb.Rodr.) R.B.Singer, S.Koehler & Carnevali	40	MO12	2.93	MO12
Brasiliorchis consanguinea	(Klotzsch) R.B.Singer, S.Koehler & Carnevali	40	PW	3.80	PW
Brasiliorchis gracilis	(Lodd., G.Lodd. & W.Lodd.) R.B.Singer, S.Koehler & Carnevali	40	MO12	2.84	MO12
Brasiliorchis kautskyi	(Pabst) R.B.Singer, S.Koehler & Carnevali	40	MO12	NA	
Brasiliorchis marginata	(Lindl.) R.B.Singer, S.Koehler & Carnevali	40	MO12	3.36	MO12
Brasiliorchis monantha	(Barb.Rodr.) Campacci	40	PW	NA	

Brasiliorchis phoenicanthera	(Barb.Rodr.) R.B.Singer, S.Koehler & Carnevali	40	MO12	3.19	MO12
Brasiliorchis picta	(Hook.) R.B.Singer, S.Koehler & Carnevali	40	TK84; MO12	3.38	MO12
Brasiliorchis porphyrostele	(Rchb.f.) R.B.Singer, S.Koehler & Carnevali	40	MO12	3.41	MO12
Brasiliorchis schunkeana	(Campacci & Kautsky) R.B.Singer, S.Koehler & Carnevali	40	MO12	4.19	MO12
Brasiliorchis ubatubana	(Hoehne) R.B.Singer, S.Koehler & Carnevali	40	MO12	3.62	MO12
***Camaridium* Lindl. (ca. 80 species)**					
Camaridium carinatum	(Barb.Rodr.) Hoehne	38	PW	1.93	PW
Camaridium densum	(Lindl.) M.A.Blanco	38	BP63	NA	
Camaridium ochroleucum	Lindl.	40	BP63	4.23	PW
Camaridium vestitum	(Sw.) Lindl.	40	BP63	1.88	PW
***Christensonella* Szlach., Mytnik, Górniak & Smiszek (11 species)**					
Christensonella echinophyta	(Barb.Rodr.) Szlach., Mytnik, Górniak & Smiszek	NA		3.94	PW
Christensonella fernandiana	(Barb.Rodr.) Szlach., Mytnik, Górniak & Smiszek	36	KO08	4.05	PW
Christensonella nardoides	(Kraenzl.) Szlach., Mytnik, Górniak & Smiszek	NA		NA	
Christensonella neowiedii	(Rchb.f.) S.Koehler	36	KO08; MO12	3.67	MO12
Christensonella pachyphylla	(Schltr. ex Hoehne) Szlach., Mytnik, Górniak & Smiszek	36	KO08, MO12	4.79	PW
Christensonella paranaensis	(Barb.Rodr.) S.Koehler	38	KO08	4.37	PW
Christensonella pumila	(Hook.) Szlach., Mytnik, Górniak & Smiszek	36	MO12	3.37	MO12
Christensonella subulata	(Lindl.) Szlach., Mytnik, Górniak & Smiszek	38	BP63; KO08; MO12	3.58	MO12
		76	KO08	NA	
Christensonella uncata	(Lindl.) Szlach., Mytnik, Górniak & Smiszek	36	MO12	1.85	MO12
***Heterotaxis* Lindl. (13 species)**					
Heterotaxis brasiliensis	(Brieger & Illg) F.Barros	42	MO16	4.32	MO16
Heterotaxis discolor	(Lodd. ex Lindl.) Ojeda & Carnevali	42	FG00, CA06, MO16	NA	
Heterotaxis equitans	(Schltr.) Ojeda & Carnevali	42	MO16	3.85	MO16
Heterotaxis superflua	(Rchb.f.) F.Barros	42	MO16	3.835	MO16
Heterotaxis valenzuelana	(A.Rich.) Ojeda & Carnevali	40	MO16	3.73	MO16
Heterotaxis villosa	(Barb.Rodr.) F.Barros	42	MO16	4.375	MO16
Heterotaxis violaceopunctata	(Rchb.f.) F.Barros	42	GO85, MO16	4.25	MO16

***Lycaste* Lindl. (ca. 30 species)**					
Lycaste aromatica	(Graham) Lindl.	40	AK88	NA	
Lycaste cruenta	(Lindl.) Lindl.	40	AK88	NA	
Lycaste macrobulbon	(Hook.) Lindl.	40	AK88; MO12	3.63	MO17
Lycaste macrophylla	(Poepp. & Endl.) Lindl.	40	MO17	3.915	MO17
Lycaste tricolor	Rchb.f.	40	AK88	NA	
***Mapinguari* Carnevali & Singer (four species)**					
Mapinguari auyantepuiensis	(Foldats) Carnevali & R.B.Singer	NA		NA	
Mapinguari desvauxianus	(Rchb.f.) Carnevali & R.B.Singer	36	BP63	NA	
		40	CA06	3.27	PW
***Maxillaria* Ruiz & Pav. (ca. 250 species)**					
Maxillaria bradei	Schltr. ex Hoehne	40	PW	2.13	PW
Maxillaria crocea	Lindl.	40	BP63	2.77	PW
Maxillaria grandiflora	(Kunth) Lindl.	40	BP63	NA	
Maxillaria kegelii	Rchb.f.	38	BP63	2.83	PW
		40	PW		
Maxillaria leucaimata	Barb.Rodr.	40	PW	2.29	PW
Maxillaria ochroleuca	Lodd. ex Lindl.	40	BP63	NA	
Maxillaria parkeri	Hook.	NA		NA	
Maxillaria setigera	Lindl.	40	BP63	NA	
***Maxillariella* Blanco & Carnevali (46 species)**					
Maxillariella alba	(Hook.) M.A.Blanco & Carnevali	40	BP63	3.45	PW
Maxillariella ponerantha	(Rchb.f.) M.A.Blanco & Carnevali	40	BP63	NA	
Maxillariella procurrens	(Lindl.) M.A.Blanco & Carnevali	40	BP63	NA	
Maxillariella robusta	(Barb.Rodr.) M.A.Blanco & Carnevali	40	PW	2.64	PW
Maxillariella tenuifolia	(Lindl.) M.A.Blanco & Carnevali	40	TK84	2.98	PW
Maxillariella variabilis	(Bateman ex Lindl.) M.A.Blanco & Carnevali	40, 42	BP63; PW	2.58	PW
***Mormolyca* Fenzl (25 species)**					

Mormolyca ringens	(Lindl.) Gentil	40	PW	1.89	PW
Mormolyca rufescens	(Lindl.) M.A.Blanco	40	BP63, FG00	2.05	PW
***Nitidobulbon* Ojeda, Carnevali & Romero (three species)**					
Nitidobulbon nasutum	(Rchb.f.) Ojeda & Carnevali	46	PW	NA	
***Ornithidium* Salisb. ex R. Br. (ca. 55 species)**					
Ornithidium aureum	Poepp. & Endl.	40	BP63	NA	
Ornithidium pendens	(Pabst) Senghas	40	BP63	NA	
Ornithidium pendulum	(Poepp. & Endl.) Cogn.	40	BP63	2.09	PW
Ornithidium semiscabrum	Lindl.	40	BP63	NA	
***Rhetinantha* Blanco (15 species)**					
Rhetinantha cerifera	(Barb.Rodr.) M.A.Blanco	38	PW	2.72	PW
Rhetinantha friedrichsthalii	(Rchb.f.) M.A.Blanco	36	PW	3.25	PW
Rhetinantha notylioglossa	(Rchb.f.) M.A.Blanco	38	CA06	NA	
Rhetinantha scorpioidea	(Kraenzl.) M.A.Blanco	38	BP63	NA	
***Rudolfiella* Hoehne (six species)**					
Rudolfiella aurantiaca	(Lindl.) Hoehne	40	MO17	3.055	MO17
***Sauvetrea* Szlach. (13 species)**					
Sauvetrea laevilabris	(Lindl.) M.A.Blanco	42	GJ94	NA	
***Scuticaria* Lindl. (nine species)**					
Scuticaria hadwenii	(Lindl.) Planch.	40	MO17	5.69	MO17
Scuticaria steelei	(Hook.) Lindl.	40	PW	6.12	PW
***Trigonidium* Lindl. (seven species)**					
Trigonidium acuminatum	Bateman ex Lindl.	40	FG00	1.59	PW
Trigonidium egertonianum	Bateman ex Lindl.	40	MO12	1.70	MO12
Trigonidium obtusum	Lindl.	40	FG00	1.72	PW
Trigonidium riopalenquense	Dodson	40	MW	NA	

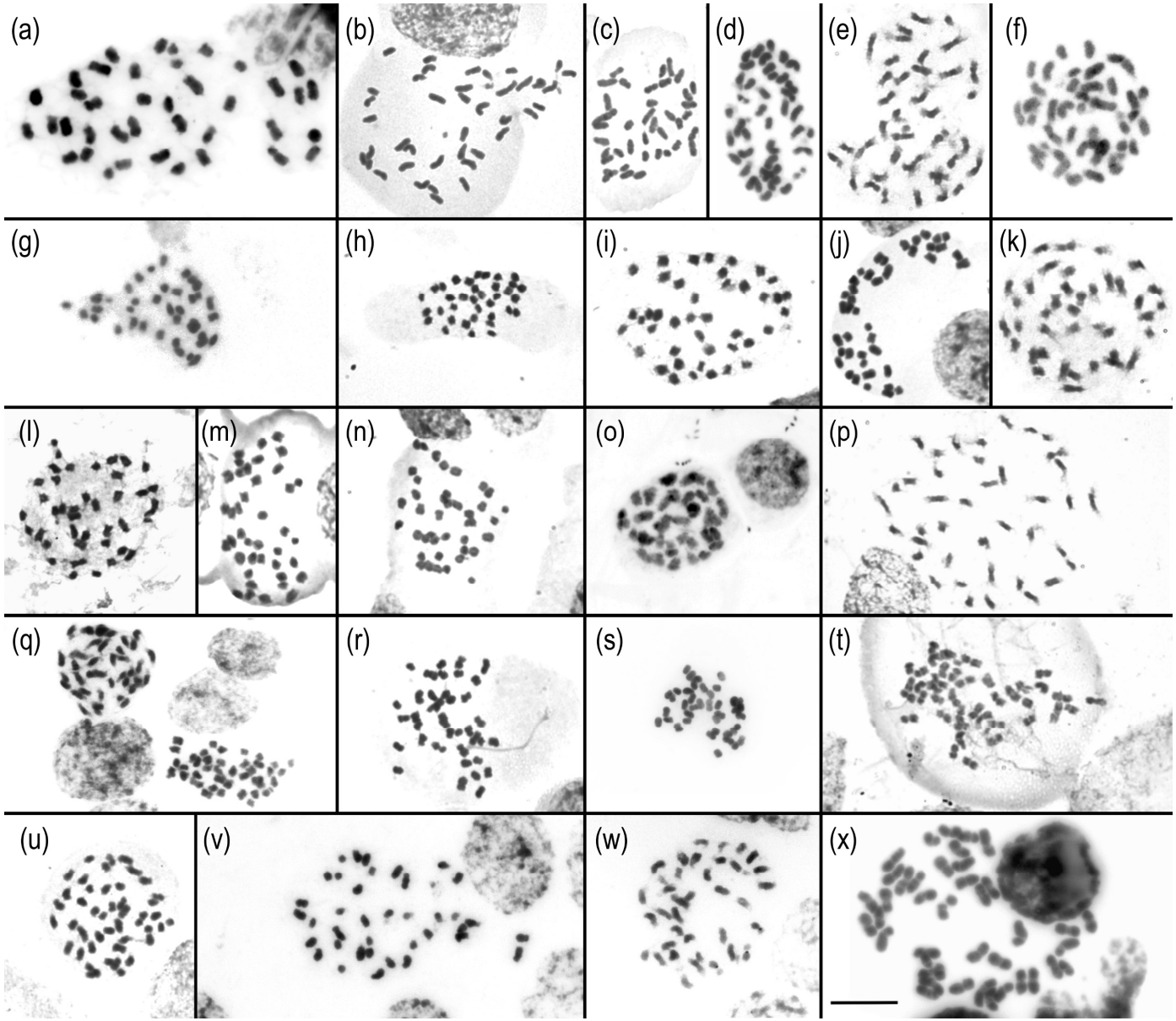
***Xylobium* Lindl. (30 species)**					
Xylobium foveatum	(Lindl.) G.Nicholson	40	FG00	4.47	MO17
Xylobium squalens	(Lindl.) Lindl.	40	GO85	NA	
Xylobium variegatum	(Ruiz & Pav.) Garay & Dunst.	40	MO17	3.74	MO17
Close related species					
Dichaea panamensis	Lindl.	52	FG00	NA	
Dichaea muricata	(Sw.) Lindl.	52	TK84	NA	
Eriopsis biloba	Lindl.	40	AO89	NA	
Koellensteinia graminea	(Lindl.) Schltr. K.	48	TK84	NA	
Peristeria elata	Hook.	40	JO98	4,7	JO98
Stanhopea ecornuta	Lem.	40	M73	NA	
Stanhopea insigins	J.Frost ex Hook.	40	TK84	NA	
Vitekorchis excavata	(Lindl.) Romowicz & Szlach.	NA		2,10	LE08
Zygopetalum maculatum	(Kunth) Garay	48	GO18	7.38	GO18
Zygopetalum maculatum	(Kunth) Garay	72	GO18	10.48	GO18
Zygopetalum maculatum	(Kunth) Garay	96	GO18	14.07 [7.035]	GO18
Zygopetalum maxillare	Lodd.	48	TK84	NA	

References: HO29, Hoffmann (1929); HO30, Hoffmann (1930); Bl60, Blumenschein (1960); BF61, Blumenschein and Flechtmann (1961); BP63, Blumenschein and Paker, 1963; TK84, Tanaka and Kamemoto (1984); GO85, Goldblatt (1985); AK88, Aoyama and Karasawa (1988); GJ94, Goldblatt e Johnson (1994); JO98, Jones et al. (1998); FG00, Felix and Guerra (2000); Ry00, Ryan et al. (2000); CA06, Cabral et al. (2006); KO08, Koehler et al. (2008); LE08, Leitch et al. (2008); MO12, Moraes et al (2012); MO16, Moraes et al. (2016); MO17, Moraes et al. (2017); GO18, Gomes et al. (2018); PW, Present work

Table 2. Summary of Phylogeny-corrected Regressions aiming to explain variation in chromosome number and genome size in Maxillariinae orchids using ecological variables (habit, elevation, and bioclimatic variables) associated with species distribution. Regressions were performed without and with polyploids in the sample. Under the model names we present estimates for intercept and the variables, followed by significance codes, according to p-value: ~0 = '***', <0.001 = '**', <0.01 = '*', <0.05 = '.'

Coefficients	Models			
	Without polyploids		With polyploids	
	Chromosome number	Genome size	Chromosome number	Genome size
Intercept	40.95967 ***	0.139529	67.4649 ***	0.43277
Number of possible Habits		0.169730 .	-13.5538 ***	
Exclusively Epiphytic			-11.5816 ***	
Elevation	0.30987 *	0.055666 *		
Temperature Seasonality				-0.28723 *
Temperature of Coldest Quarter			1.8322 .	
Mean Diurnal Temperature Range				
Precipitation of the Coldest Quarter			-4.2069 ***	-0.44616 ***
Precipitation of the Warmest Quarter		-0.141759 *		
Precipitation of the Driest Quarter				
Model Summary				
lambda [Maximum Likelihood]	0.921	1.000	0.332	0.740
Residual standard error	12.69	5.286	44.98	5.424
Degrees of freedom	90	58	91	62
Adjusted R-squared	0.03415	0.1243	0.3266	0.1456
F-statistic	4.217 on 1	3.887 on 3	12.52 on 4	6.455 on 2
p-value	0.04292	0.01337	3.717e-08	0.002842
Best Model AICc	322.8996	89.28603	656.7690	145.5251
Intercept-only Model AICc	324.5901	94.00995	689.0831	153.1811
ΔAICc	1.6905	47.2392	32.3141	7.6560

Figure 1. Chromosome number in Maxillariinae. (a) *Anguloa virginalis* (2*n*=40), (b) *Brasiliorchis barbosae* (2*n*=40), (c) *Bra. chrysantha* (2*n*=40), (d) *Bra. consanguinea* (2*n*=40), (e) *Bra. marginata* (2*n*=40), (f) *Bra. monantha* (2*n*=40), (g) *Camaridium carinatum* (2*n*=38), (h) *Christensonella pumila* (2*n*=36), (i) *Heterotaxis equitans* (2*n*=42), (j) *Het. superflua* (2*n*=42), (k) *Mapinguari desvauxianus* (2*n*=40), (l) *Maxillaria bradei* (2*n*=40), (m) *Max. kegelii* (2n=40), (n) *Max. leucaimata* (2*n*=40), (o) *Max. parkeri* (2*n*=40), (p) *Max. setigera* (2*n*=40), (q) *Maxillariella robusta* (2*n*=40), (r) *Maxillariella tenuifolia* (2*n*=40), (s) *Mormolyca ringes* (2*n*=40), (t) *Mor. rufescens* (2*n*=40), (u) *Nitidobulbon nasutum* (2*n*=46), (v) *Rhetinantha cerifera* (2*n*=38), (w) *Rhe. friedrichsthalii* (2*n*=36), (x) *Scuticaria steelei* (2*n*=40). Bar=10 um.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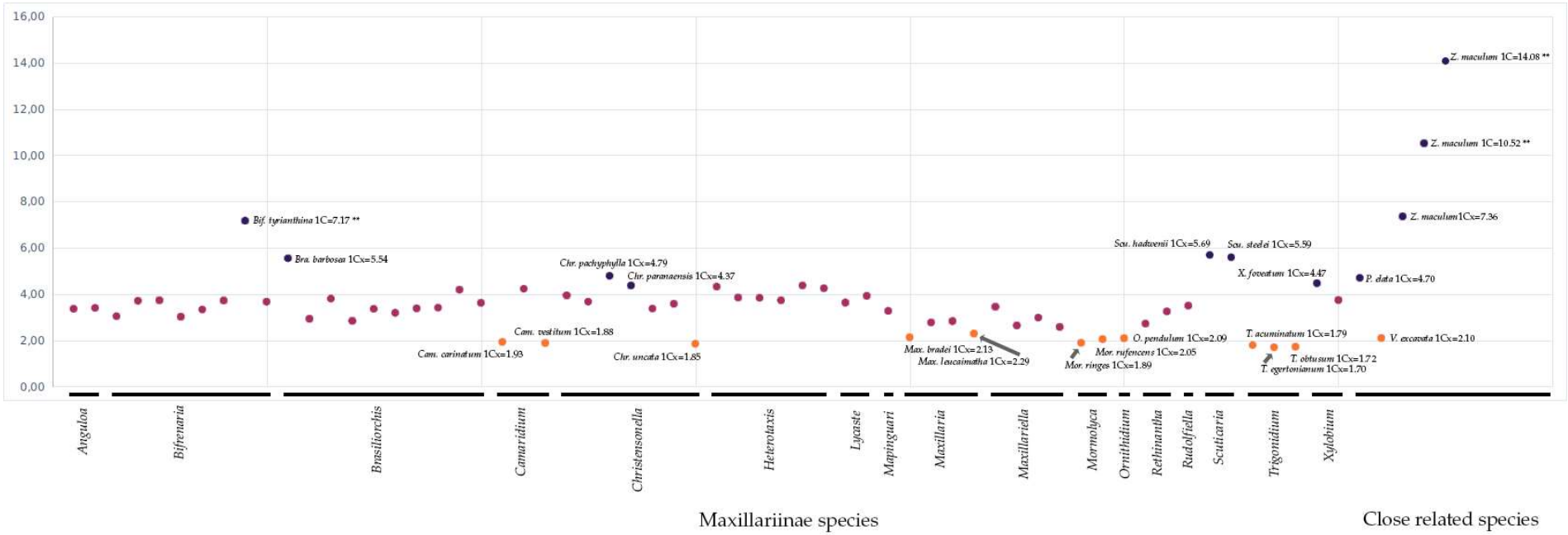


Figure 2. Genome size in Maxillariinae and closely related species. The genome size of 62 species is presented here with average 1Cx=3.38 +/- 0.94. The large genome sizes are indicated in dark, average genome sizes in purple and the small genome sizes in orange. The large and small genome sizes are also indicated by its species and monoploid genome size. The polyploid cytotypes (*Bif. tyrianthina* and *Z. maculum*) are indicated by '**' and the haploid genome size (1C-value).

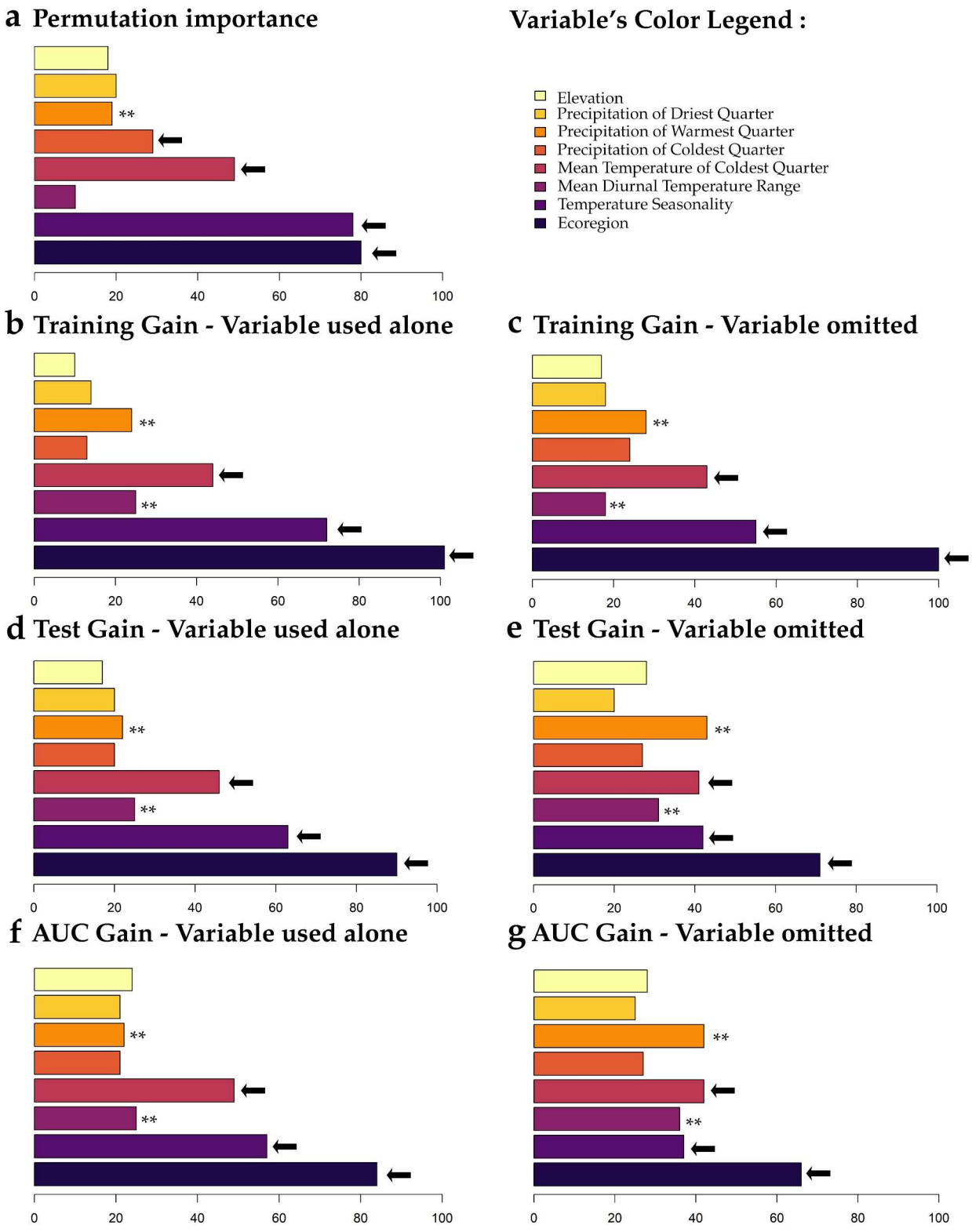


Figure 3. Variables importance in the SDM of Maxillariinae species. The variables importance are presented in each (a) Permutation Importance, (b, c) Training Gain, (d, e) Test Gain and (f, g) AUC, both with variable omitted (b, d, and f) and used alone (c, e, and g). The most important variables are indicated by an arrow and the secondary important variable by asterisks. Each bar corresponds to one variable following the variable's colour legend.

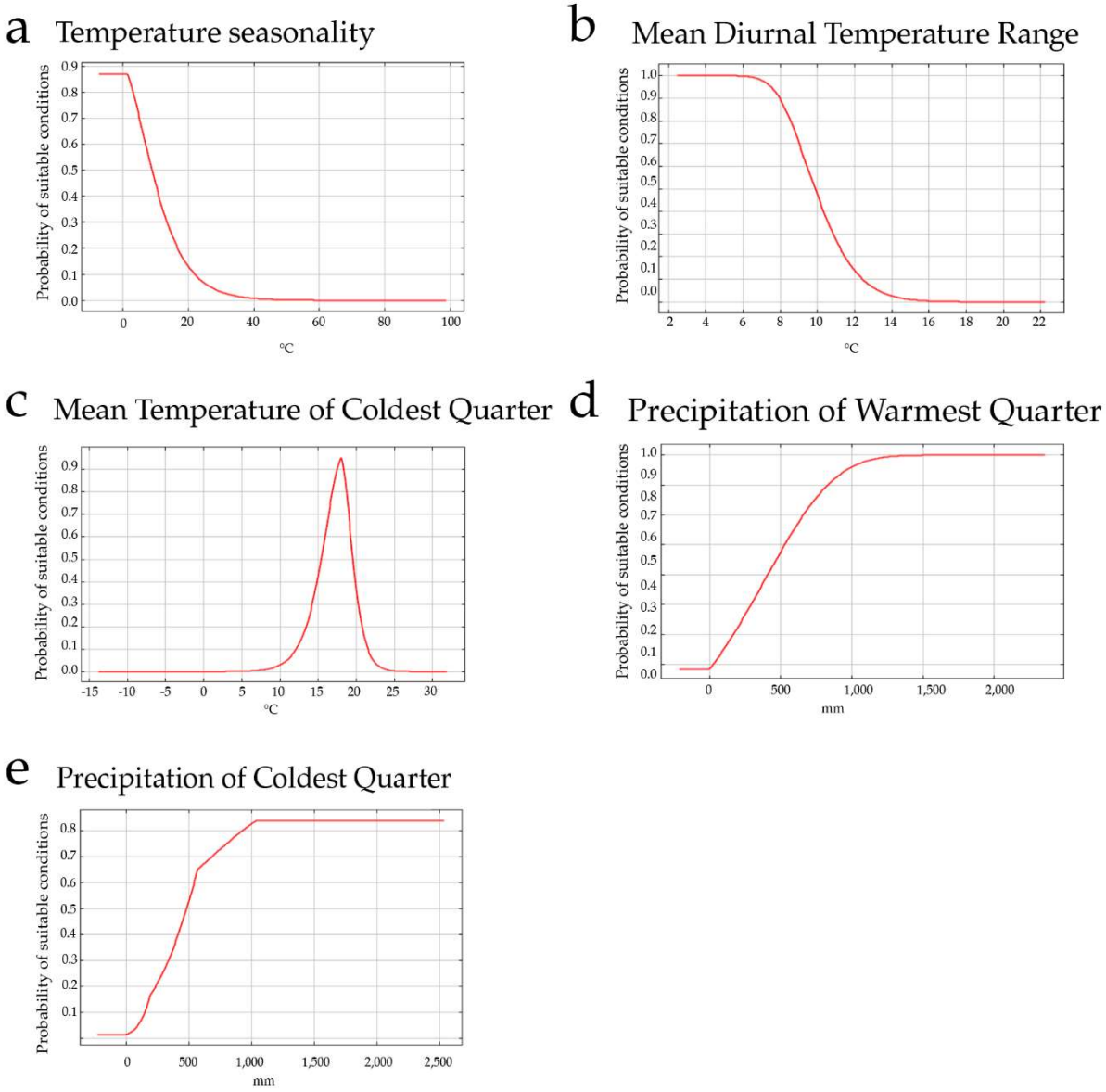


Figure 4. Performance of variables represented by the curve response. The curves show how the estimated probability of suitable occurrence changes in response to variation in an environmental variable. Each curve represents a model fitted using only one variable: (a) Temperature seasonality, (b) Mean Diurnal Temperature Range, (c) Isothermality, (d) Mean Temperature of Coldest Quarter, (e) Precipitation of Warmest quarter, and (f) Precipitation of Coldest Quarter.

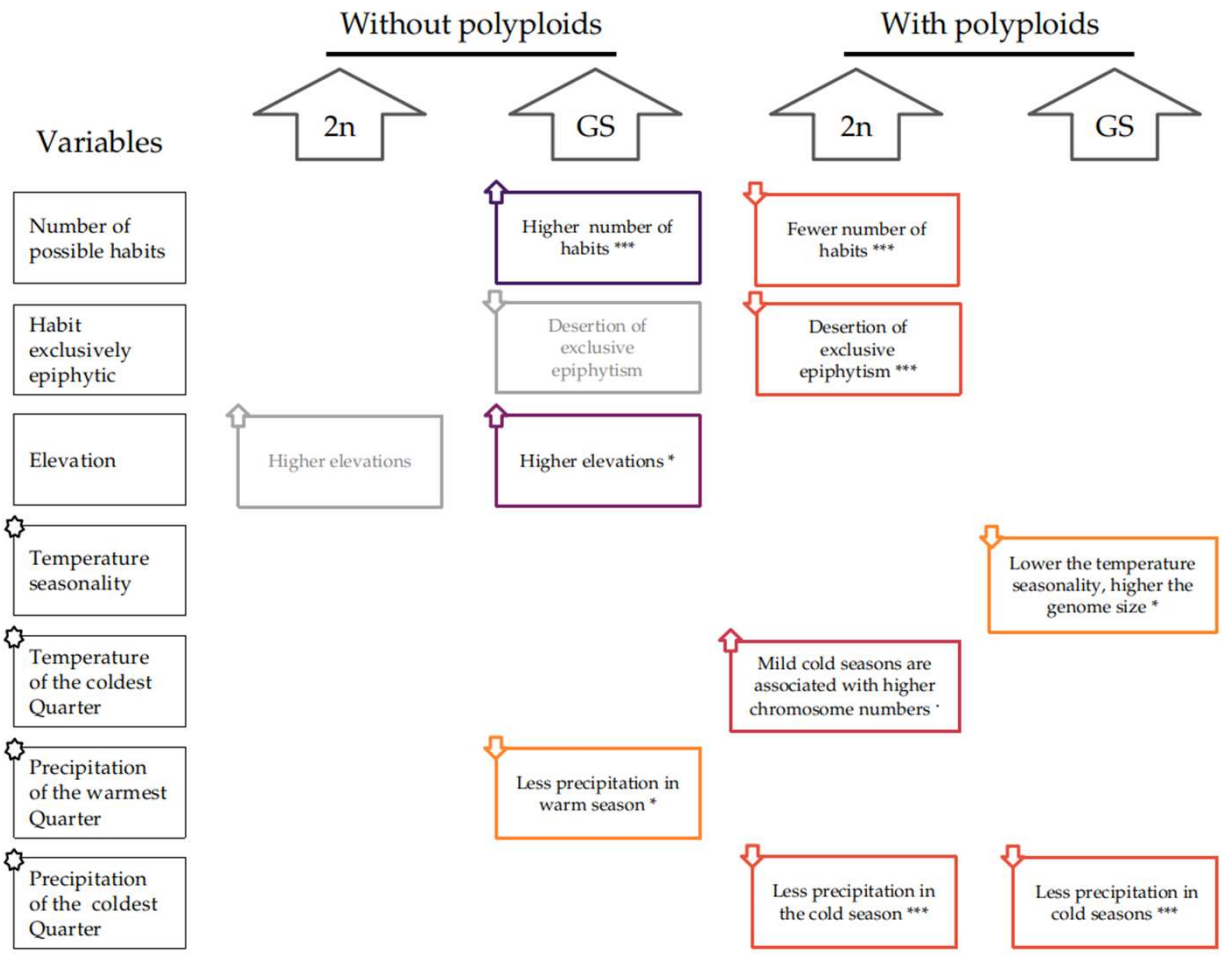


Figure 5. Genomic traits correlations: chromosome number (2n) and genome size (GS) increase with environmental and ecological variables according to the PGLS analyses. The first column presents the seven variables in the Maximum Model, with the variables indicated in SDM as the most important to Maxillariinae occurrence indicated by asterisks. The next columns represent each PGLS model, indicating the variables retained in the Best Model. Arrows in the variable boxes in the models indicate the direction of the correlation with the variation of the genomic traits. Positive correlations are indicated by up arrows and purple boxes, while down arrows indicate negative correlations in orange boxes. The darker the shade of colour, the greater the importance of this variable in the model, being the light shades the least important variables. Inside each box, we present the significance codes according to p-value: ~0 = '***', <0.001 = '**', <0.01 = '*', <0.05 = '·' .

***New Phytologist* Supporting Information**

Article title: Are chromosome number and genome size associated with habit and environmental niche variables? Insights from the Neotropical Maxillariinae (Orchidaceae)

Authors: Thaissa Brogliato Junqueira Engel[1], Eliana R. Forni-Martins[1], Leonardo P. Félix[2], Marcelo Guerra[3], Juliano Sarmento Cabral[4*], Ana Paula Moraes[5*]

Article acceptance date: Click here to enter a date.

The following Supporting Information is available for this article (*Short legends/titles*):

Fig. S1 Maximum Likelihood and Bayesian Inference phylogenetic trees.

Fig. S2 Heatmaps of variables importance to the model's performance.

Fig. S3 Best Model candidates according to PGLS of environmental and ecological explanatory variables against the genomic traits.

Table S1 Maxillariinae's new chromosome number and genome size records with origin and voucher information.

Table S2 GenBank accessions for DNA sequences used in the phylogenetic analyses.

Table S3 Habit and elevation information.

Table S4 Species Distribution Models for Maxillariinae and close related species.

Table S5 Evolution model test for chromosome number and genome size.

Table S6 Regressions summary between the genomic traits with and without polyploids in the sample.

Methods S1 Variable selection.

Methods S2 Methods on species distribution models (SDM) performed by MaxEnt.

Notes S1 Notes on Maxillariinae SDM.

The following Supporting Information is available for this article (*Full legend/titles*):

Fig. S1 Maximum Likelihood (a) and Bayesian Inference (b) phylogenetic trees based on matK, ycf1 and ITS markers. Branches support, Bootstrap and Posterior Probability, are indicated by the branch colour according to the colour scale of each phylogenetic tree. The dashed branches in black represent topology differences among species.

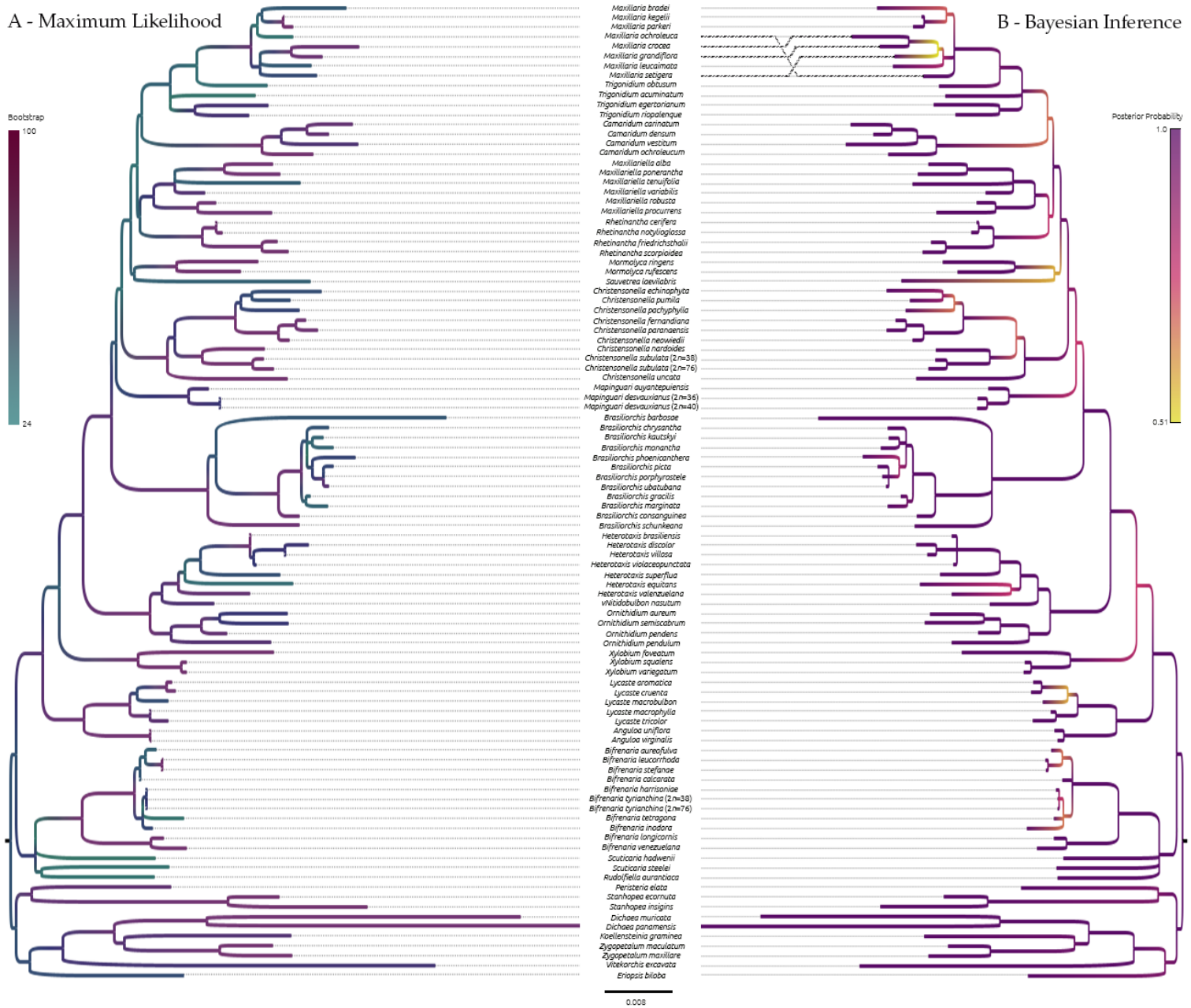

A - Maximum Likelihood
B - Bayesian Inference
Bootstrap
100
24
Posterior Probability
1.0
0.51
Maxillaria bradei
Maxillaria kegelii
Maxillaria parkeri
Maxillaria ochroleuca
Maxillaria crocea
Maxillaria grandiflora
Maxillaria leucaimata
Maxillaria setigera
Trigonidium obtusum
Trigonidium acuminatum
Trigonidium egertonianum
Trigonidium riopalenque
Camaridium carinatum
Camaridium densum
Camaridium vesitum
Camaridium ochroleucum
Maxillariella alba
Maxillariella ponerantha
Maxillariella tenuifolia
Maxillariella variabilis
Maxillariella robusta
Maxillariella procurrens
Rhetinantha cerifera
Rhetinantha notyioglossa
Rhetinantha friedrichsthalii
Rhetinantha scorpioidea
Mormolyca ringens
Mormolyca rufescens
Sauvetrea laevilabris
Christensonella echinophyta
Christensonella pumila
Christensonella pachyphylla
Christensonella fernandiana
Christensonella paranaensis
Christensonella neowiedii
Christensonella nardoides
Christensonella subulata (2n=38)
Christensonella subulata (2n=76)
Christensonella uncata
Mapinguari auyantepuiensis
Mapinguari desvauxianus (2n=36)
Mapinguari desvauxianus (2n=40)
Brasiliorchis barbosae
Brasiliorchis chrysantha
Brasiliorchis kautskyi
Brasiliorchis monantha
Brasiliorchis phoenicanthera
Brasiliorchis picta
Brasiliorchis porphyrostele
Brasiliorchis ubatubana
Brasiliorchis gracilis
Brasiliorchis marginata
Brasiliorchis consanguinea
Brasiliorchis schunkeana
Heterotaxis brasiliensis
Heterotaxis discolor
Heterotaxis villosa
Heterotaxis violaceopunctata
Heterotaxis superflua
Heterotaxis equitans
Heterotaxis valenzuelana
Wilsonbulbon nasutum
Ornithidium aureum
Ornithidium semiscabrum
Ornithidium pendens
Ornithidium pendulum
Xylobium foveatum
Xylobium squalens
Xylobium variegatum
Lycaste aromatica
Lycaste cruenta
Lycaste macrobulbon
Lycaste macrophylla
Lycaste tricolor
Anguloa uniflora
Anguloa virginalis
Bifrenaria aureofulva
Bifrenaria leucorrhoda
Bifrenaria stefanae
Bifrenaria calcarata
Bifrenaria harrisoniae
Bifrenaria tyrianthina (2n=38)
Bifrenaria tyrianthina (2n=76)
Bifrenaria tetragona
Bifrenaria inodora
Bifrenaria longicornis
Bifrenaria venezuelana
Scuticaria hadwenii
Scuticaria steelei
Rudolfiella aurantiaca
Peristeria elata
Stanhopea ecornuta
Stanhopea insignis
Dichaea muricata
Dichaea panamensis
Koellensteinia graminea
Zygopetalum maculatum
Zygopetalum maxillare
Vitekorchis excavata
Eriopsis biloba
0.008

Fig. S2 Heatmaps of variables importance to the model's performance. (a) Permutation Importance, (b, c) Training Gain, (d, e) Test Gain and (f, g) AUC, both with variables omitted (b, d, and f) and used alone (c, e, and g). For each heatmap, a colour scale is provided. The darker the colour shade of a cell, the higher the contribution of a variable to the model for the species represented by that cell. The training, test and AUC gain tests aim to analyse how much a model's gain decreases or increases when a given variable is omitted. All the other variables are kept and when the variable is used alone to fit the model. In each test, when performed without a variable ('variable omitted'), lighter shades indicate that without that variable the gain decreases, meaning that variable has more information than the other variables. In each test, when performed with only one variable ('variable used'), the darker the colour, the higher the gain that variable provides alone, meaning it might have the most useful information by itself.

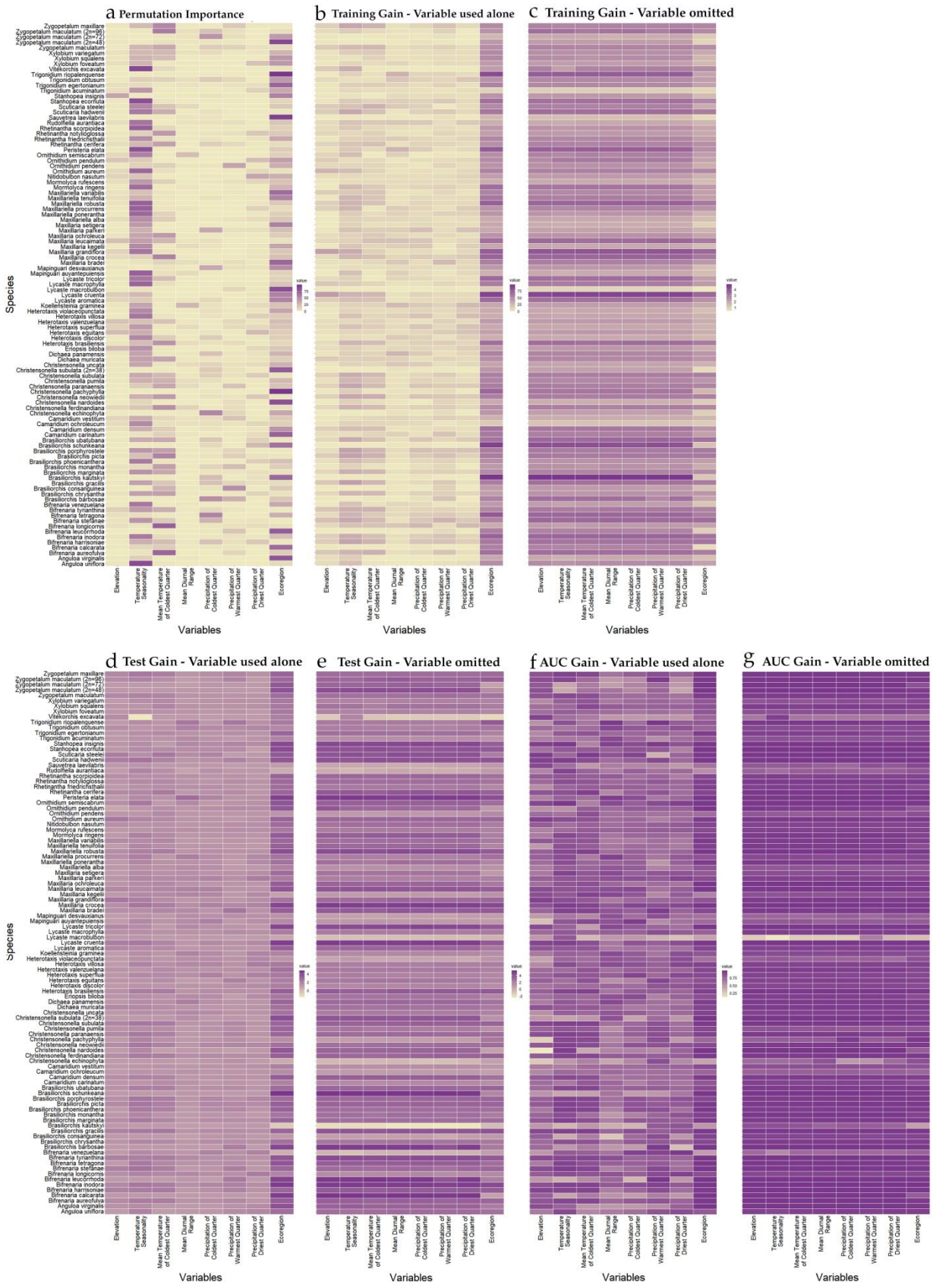

a Permutation Importance
b Training Gain - Variable used alone
c Training Gain - Variable omitted
d Test Gain - Variable used alone
e Test Gain - Variable omitted
f AUC Gain - Variable used alone
g AUC Gain - Variable omitted
Species
Variables
Elevation
Temperature Seasonality
Mean Temperature of Coldest Quarter
Mean Diurnal Range
Precipitation of Coldest Quarter
Precipitation of Warmest Quarter
Precipitation of Driest Quarter
Ecoregion
value

Fig. S3 Model Selection showing Akaike Information Criterion corrected for small sampling sizes (AICc) cumulative weights of the Best Model candidates (ΔAICc<2) according to PGLS regression of environmental and ecological explanatory variables. Figures present the results of Best Models (rows) for each dependent variable, chromosome number and genome size, without (a and b) and with polyploids (c and d) in the sample. Images in a and c present the result for chromosome number, and c and d, for genome size variation. Each Best Model is represented by one row; variables are shown in the columns, with colour columns alternating between grey and blue (for the sake of visual clarity). Whenever a variable is present in a model, the respective cell appears filled in grey or blue. Darker the colour shade, the higher the variable importance to the model.

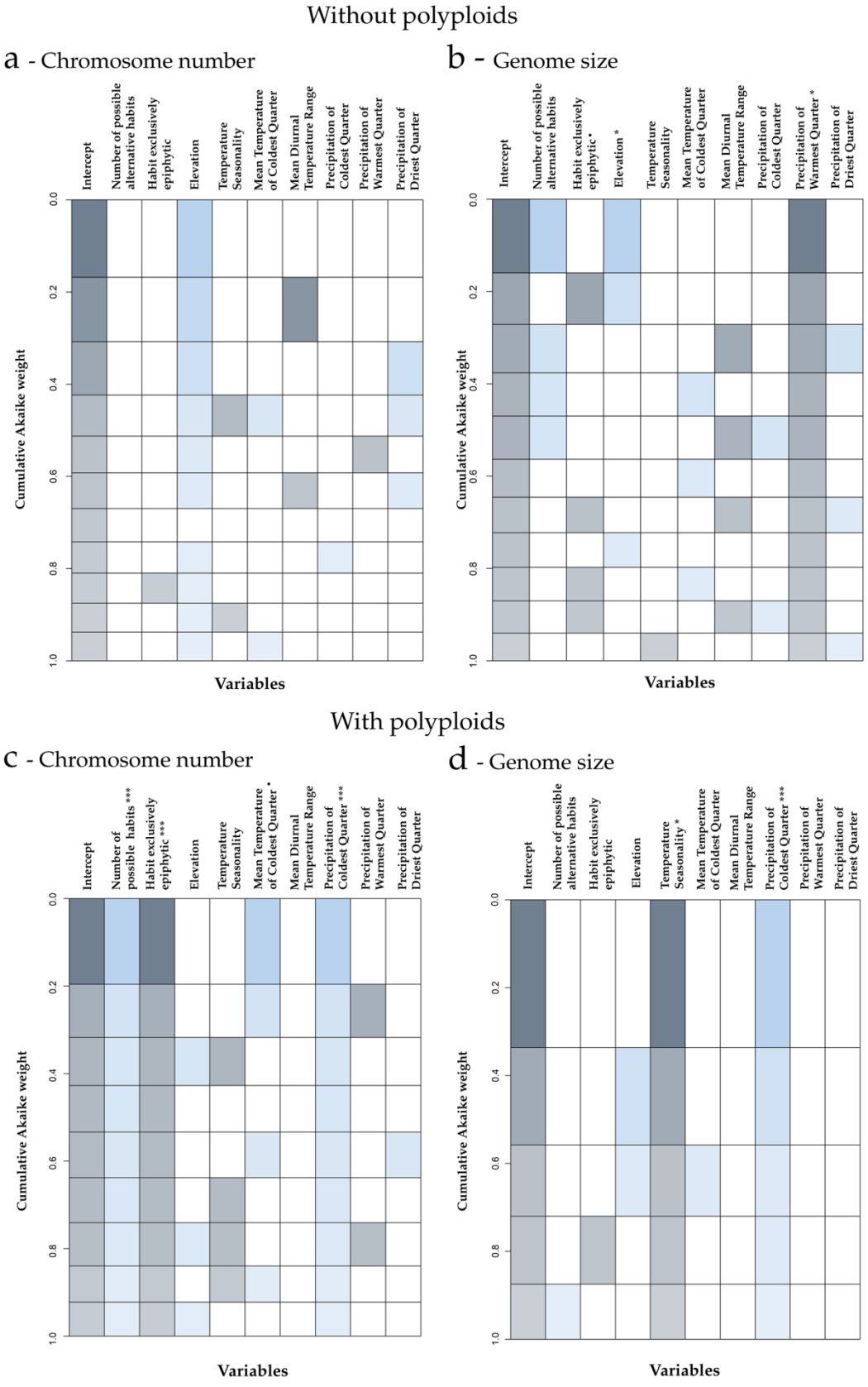

Without polyploids
a - Chromosome number
b - Genome size
With polyploids
c - Chromosome number
d - Genome size
Intercept
Number of possible alternative habits
Habit exclusively epiphytic
Elevation
Temperature Seasonality
Mean Temperature of Coldest Quarter
Mean Diurnal Temperature Range
Precipitation of Coldest Quarter
Precipitation of Warmest Quarter
Precipitation of Driest Quarter
Number of possible habits ***
Habit exclusively epiphytic ***
Mean Temperature of Coldest Quarter •
Precipitation of Coldest Quarter ***
Habit exclusively epiphytic •
Elevation *
Temperature Seasonality *
Precipitation of Warmest Quarter *
Cumulative Akaike weight
0.0
0.2
0.4
0.6
0.8
1.0
Variables

Table S1 Maxillariinae's chromosome number and genome size records. The voucher information (collector name, number, and date; Herbarium voucher; and number in the living collection, when available, is provided) along with specimem origin are presented. For each chromosome number determined, the correspondent metaphase image in 'Figure1' are indicated. The genome size record is accmpained by the standart deviation (SD) and the average coefficient of variation (CV) for each species.

Table S1 is available in xls format.

Table S2 GenBank accessions for DNA sequences used in the phylogenetic analyses. GenBank coded starting with MZ were produced in the present study.

Table S2 is available in xls format.

Table S3 Habit and elevation for Maxillariinae and close related species. Species were classified according the habit type (epiphytic, litophytic and/or terrestrial). Following such classification, two variables were determined for the macroevolutive analysis. Elevation data was obtained from Global Multi-resolution Terrain Elevation Data 2010 (GMTED2010), compiled from observation points.

Table S3 is available in xls format.

Table S4 MaxEnt's Species Distribution Models (SDM) results for Maxillariinae species. Number of training and testing occurrence points and parameters for the overall model evaluation for each species are presented: Regularized training gain, training AUC, Regularized test gain, test AUC.

Table S4 is available in xls format.

Table S5 Evolution model test. For each trait, with and without polyploids, three evolutive

models were tested, Brownian Motion (BM), Ornstein–Uhlenbeck (OU), and Early Burst (EB). The AICc value for each model is presented and the lowest value for each trait is presented in bold. Model parameters: "Pagel's Lambda"(for BM), "alpha" rate of adaptation (for OU), "a" rate change (for EB), rate of evolution (sigsq) and root state (z0).

Table S5 is available in xls format.

Table S6 Regressions summary between the genomic traits with and without polyploids in the sample.

Table S6 is available in xls format.

Methods S1. Selection of variables. For both SDM and the phylogenetic multivariate regressions, the set of 19 bioclimatic variables, representing annual trends, seasonality and extreme environmental factors, plus elevation was tested for multicollinearity. Nine groups of collinear variables were identified, considering Pearson's r=0.7 cutoff (Fig. 1).

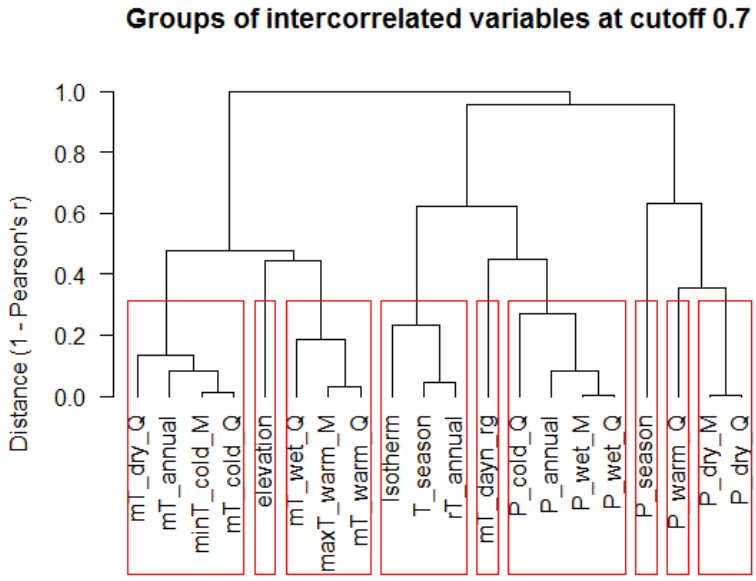
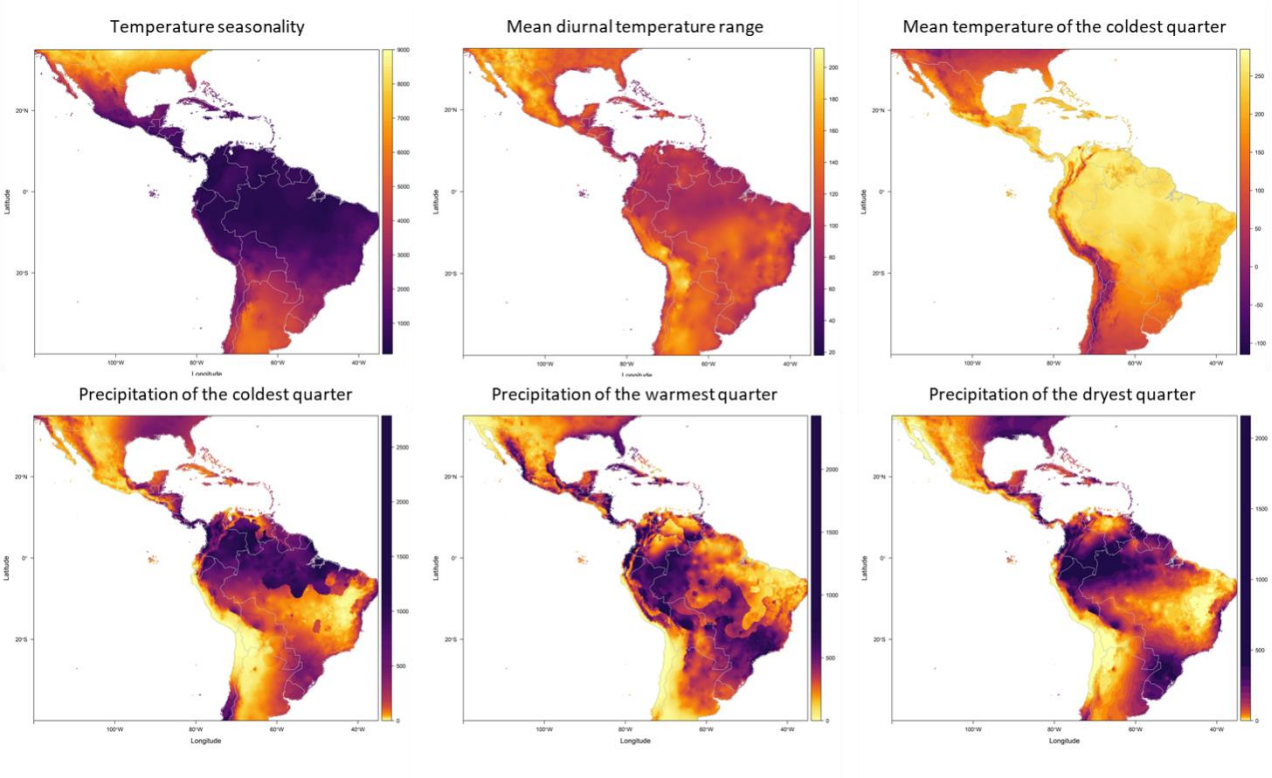


Fig. 1 - Groups of intercorrelated variables at a Pearson's r 0.7 cutoff using the 'virtualspecies' R package. Red boxes are placed around the intercorrelated variables. mT = mean Temperature, minT = minimum temperature, maxT = maximum temperature, P = precipitation, r = range, M = month, Q = quarter.Y-axis represents the distance estimated by 1- Pearson's r.

To choose which variables to keep within each multicollinear group, we used information on variables weight and importance according to the AICc values in a preliminary model fitting (Zurell *et al.*, 2020), using species presence as a response variable. We took an extra step to ensure low collinearity among the bioclimatic variables, calculating the variance inflation factor (VIF, being VIF = $1/(1-R^2)$; Fox & Monette, 1992), fitting models with each variable as a response against the other variables as predictors and keeping only variables with VIF<10 (Mundry, 2014). As a result we kept elevation and six bioclimatic variables (Fig. 2): (1) Temperature Seasonality, (2) Mean Temperature during the Coldest Quarter, (3) Mean Diurnal Temperature Range,



Precipitation during the (4) Coldest Quarter, (5) Warmest Quarter, and (6) Driest Quarter.

Fig. 2 - Maps representing the bioclimatic variables selected for the present study.

Methods S2 - MaxEnt

MaxEnt parameters and Threshold selection. For MaxEnt SDM, we produced 20,000 background points for each species, seeded randomly within the study area extent (120° to 35°W, 40°S to 35°N for all species). For both background and presence data, we set aside 20% of the points to test the models (See Total sample, Training sample and Test sample numbers per species in Table S4). The MaxEnt models were performed with statistics based on linear, quadratic and product features allowed, and up to five million iterations for the training algorithm to find the model parameters. We used only species with at least five observation points, a sample size MaxEnt was shown to produce reasonable predictions for (Pearson *et al.*, 2007; Wisz *et al.*, 2008). Hence, we produced SDM for 13 outgroup specimens (*Zygopetalum maculatum* (Kunth) Garay generated four models because it has three cytotypes) and 88 models for 87 Maxillariinae species (*Christensonella subulata* (Lindl.) Szlach., Mytnik, Górniak & Smiszek has two cytotypes and generated two models; Table S4). We selected 'cloglog' as MaxEnt SDM output (Phillips *et al.*, 2017), which provides a representation of the probability of environmental suitability for species occurrence at a locality (which we will refer from now on as "occurrence probability" only, for the sake of simplicity), ranging from 0 to 1. In order to derive the MaxEnt output map, a representation of probabilities, into a binary representation of species occurrence (*i.e.*, a polygon of occurrence), it is necessary to define a threshold value, and all cells in the map whose predicted presence probability value are greater than such threshold, are merged into a polygon of estimated species occurrence. The 'spec-sens' threshold was calculated for each model and corresponded to the value at which the sum of the sensitivity (true positive rate) and specificity (true negative rate) was the highest.

Evaluation of models. To evaluate the performance of the models, we analysed them by two methods aiming to overcome each one fragility: the receiver operating characteristic (ROC) curve (AUC; Bradley, 1997) and the True Skill Statistics (TSS; Allouche *et al.*, 2006). All SDM performed better than random, with AUC scores for training sets remaining greater than 0.9 for all species (Table S4). The AUC scores for testing sets were greater than 0.7 for 96 out of the 97 species, with only one model scoring less than 0.5 (i.e., no better than random; see '*' in Table S4). Considering TSS values, all models performed better than random (TSS>0; Table S4). As a

result of such models, Maxillariinae species distribution is predicted to range from south of California to northern Argentina and Uruguay coast and predicted occurrence fits the occurrence points (see Fig. 1). The highest richness is predicted at the region stretching from Central America to Bolivia, mainly along the tropical Andes, and for the Brazilian Atlantic coast along with the extension of the Atlantic Forest.

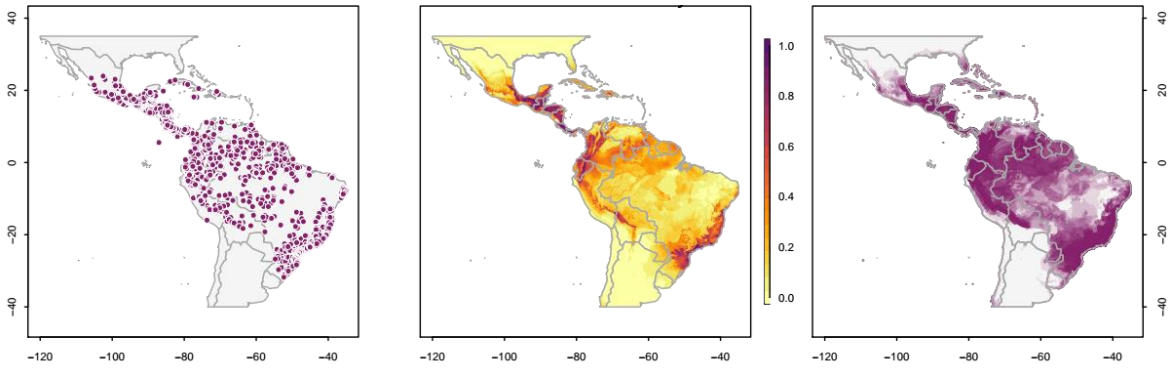


Fig. 1 - Maxillariinae distribution. Observed points (left), projection for the entire subtribe in a single model (centre) and superposed projections of each species (right) are presented to the subtribe Maxillariinae. For the single purpose of illustrating the predicted probability of occurrence of the entire subtribe, a model was fitted using the observation points of all species in a single model. However, for the estimated potential distribution, individual occurrence polygons for each species were superposed because models of single species are more precise than a model for the entire subtribe, providing a better representation.

Evaluation of variables. The MaxEnt output comprises a series of statistics on the importance and contribution of the imputed predictor variables to the model, such as Permutation importance and the Jackknife tests for regularised training gain, test gain and AUC. To better visualize the data for all species, we compiled the raw numbers for each species in seven tables, one for Permutation Importance and six for the jackknife tests of regularized trainning gain, test gain and AUC, with variables used alone and omitted (Figure S2). These compilations were then transformed from tables into heatmaps, making the comprehension of the gain values when using or removing a variable, very intuitive. To avoid putative doubts on the reliability of such measures of importance, we followed all the criteria suggested in (Smith & Santos, 2020) but

one, which was partially attended: the minimum sample size of 32 points for training sets was not possible for all species (see Table S4). To assess which variables affect species distribution, we ranked the variables according to their performance in the model statistics and accounted for those most frequently high ranking across the 97 species modelled.

Note S1. The variable Terrestrial Ecoregions proved to be central in species distribution, presenting, by itself, the most useful and unique information. The delimitation of ecoregions takes under consideration a wide group of variables like climate, vegetation types, habitat and guild structures, as well as species composition (faunas and floras) and biotic interactions (Dinerstein *et al.*, 1995, 2017; Olson & Dinerstein, 1998). Large scale distributions are mainly determined by climate, but concerning smaller scales, *e.g.* within a vegetation type, non-climate variables such as topography, community dynamics, fauna, hosts availability and ecoregions may play a more significant role than climate variables (Woodward & Williams, 1987; Hijmans, 2012). As this variable was coded as factor within the arguments of MaxEnt, it does not produce a response curve of intuitive interpretation, but the change in species occurrence in response to the ecoregions can be perceived by comparing maps of species occurrence with the incidence of the ecoregions.

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Table S1. Maxillariinae's chromosome number and genome size records. The voucher information (collector name, number, and date; Herbarium voucher; and number in the living collection, when available, is provided) along with specimem origin are presented. For each chromosome number determined, the correspondent metaphase image in 'Figure1' are indicated. The genome size record is accmpained by the standart deviation (SD) and the average coefficient of variation (CV) for each species.

Species	Chromosome number				Genome size				
	2n	Voucher [Collector, Herbarium, Live collection]*	Origin	Figure 1	1C	SD	CV	Voucher [Collector, Herbarium, Live collection]*	Origin
Anguloa Ruiz & Pav. (11 species)									
Anguloa uniflora Ruiz & Pav.					3.36	0.01	3.36	Hannover Botanic Garden n. 1997-G-17	Commercial Orchidarium
Anguloa virginalis Linden ex B.S.Williams	40	APMoraes 171	Commercial Orchidarium	(a)	3.4	0.01	2.92	AP. Moraes 171	Commercial Orchidarium
Brasiliorchis Singer, Koehler & Carnevali (13 species)									
Brasiliorchis barbosae (Loefgr.) R.B.Singer, S.Koehler & Carnevali	40	L. Félix s.n.	Domingos Martins, ES	(b)	5.54	0.02	2.83	VL. Gil & al. s.n., São Paulo Institute of Botany n. 12159	Campos do Jordão, SP
								F. Pinheiro 597, HUFABC 929, São Paulo Institute of Botany n. 1374	Pedra Grande, SP
Brasiliorchis chrysantha (Barb.Rodr.) R.B.Singer, S.Koehler & Carnevali	40	L. Félix s.n.	Una, BA	(c)					
Brasiliorchis consanguinea (Klotzsch) R.B.Singer, S.Koehler & Carnevali	40	FPinheiro 324, SP 388212, São Paulo Institute of Botany n. 1303	Paranapiacaba Reserve, Santo André, SP	(d)	3.80	0.08	2.97	F. Pinheiro 324, SP 388212, São Paulo Institute of Botany n. 1303	Paranapiacaba Biological Reserve, Santo André, SP
Brasiliorchis marginata (Lindl.) R.B.Singer, S.Koehler & Carnevali	40	GFerreira s.n.	Commercial Orchidarium	(e)					
Brasiliorchis monantha (Barb.Rodr.) Campacci	40	HDBicalho s.n., São Paulo Institute of Botany n. 5442	Córrego Fundo, MG	(f)					
***Camaridium* Lindl. (ca. 80 species)**									
Camaridium carinatum (Barb.Rodr.) Hoehne	38	HDBicalho s.n. in 02/10/1961, São Paulo Institute of Botany n. 911	Ilha Comprida, SP	(g)				HD.Bicalho in 02/10/1961, São Paulo Institute of Botany n. 911	Ilha Comprida, SP
					1.93	0.06	2.85	F. Pinheiro & al in 10/08/2000, São Paulo Institute of Botany n. 17339	Parque Estadual Serra do Mar (Trilha da Mococa), Caraguatatuba, SP
								EP. Chu in 01/01/1996, São Paulo Institute of Botany n. 16781	Cananéia (Ilha do Cardoso), SP
Camaridium ochroleucum Lindl.					4.23	0.06	2.57	AP. Moraes 242, HUFABC 2293	Commercial Orchidarium
Camaridium vestitum (Sw.) Lindl.					1.88	0.03	3.39	AP. Moraes & al. 80, HUFABC 968, São Paulo Institute of Botany s.n.	Manaus, AM
***Christensonella* Szlach., Mytnik, Górniak & Smiszek (11 species)**									
Christensonella echinophyta (Barb.Rodr.) Szlach., Mytnik, Górniak & Smiszek	NA				3.94	0.01	3.17	SC. Francisco s.n., HUFABC 1168, São Paulo Institute of Botany n. 11738	Paranapiacaba Biological Reserve, Santo André, SP
								HUFABC 971, São Paulo Institute of Botany n. 1492	no data
Christensonella fernandiana (Barb.Rodr.) Szlach., Mytnik, Górniak & Smiszek					4,05	0.29	3.59	HD.Bicalho in 17-19/09/1965, São Paulo Institute of Botany n. 5056	Sant'Ana, MG
								HD.Bicalho in 19-22/03/1962, São Paulo Institute of Botany n. 1487	Camanducaia, MG

Christensonella pachyphylla (Schltr. ex Hoehne) Szlach., Mytnik, Górniak & Smiszek								HD. Bicalho s.n., HUFABC 972, São Paulo Institute of Botany n. 901	Cananéia, SP
					4.79	0.02	3.26	P. Brólio s.n., HUFABC 973, São Paulo Institute of Botany n. 3876	Juréia, SP
								AS. Pires s.n., HUFABC 1171, São Paulo Institute of Botany n. 2153	São Paulo, SP
Christensonella paranaensis (Barb.Rodr.) S.Koehler								São Paulo Institute of Botany n. 17096	
					4.37	0.06	3.68	São Paulo Institute of Botany n.13075	
								VL. Gil & al. s.n. in 26 - 27/10/1981, São Paulo Institute of Botany n. 12153	Campos do Jordão, SP
Christensonella pumila (Hook.) Szlach., Mytnik, Górniak & Smiszek	36	LPFélix 9675, EAN 9408	Morro do Chapéu, Chapada Diamantina, BA	(h)					
***Heterotaxis* Lindl. (13 species)**									
Heterotaxis equitans (Schltr.) Ojeda & Carnevali	42	G. Ferreira s.n. (under cultivation)	Commercial Orchidarium	(i)					
Heterotaxis superflua (Rchb.f.) F.Barros	42	LP. Félix & GV. Dornelas s.n., EAN 10832	São Félix do Xingu, PA	(j)					
***Mapinguari* Carnevali & Singer (four species)**									
Mapinguari desvauxianus (Rchb.f.) Carnevali & R.B.Singer	40	LP. Félix & GV. Dornelas s.n., EAN 3835	Una, BA	(k)				ELM. Catharino s.n., HUFABC 952, São Paulo Institute of Botany n. 16285	Salesópolis, SP
					3.27	0.05	3.42	HD. Bicalho s.n., HUFABC 1174, São Paulo Institute of Botany n. 704	Pedro de Toledo, SP
								São Paulo Institute of Botany n. 207	No data
***Maxillaria* Ruiz & Pav. (200-250 species)**									
Maxillaria bradei Schltr. ex Hoehne	40	São Paulo Institute of Botany n. 18077	Peruíbe, SP	(l)				São Paulo Institute of Botany n. 18077	Peruíbe, SP
					2.13	0.14	3.354	B. Guillany s.n., HUFABC 956, São Paulo Institute of Botany n. 8392	Miracatu, SP
								F. Barros s.n., HUFABC 934, São Paulo Institute of Botany n. 15665	Cananéia, SP
Maxillaria crocea Lindl.								P. Martuscelli s.n., HUFABC 1206, São Paulo Institute of Botany n. 15040	Ji-Paraná, Rondônia
					2.77	0.04	3.22	P. Martuscelli s.n., São Paulo Institute of Botany n. 15041	Ji-Paraná, Rondônia
								P. Martuscelli s.n., HUFABC 1203, São Paulo Institute of Botany n. 15044	Ji-Paraná, Rondônia
Maxillaria kegelii Rchb.f.	40	LP. Felix 9484, EAN 10822	Amaraji, PE	(m)	2.83	0.01	2.84	APMoraes & al 77, São Paulo Institute of Botany n. P6286	Manaus, AM
Maxillaria leucaimata Barb.Rodr.	40	LP. Felix s.n. (under cultivation)	Maranguape, CE	(n)				F.Pinheiro & al in 10/08/2000, São Paulo Institute of Botany n. 17337	Parque Estadual Serra do Mar (Trilha da Mococa), Caraguatatuba, SP
					2.29	0.01	2.94	E. Mariano and M. Sugiyama s.n. in 20/12/1995, São Paulo Institute of Botany n. 16766	Cananéia (Ilha do Cardoso), SP

								São Paulo Institute of Botany n. 17563	No data
Maxillaria parkeri Hook.	40	LPFelix s.n. (under cultivation)	Commercial Orchidarium	(o)					
Maxillaria setigera Lindl.	40	LPFelix s.n. (under cultivation)	São Félix do Xingu, PA	(p)					
***Maxillariella* Blanco & Carnevali (46 species)**									
Maxillariella alba (Hook.) M.A.Blanco & Carnevali					3.45	0.09	2.27	F. Barros s.n., São Paulo Institute of Botany n. P3930	Belém, Pará
Maxillariella robusta (Barb.Rodr.) M.A.Blanco & Carnevali	40	J. Rodrigues s.n., São Paulo Institute of Botany n. 8515	Rio Sepotuba, MT	(q)				J. Rodrigues s.n., São Paulo Institute of Botany n. 8510	Rio Sepotuba, MT
					2.64	0.07	3.76	J. Rodrigues s.n., São Paulo Institute of Botany n. 8515	Rio Sepotuba, MT
								B. Guillany s.n., SP 343345, São Paulo Institute of Botany n. 8452	Boracéia, SP
Maxillariella tenuifolia (Lindl.) M.A.Blanco & Carnevali	40	Gomes Ferreira s.n. (under cultivation)	Mexico	(r)	2.98	0.06	2.87	AP. Moraes 09	Ubatuba, SP
Maxillariella variabilis (Bateman ex Lindl.) M.A.Blanco & Carnevali					2.58	0.09	4.96	HUFABC 1208, São Paulo Institute of Botany s.n.	Belém, Pará
***Mormolyca* Fenzl (25 species)**									
Mormolyca ringens (Lindl.) Gentil	40	HUFABC 955, São Paulo Institute of Botany n. 16891	Ubatuba, SP	(s)	1.89	0.01	2.72	HUFABC 955, São Paulo Institute of Botany n. 16981	Ubatuba, SP
Mormolyca rufescens (Lindl.) M.A.Blanco	40	LPFelix s.n. (under cultivation)	São Vicente Férrer, PE	(t)				P. Brólio s.n., HUFABC 939, São Paulo Institute of Botany n. 3828	Juréia, SP
					2.05	0.07	2.24	HD. Bicalho s.n., HUFABC 938, São Paulo Institute of Botany n. 5410	Córrego Fundo, MG
								LA. Pereira s.n., HUFABC 951, São Paulo Institute of Botany n. 12547	Joaquim Gomes, AL
***Nitidobulbon* Ojeda, Carnevali & Romero (three species)**									
Nitidobulbon nasutum (Rchb.f.) Ojeda & Carnevali	46	Siqueira-Filo & Vicente 984, UFP 24932	Jaqueira, PE	(u)					
***Ornithidium* Salisb. ex R. Br. (ca. 55 species)**									
Ornithidium pendulum (Poepp. & Endl.) Cogn.	40				2.08	0.04	2.69	SAC. Chica s.n., São Paulo Institute of Botany n. 16229	São Bernardo do Campo, SP
								P. Brólio s.n., HUFABC 945, São Paulo Institute of Botany n. 15679	State Reserve of Serra do Mar, SP
***Rhetinantha* Blanco (15 species)**									
Rhetinantha cerifera (Barb.Rodr.) M.A.Blanco	38	F. de Barros s.n., HUFABC 1164, São Paulo Institute of Botany n. 14099	Ilha do Cardoso, Cananéia, SP	(v)				F. Barros s.n., HUFABC 1164, São Paulo Institute of Botany n. 14099	Cananéia (Ilha do Cardoso), SP
					2.72	0.15	3.17	HJ. Targa s.n., HUFABC 1205, São Paulo Institute of Botany n. 3324	Paraty, RJ
								W. Hoehne s.n., São Paulo Institute of Botany n. 6103	Paraty, RJ
Rhetinantha friedrichsthalii (Rchb.f.) M.A.Blanco	36	HUFABC 1165, São Paulo Institute of Botany n. 15196	Machadinho, RO	(w)	3.25	0.01	2.95	P. Martuscelli s.n., SP 251666, São Paulo Institute of Botany n. 15173	Ji-Paraná, RO

								HUFABC 1165, São Paulo Institute of Botany n. 15196	Machadinho, RO
***Scuticaria* Lindl. (nine species)**									
Scuticaria steelei (Hook.) Lindl.	40	AP. Moraes 81, São Paulo Institute of Botany n. P6287	Manaus, AM	(x)	6,12	0.17	1.84	AP. Moraes & al. 75, São Paulo Institute of Botany n. P6287	Manaus, AM
								AP. Moraes & al. 81	Manaus, AM
***Trigonidium* Lindl. (seven species)**									
Trigonidium acuminatum Bateman ex Lindl.								São Paulo Institute of Botany n. 8390	Amazonas
					1.59	0.02	2.43	MB. da Silva and P. Brólio in 07/08/1971, São Paulo Institute of Botany n. 9252	Itagimirim, BA
								HD. Bicalho in 07/11/1964, São Paulo Institute of Botany n. 4709	Faxinal, SC
Trigonidium obtusum Lindl.								HD. Bicalho in 01/10/1961, SP 340099, São Paulo Institute of Botany n. 850	Cananéia, SP
					1.72	0.12	2.50	MB. da Silva & al. in 07/04/1979, SP 340100, São Paulo Institute of Botany n.10822	Boráceia, SP
								HUFABC 1160, São Paulo Institute of Botany n.11340	No data

* Herbarium following the acronym of Thiers (2020); Living collection: Orchidarium Frederico Hoehne in the São Paulo Botanical Garden or Hannover Botanic Garden.

Table S2. GenBank accessions for DNA sequences used in the phylogenetic analyses GenBank coded starting with MZ were produced in the present study.

Species	Authority	Phylogeny		
		GenBank		
		chloroplast		nuclear
		matK	ycf1	ITS
***Anguloa* Ruiz & Pav. (two out of 11 species)**				
Anguloa uniflora	Ruiz & Pav.	KF660280	KF660364	x
Anguloa virginalis	Linden ex B.S.Williams	MZ334594	x	MZ268386
Bifrenaria Lindl. (ten out of 18 species)				
Bifrenaria aureofulva	Lindl.	MZ334595	x	MZ268387
Bifrenaria calcarata	Barb.Rodr.	MZ334596	x	x
Bifrenaria harrisoniae	(Hook.) Rchb.f.	MZ334597	x	MZ268388
Bifrenaria inodora	Lindl.	DQ210744	KF660365	DQ210217
Bifrenaria leucorrhoda	Rchb. f.	MZ334598	x	MZ268389
Bifrenaria longicornis	Lindl.	MZ334599	x	MZ268390
Bifrenaria stefanae	V.P.Castro	MZ334600	x	MZ268391
Bifrenaria tetragona	(Lindl.) Schltr.	DQ210751	KF660529	AF239335
Bifrenaria tyrianthina	(Lodd. ex Loudon) Rchb.f.	MZ334601	EU490721	MZ268392
Bifrenaria tyrianthina	(Lodd. ex Loudon) Rchb.f.	MZ334602	KF660379	MZ268393
Bifrenaria venezuelana	C.Schweinf.	MZ334603	x	MZ268394
Brasiliorchis Singer, Koehler & Carnevali (12 out of 13 species)				
Brasiliorchis barbosae	(Loefgr.) R.B.Singer, S.Koehler & Carnevali	DQ210682	x	DQ210150
Brasiliorchis chrysantha	(Barb.Rodr.) R.B.Singer, S.Koehler & Carnevali	DQ210644	x	DQ210113
Brasiliorchis consanguinea	(Klotzsch) R.B.Singer, S.Koehler & Carnevali	DQ210718	x	DQ210188
Brasiliorchis gracilis	(Lodd., G.Lodd. & W.Lodd.) R.B.Singer, S.Koehler & Carnevali	DQ210686	KF660426	DQ210154
Brasiliorchis kautskyi	(Pabst) R.B.Singer, S.Koehler & Carnevali	DQ210646	x	DQ210115
Brasiliorchis marginata	(Lindl.) R.B.Singer, S.Koehler & Carnevali	DQ210688	x	DQ210156
Brasiliorchis monantha	(Barb.Rodr.) Campacci	MZ334604	x	MZ268395
Brasiliorchis phoenicanthera	(Barb.Rodr.) R.B.Singer, S.Koehler & Carnevali	DQ210703	x	DQ210173

Brasiliorchis picta	(Hook.) R.B.Singer, S.Koehler & Carnevali	DQ210637	x	DQ210190
Brasiliorchis porphyrostele	(Rchb.f.) R.B.Singer, S.Koehler & Carnevali	DQ210691	x	DQ210159
Brasiliorchis schunkeana	(Campacci & Kautsky) R.B.Singer, S.Koehler & Carnevali	DQ210799	KF660421	DQ210300
Brasiliorchis ubatubana	(Hoehne) R.B.Singer, S.Koehler & Carnevali	DQ210735	x	DQ210205
Camaridium Lindl. (four out of ca. 80 species)				
Camaridium carinatum	(Barb.Rodr.) Hoehne	DQ210828	KP269155	DQ210329
Camaridium densum	(Lindl.) M.A.Blanco	DQ210629	x	DQ210091
Camaridium ochroleucum	Lindl.	DQ210626	KF660312	DQ210086
Camaridium vestitum	(Sw.) Lindl.	DQ210643	KF660304	DQ210112
Christensonella Szlach., Mytnik, Górniak & Smiszek (nine out of 11 species)				
Christensonella echinophyta	(Barb.Rodr.) Szlach., Mytnik, Górniak & Smiszek	DQ210727	x	DQ210197
Christensonella fernandiana	(Barb.Rodr.) Szlach., Mytnik, Górniak & Smiszek	DQ210660	KF660353	DQ210129
Christensonella nardoides	(Kraenzl.) Szlach., Mytnik, Górniak & Smiszek	DQ210890	KF660452	DQ210403
Christensonella neowiedii	(Rchb.f.) S.Koehler	DQ210661	x	DQ210130
Christensonella pachyphylla	(Schltr. ex Hoehne) Szlach., Mytnik, Górniak & Smiszek	DQ210733	x	DQ210203
Christensonella paranaensis	(Barb.Rodr.) S.Koehler	DQ210651	x	DQ210120
Christensonella pumila	(Hook.) Szlach., Mytnik, Górniak & Smiszek	DQ210696	x	DQ210166
Christensonella subulata	(Lindl.) Szlach., Mytnik, Górniak & Smiszek	DQ210693	x	DQ210161
Christensonella subulata	(Lindl.) Szlach., Mytnik, Górniak & Smiszek	DQ210650	x	DQ210119
Christensonella uncata	(Lindl.) Szlach., Mytnik, Górniak & Smiszek	DQ210654	KP269116	DQ210123
Heterotaxis Lindl. (seven out of 13 species)				
Heterotaxis brasiliensis	(Brieger & Illg) F.Barros	DQ210687	x	DQ210155
Heterotaxis discolor	(Lodd. ex Lindl.) Ojeda & Carnevali	DQ210711	x	DQ210181
Heterotaxis equitans	(Schltr.) Ojeda & Carnevali	DQ210683	KF660448	DQ210151
Heterotaxis superflua	(Rchb.f.) F.Barros	DQ210705	x	DQ210175
Heterotaxis valenzuelana	(A.Rich.) Ojeda & Carnevali	DQ210700	KF660510	DQ210170
Heterotaxis villosa	(Barb.Rodr.) F.Barros	DQ210732	KP269154	DQ210202
Heterotaxis violaceopunctata	(Rchb.f.) F.Barros	DQ210678	EU123795	DQ210146

Lycaste Lindl. (five out of ca. 30 species)				
Lycaste aromatica	(Graham) Lindl.	AF263669	KF660322	MH762937
Lycaste cruenta	(Lindl.) Lindl.	AF239438	x	AF239342
Lycaste macrobulbon	(Hook.) Lindl.	MZ334605	x	KX434448
Lycaste macrophylla	(Poepp. & Endl.) Lindl.	EU214178	MG490363	AM162259
Lycaste tricolor	Rchb.f.	EU214513	x	x
Mapinguari Carnevali & Singer (two out of four species)				
Mapinguari auyantepuiensis	(Foldats) Carnevali & R.B.Singer	DQ210834	KF660432	DQ210336
Mapinguari desvauxianus	(Rchb.f.) Carnevali & R.B.Singer	DQ210736	x	x
Mapinguari desvauxianus	(Rchb.f.) Carnevali & R.B.Singer	KJ472340	x	DQ210206
Maxillaria Ruiz & Pav. (eight out of ca. 250 species)				
Maxillaria bradei	Schltr. ex Hoehne	DQ210681	x	DQ210149
Maxillaria crocea	Lindl.	DQ210634	x	DQ210103
Maxillaria grandiflora	(Kunth) Lindl.	DQ210938	x	DQ210454
Maxillaria kegelii	Rchb.f.	MZ334606	x	MZ268396
Maxillaria leucaimata	Barb.Rodr.	DQ210638	x	DQ210107
Maxillaria ochroleuca	Lodd. ex Lindl.	DQ210844	x	DQ210346
Maxillaria parkeri	Hook.	DQ210675	x	DQ210144
Maxillaria setigera	Lindl.	DQ210674	x	DQ210143
Maxillariella Blanco & Carnevali (six out of 46 species)				
Maxillariella alba	(Hook.) M.A.Blanco & Carnevali	DQ210814	x	DQ210315
Maxillariella ponerantha	(Rchb.f.) M.A.Blanco & Carnevali	DQ210905	KF660474	DQ210418
Maxillariella procurrens	(Lindl.) M.A.Blanco & Carnevali	DQ210782	KF660438	DQ210272
Maxillariella robusta	(Barb.Rodr.) M.A.Blanco & Carnevali	DQ210679	x	DQ210147
Maxillariella tenuifolia	(Lindl.) M.A.Blanco & Carnevali	DQ210787	x	DQ210282
Maxillariella variabilis	(Bateman ex Lindl.) M.A.Blanco & Carnevali	DQ210717	KF660481	DQ210187
Mormolyca Fenzl (two out of 25 species)				
Mormolyca ringens	(Lindl.) Gentil	DQ210680	KP269158	DQ210148
Mormolyca rufescens	(Lindl.) M.A.Blanco	DQ210721	KP269145	DQ210191

***Nitidobulbon* Ojeda, Carnevali & Romero (one out of three species)**				
Nitidobulbon nasutum	(Rchb.f.) Ojeda & Carnevali	DQ210699	KF660419	DQ210169
***Ornithidium* Salisb. ex R. Br. (four out of ca. 55 species)**				
Ornithidium aureum	Poepp. & Endl.	DQ210817	x	DQ210318
Ornithidium pendens	(Pabst) Senghas	DQ210635	x	DQ210104
Ornithidium pendulum	(Poepp. & Endl.) Cogn.	DQ210892	x	DQ209690
Ornithidium semiscabrum	Lindl.	KP278300	KP898885	KP323339
Rhetinantha Blanco (four out of 15 species)				
Rhetinantha cerifera	(Barb.Rodr.) M.A.Blanco	MZ334607	KP269133	MZ268397
Rhetinantha friedrichsthalii	(Rchb.f.) M.A.Blanco	DQ209923	x	DQ210210
Rhetinantha notylioglossa	(Rchb.f.) M.A.Blanco	DQ210645	KF660351	DQ210114
Rhetinantha scorpioidea	(Kraenzl.) M.A.Blanco	DQ209905	x	DQ210058
Rudolfiella Hoehne (one out of six species)				
Rudolfiella aurantiaca	(Lindl.) Hoehne	MZ334608	x	MZ268398
Sauvetrea Szlach. (one out of 13 species)				
Sauvetrea laevilabris	(Lindl.) M.A.Blanco	DQ210832	KF660433	DQ210334
Scuticaria Lindl. (two out of nine species)				
Scuticaria hadwenii	(Lindl.) Planch.	MZ334609	KF660370	MZ268399
Scuticaria steelei	(Hook.) Lindl.	MZ334610	x	MZ268400
Trigonidium Lindl. (four out of seven species)				
Trigonidium acuminatum	Bateman ex Lindl.	DQ210731	KF660358	DQ210201
Trigonidium egertonianum	Bateman ex Lindl.	DQ210714	KF660357	DQ210184
Trigonidium obtusum	Lindl.	DQ210641	x	DQ210110
Trigonidium riopalenquense	Dodson	DQ210766	x	DQ210252
Xylobium Lindl. (three out of 30 species)				
Xylobium foveatum	(Lindl.) G.Nicholson	MZ334611	x	KX434452
Xylobium squalens	(Lindl.) Lindl.	EF079255	x	EF079427
Xylobium variegatum	(Ruiz & Pav.) Garay & Dunst.	MZ334612	x	MZ268401

Outgroup				
Dichaea panamensis	Lindl.	AY869981	EU123772	EU123586
Dichaea muricata	(Sw.) Lindl.	EU214160	x	AF239319
Eriopsis biloba	Lindl.	DQ210866	KF660441	DQ210374
Koellensteinia graminea	(Lindl.) Schltr. K.	AY870003	KF660429	AY870102
Peristeria elata	Hook.	AF239442	EU490761	AF239346
Stanhopea ecornuta	Lem.	AF239445	KF660362	AF239349
Stanhopea insigins	J.Frost ex Hook.	KM458431	x	KM458412
Vitekorchis excavata	(Lindl.) Romowicz & Szlach.	FJ563949	FJ562813	FJ565398
Zygopetalum maculatum	(Kunth) Garay	AY869998	FJ562864	AY870097
Zygopetalum maxillare	Lodd.	EF079242	EU123799	AY870095

Table S3. Habit and elevation for Maxillariinae and close related species. Species were classified according the habit type (epiphytic, litophytic and/or terrestrial). Following such classification, two variables were determined for the macroevolutive analysis. Elevation data was obtained from Global Multi-resolution Terrain Elevation Data 2010 (GMTED2010), compiled from observation points.

Species	Authority	Habit			Variables		Elevation		
		Epiphytic	Lithophytic	Terrestrial	Number of possible habits	Exclusively epiphytic	Minimum	Maximun	Mean
Anguloa **Ruiz & Pav. (11 species)**									
Anguloa uniflora	Ruiz & Pav.			x	1		110	3841	1290,1
Anguloa virginalis	Linden ex B.S.Williams			x	1		10	5240	1777,8
Bifrenaria **Lindl. (18 species)**									
Bifrenaria aureofulva	Lindl.	x	x		2		0	1757	762,4
Bifrenaria calcarata	Barb.Rodr.	x	x		2		713	1061	788,3
Bifrenaria harrisoniae	(Hook.) Rchb.f.	x	x		2		0	1552	652,9
Bifrenaria inodora	Lindl.	x	x		2		4	850	350,0
Bifrenaria leucorrhoda	Rchb. f.	x			1	x	4	1795	644,4
Bifrenaria longicornis	Lindl.	x			1	x	12	800	127,3
Bifrenaria stefanae	V.P.Castro	x	x		2		411	1592	924,5
Bifrenaria tetragona	(Lindl.) Schltr.	x	x		2		5	986	378,0
Bifrenaria tyrianthina	(Lodd. ex Loudon) Rchb.f.	x	x		2		4	1795	1032,8
Bifrenaria venezuelana	C.Schweinf.	x			1	x	35	2330	514,2
Brasiliorchis **Singer, Koehler & Carnevali (13 species)**									
Brasiliorchis barbosae	(Loefgr.) R.B.Singer, S.Koehler & Carnevali	x			1	x	11	1739	1114,4
Brasiliorchis chrysantha	(Barb.Rodr.) R.B.Singer, S.Koehler & Carnevali	x			1	x	8	1226	488,9
Brasiliorchis consanguinea	(Klotzsch) R.B.Singer, S.Koehler & Carnevali	x	x		2		580	836	729,7
Brasiliorchis gracilis	(Lodd., G.Lodd. & W.Lodd.) R.B.Singer, S.Koehler & Carnevali	x			1	x	3	1658	713,6
Brasiliorchis kautskyi	(Pabst) R.B.Singer, S.Koehler & Carnevali	x			1	x	70	913	641,9
Brasiliorchis marginata	(Lindl.) R.B.Singer, S.Koehler & Carnevali	x			1	x	0	1795	554,3
Brasiliorchis monantha	(Barb.Rodr.) Campacci	x	x		2		0	1623	753,5
Brasiliorchis phoenicanthera	(Barb.Rodr.) R.B.Singer, S.Koehler & Carnevali	x	x		2		5	1291	427,4
Brasiliorchis picta	(Hook.) R.B.Singer, S.Koehler & Carnevali	x	x		2		0	1650	705,3
Brasiliorchis porphyrostele	(Rchb.f.) R.B.Singer, S.Koehler & Carnevali	x			1	x	6	1341	536,7
Brasiliorchis schunkeana	(Campacci & Kautsky) R.B.Singer, S.Koehler & Carnevali	x			1	x	49	816	375,0
Brasiliorchis ubatubana	(Hoehne) R.B.Singer, S.Koehler & Carnevali	x			1	x	4	1795	780,5
Camaridium **Lindl. (ca. 80 species)**									
Camaridium carinatum	(Barb.Rodr.) Hoehne	x			1	x	0	2778	441,9
Camaridium densum	(Lindl.) M.A.Blanco	x			1	x	8	2398	960,6
Camaridium ochroleucum	Lindl.	x	x		2		1	2331	263,1
Camaridium vestitum	(Sw.) Lindl.	x			1	x	0	2224	376,9
Christensonella **Szlach., Mytnik, Górniak & Smiszek (11 species)**									
Christensonella echinophyta	(Barb.Rodr.) Szlach., Mytnik, Górniak & Smiszek	x			1	x	12	920	494,7
Christensonella fernandiana	(Barb.Rodr.) Szlach., Mytnik, Górniak & Smiszek	x			1	x	4	1264	566,2
Christensonella nardoides	(Kraenzl.) Szlach., Mytnik, Górniak & Smiszek	x			1	x	195	1635	647,6
Christensonella neowiedii	(Rchb.f.) S.Koehler	x			1	x	6	1656	742,7
Christensonella pachyphylla	(Schltr. ex Hoehne) Szlach., Mytnik, Górniak & Smiszek	x	x		2		4	1097	543,6
Christensonella paranaensis	(Barb.Rodr.) S.Koehler	x	x		2		8	1623	734,1
Christensonella pumila	(Hook.) Szlach., Mytnik, Górniak & Smiszek	x			1	x	0	1623	481,8

Christensonella subulata	(Lindl.) Szlach., Mytnik, Górniak & Smiszek	x	x		2		0	1795	712,2
Christensonella uncata	(Lindl.) Szlach., Mytnik, Górniak & Smiszek	x			1	x	0	2010	329,9
***Heterotaxis* Lindl. (13 species)**									
Heterotaxis brasiliensis	(Brieger & Illg) F.Barros	x	x		2		0	1795	401,4
Heterotaxis discolor	(Lodd. ex Lindl.) Ojeda & Carnevali	x			1	x	6	3317	593,0
Heterotaxis equitans	(Schltr.) Ojeda & Carnevali	x			1	x	17	1192	432,8
Heterotaxis superflua	(Rchb.f.) F.Barros	x			1	x	7	942	147,8
Heterotaxis valenzuelana	(A.Rich.) Ojeda & Carnevali	x			1	x	0	1867	640,7
Heterotaxis villosa	(Barb.Rodr.) F.Barros	x			1	x	10	4123	303,5
Heterotaxis violaceopunctata	(Rchb.f.) F.Barros	x			1	x	35	1617	366,7
***Lycaste* Lindl. (ca. 30 species)**									
Lycaste aromatica	(Graham) Lindl.	x			1	x	17	2499	1036,2
Lycaste cruenta	(Lindl.) Lindl.	x	x		2		408	2477	1625,0
Lycaste macrobulbon	(Hook.) Lindl.	x			1	x	9	2342	1047,5
Lycaste macrophylla	(Poepp. & Endl.) Lindl.	x			1	x	27	3737	1318,4
Lycaste tricolor	Rchb.f.	x			1	x	15	1725	1097,9
***Mapinguari* Carnevali & Singer (four species)**									
Mapinguari auyantepuiensis	(Foldats) Carnevali & R.B.Singer		x	x	2		24	2925	709,9
Mapinguari desvauxianus	(Rchb.f.) Carnevali & R.B.Singer	x	x		2		0	2378	483,3
***Maxillaria* Ruiz & Pav. (ca. 250 species)**									
Maxillaria bradei	Schltr. ex Hoehne	x			1	x	0	2583	304,6
Maxillaria crocea	Lindl.	x			1	x	6	1232	362,8
Maxillaria grandiflora	(Kunth) Lindl.	x			1	x	180	4199	2431,3
Maxillaria kegelii	Rchb.f.	x			1	x	18	2019	309,1
Maxillaria leucaimata	Barb.Rodr.	x	x		2		0	970	270,4
Maxillaria ochroleuca	Lodd. ex Lindl.	x	x		2		0	1894	415,3
Maxillaria parkeri	Hook.	x			1	x	18	2330	221,2
Maxillaria setigera	Lindl.	x			1	x	24	2744	431,2
***Maxillariella* Blanco & Carnevali (46 species)**									
Maxillariella alba	(Hook.) M.A.Blanco & Carnevali	x			1	x	0	1808	427,3
Maxillariella ponerantha	(Rchb.f.) M.A.Blanco & Carnevali	x			1	x	10	2208	626,1
Maxillariella procurrens	(Lindl.) M.A.Blanco & Carnevali	x			1	x	137	3438	1509,5
Maxillariella robusta	(Barb.Rodr.) M.A.Blanco & Carnevali	x			1	x	0	1226	541,7
Maxillariella tenuifolia	(Lindl.) M.A.Blanco & Carnevali	x			1	x	3	3833	457,8
Maxillariella variabilis	(Bateman ex Lindl.) M.A.Blanco & Carnevali	x	x	x	3		6	2728	1030,2
***Mormolyca* Fenzl (25 species)**									
Mormolyca ringens	(Lindl.) Gentil	x			1	x	6	2539	463,8
Mormolyca rufescens	(Lindl.) M.A.Blanco	x			1	x	0	3825	506,3
***Nitidobulbon* Ojeda, Carnevali & Romero (three species)**									
Nitidobulbon nasutum	(Rchb.f.) Ojeda & Carnevali	x			1	x	49	4331	869,0
***Ornithidium* Salisb. ex R. Br. (ca. 55 species)**									
Ornithidium aureum	Poepp. & Endl.	x		x	2		164	4331	1950,1
Ornithidium pendens	(Pabst) Senghas	x			1	x	0	1808	577,0
Ornithidium pendulum	(Poepp. & Endl.) Cogn.	x			1	x	21	2862	823,7
Ornithidium semiscabrum	Lindl.	x			1	x	584	3390	2109,0
***Rhetinantha* Blanco (15 species)**									

Rhetinantha cerifera	(Barb.Rodr.) M.A.Blanco	x			1	x	0	1808	669,2
Rhetinantha friedrichsthalii	(Rchb.f.) M.A.Blanco	x			1	x	0	2545	471,1
Rhetinantha notylioglossa	(Rchb.f.) M.A.Blanco	x			1	x	0	4529	826,5
Rhetinantha scorpioidea	(Kraenzl.) M.A.Blanco	x			1	x	35	2872	892,1
Rudolfiella **Hoehne (six species)**									
Rudolfiella aurantiaca	(Lindl.) Hoehne	x			1	x	14	925	138,3
Sauvetrea **Szlach. (13 species)**									
Sauvetrea laevilabris	(Lindl.) M.A.Blanco	x			1	x	12	3070	1035,3
Scuticaria **Lindl. (nine species)**									
Scuticaria hadwenii	(Lindl.) Planch.	x			1	x	4	1516	820,0
Scuticaria steelei	(Hook.) Lindl.	x			1	x	18	153	45,4
Trigonidium **Lindl. (seven species)**									
Trigonidium acuminatum	Bateman ex Lindl.	x			1	x	3	4008	300,8
Trigonidium egertonianum	Bateman ex Lindl.	x			1	x	0	2197	306,8
Trigonidium obtusum	Lindl.	x			1	x	0	1068	174,4
Trigonidium riopalenquense	Dodson	x			1	x	16	1925	341,3
Xylobium **Lindl. (30 species)**									
Xylobium foveatum	(Lindl.) G.Nicholson	x			1	x	0	2811	558,1
Xylobium squalens	(Lindl.) Lindl.	x			1	x	0	1873	478,6
Xylobium variegatum	(Ruiz & Pav.) Garay & Dunst.	x			1	x	0	1808	388,6
Close related species									
Dichaea panamensis	Lindl.	x			1	x	8	4123	1411,0
Dichaea muricata	(Sw.) Lindl.	x			1	x	0	1636	298,0
Eriopsis biloba	Lindl.		x	x	2		0	1813	813,9
Koellensteinia graminea	(Lindl.) Schltr. K.	x			1	x	0	4055	214,3
Peristeria elata	Hook.			x	1		4	1599	521,6
Stanhopea ecornuta	Lem.	x			1	x	29	1725	635,0
Stanhopea insigins	J.Frost ex Hook.	x			1	x	4	759	137,7
Vitekorchis excavata	(Lindl.) Romowicz & Szlach.			x	1		1512	2967	2317,0
Zygopetalum maculatum	(Kunth) Garay	x			1	x	0	2666	863,3

Table S4. MaxEnt's Species Distribution Models (SDM) results for Maxillariinae species. Number of training and testing occurrence points and parameters for the overall model evaluation for each species are presented: Regularized training gain, training AUC, Regularized test gain, test AUC.

Species	Authority	Presence sample	Training sample	Regularized training gain	Training AUC	Test sample	Test gain	Test AUC	TSS
Anguloa **Ruiz & Pav. (11 species)**									
Anguloa uniflora	Ruiz & Pav.	19.00	16.00	2.72	0.99	3.00	03,04	0.96	0.63
Anguloa virginalis	Linden ex B.S.Williams	12.00	10.00	1.88	0.99	2.00	3.39	0.99	0.74
Bifrenaria **Lindl. (18 species)**									
Bifrenaria aureofulva	Lindl.	85.00	68.00	3.63	0.99	17.00	3.60	0.99	0.92
Bifrenaria calcarata	Barb.Rodr.	8.00	7.00	2.96	0.99	1.00	5.40	1.00	0.75
Bifrenaria harrisoniae	(Hook.) Rchb.f.	77.00	62.00	3.49	0.99	15.00	3.82	0.99	0.95
Bifrenaria inodora	Lindl.	21.00	17.00	3.89	0.99	4.00	5,11	1.00	0.90
Bifrenaria leucorrhoda	Rchb. f.	9.00	8.00	2.98	1.00	1.00	5.21	1.00	0.78
Bifrenaria longicornis	Lindl.	39.00	32.00	2.33	0.98	7.00	1.53	0.94	0.84
Bifrenaria stefanae	V.P.Castro	23.00	19.00	3.80	1.00	4.00	03,09	0.99	0.90
Bifrenaria tetragona	(Lindl.) Schltr.	18.00	15.00	3.80	1.00	3.00	3.67	0.99	0.93
Bifrenaria tyrianthina	(Lodd. ex Loudon) Rchb.f. (2n=38)	34.00	28.00	3.33	0.99	6.00	4.48	1.00	0.90
Bifrenaria venezuelana	C.Schweinf.	11.00	9.00	2.21	0.99	2.00	-0.30	0.79	0.67
Brasiliorchis **Singer, Koehler & Carnevali (13 species)**									
Brasiliorchis barbosae	(Loefgr.) R.B.Singer, S.Koehler & Carnevali	10.00	8.00	3.57	1.00	2.00	5,10	1.00	0.90
Brasiliorchis chrysantha	(Barb.Rodr.) R.B.Singer, S.Koehler & Carnevali	43.00	35.00	2.53	0.98	8.00	2.67	0.98	0.93
Brasiliorchis consanguinea	(Klotzsch) R.B.Singer, S.Koehler & Carnevali	6.00	5.00	2.33	0.99	1.00	0.67	0.95	0.79
Brasiliorchis gracilis	(Lodd., G.Lodd. & W.Lodd.) R.B.Singer, S.Koehler & Carnevali	37.00	30.00	3.54	0.99	7.00	4,10	0.99	0.97
Brasiliorchis kautskyi	(Pabst) R.B.Singer, S.Koehler & Carnevali	7.00	6.00	5.18	1.00	1.00	-2.04	0.90	0.57
Brasiliorchis marginata	(Lindl.) R.B.Singer, S.Koehler & Carnevali	71.00	57.00	3.00	0.99	14.00	2.99	0.98	0.93
Brasiliorchis monantha	(Barb.Rodr.) Campacci	13.00	11.00	2.61	0.99	2.00	2.96	0.99	0.76
Brasiliorchis phoenicanthera	(Barb.Rodr.) R.B.Singer, S.Koehler & Carnevali	20.00	16.00	2.55	0.98	4.00	2.93	0.98	0.88
Brasiliorchis picta	(Hook.) R.B.Singer, S.Koehler & Carnevali	138.00	111.00	3.30	0.99	27.00	3.40	0.99	0.93
Brasiliorchis porphyrostele	(Rchb.f.) R.B.Singer, S.Koehler & Carnevali	42.00	34.00	3.29	0.99	8.00	3.32	0.98	0.86
Brasiliorchis schunkeana	(Campacci & Kautsky) R.B.Singer, S.Koehler & Carnevali	5.00	4.00	4.45	1.00	1.00	5.43	1.00	0.60
Brasiliorchis ubatubana	(Hoehne) R.B.Singer, S.Koehler & Carnevali	33.00	27.00	3.76	1.00	6.00	3.34	0.98	0.81
Camaridium **Lindl. (ca. 80 species)**									
Camaridium carinatum	(Barb.Rodr.) Hoehne	37.00	30.00	3,10	0.99	7.00	1.69	0.95	0.88
Camaridium densum	(Lindl.) M.A.Blanco	246.00	197.00	3.35	0.99	49.00	3.60	0.97	0.96
Camaridium ochroleucum	Lindl.	140.00	112.00	1.18	0.91	28.00	0.86	0.83	0.61
Camaridium vestitum	(Sw.) Lindl.	156.00	125.00	1.79	0.94	31.00	1.43	0.90	0.74
Christensonella **Szlach., Mytnik, Górniak & Smiszek (11 species)**									
Christensonella echinophyta	(Barb.Rodr.) Szlach., Mytnik, Górniak & Smiszek	8.00	7.00	2.19	0.98	1.00	-0.47	0.78	0.50
Christensonella fernandiana	(Barb.Rodr.) Szlach., Mytnik, Górniak & Smiszek	37.00	30.00	3.16	0.99	7.00	3.39	0.98	0.87
Christensonella nardoides	(Kraenzl.) Szlach., Mytnik, Górniak & Smiszek	8.00	7.00	1.73	0.99	1.00	3.34	1.00	0.25
Christensonella neowiedii	(Rchb.f.) S.Koehler	74.00	60.00	3.19	0.99	14.00	2.92	0.98	0.95
Christensonella pachyphylla	(Schltr. ex Hoehne) Szlach., Mytnik, Górniak & Smiszek	12.00	10.00	3.36	1.00	2.00	0.74	0.84	0.74
Christensonella paranaensis	(Barb.Rodr.) S.Koehler	67.00	54.00	3.15	0.99	13.00	3.16	0.98	0.89
Christensonella pumila	(Hook.) Szlach., Mytnik, Górniak & Smiszek	30.00	24.00	2.89	0.99	6.00	2.20	0.87	0.90

Christensonella subulata	(Lindl.) Szlach., Mytnik, Górniak & Smiszek (2n=38)	111.00	89.00	3.22	0.99	22.00	3.52	0.99	0.90
Christensonella subulata	(Lindl.) Szlach., Mytnik, Górniak & Smiszek (2n=76)	10.00	8.00	2.13	0.99	2.00	04,01	1.00	0.69
Christensonella uncata	(Lindl.) Szlach., Mytnik, Górniak & Smiszek	332.00	266.00	02,02	0.95	66.00	2.30	0.96	0.78
***Heterotaxis* Lindl. (13 species)**									
Heterotaxis brasiliensis	(Brieger & Illg) F.Barros	82.00	66.00	3.64	0.99	16.00	3.81	0.99	0.95
Heterotaxis discolor	(Lodd. ex Lindl.) Ojeda & Carnevali	59.00	48.00	1.88	0.97	11.00	1.83	0.94	0.79
Heterotaxis equitans	(Schltr.) Ojeda & Carnevali	27.00	22.00	2.38	0.98	5.00	2.63	0.96	0.62
Heterotaxis superflua	(Rchb.f.) F.Barros	42.00	34.00	1.96	0.97	8.00	02,05	0.94	0.72
Heterotaxis valenzuelana	(A.Rich.) Ojeda & Carnevali	47.00	38.00	2.36	0.98	9.00	2.46	0.97	0.85
Heterotaxis villosa	(Barb.Rodr.) F.Barros	65.00	52.00	1.98	0.97	13.00	2.20	0.96	0.76
Heterotaxis violaceopunctata	(Rchb.f.) F.Barros	22.00	18.00	1.95	0.97	4.00	0.34	0.82	0.74
***Lycaste* Lindl. (ca. 30 species)**									
Lycaste aromatica	(Graham) Lindl.	110.00	88.00	3.52	0.99	22.00	3.26	0.96	0.93
Lycaste cruenta	(Lindl.) Lindl.	44.00	36.00	4.78	1.00	8.00	5,10	1.00	0.91
Lycaste macrobulbon	(Hook.) Lindl.	5.00	4.00	0.68	0.99	1.00	-0.75	0.4025*	0.39
Lycaste macrophylla	(Poepp. & Endl.) Lindl.	27.00	22.00	3.22	0.99	5.00	2.33	0.97	0.93
Lycaste tricolor	Rchb.f.	14.00	12.00	3.63	1.00	2.00	4.66	1.00	0.78
***Mapinguari* Carnevali & Singer (four species)**									
Mapinguari auyantepuiensis	(Foldats) Carnevali & R.B.Singer	13.00	11.00	1.89	0.99	2.00	01,04	0.85	0.75
Mapinguari desvauxianus	(Rchb.f.) Carnevali & R.B.Singer	31.00	25.00	2.41	0.98	6.00	0.62	0.81	0.78
***Maxillaria* Ruiz & Pav. (ca. 250 species)**									
Maxillaria bradei	Schltr. ex Hoehne	34.00	28.00	3,11	0.99	6.00	3.85	0.98	0.92
Maxillaria crocea	Lindl.	33.00	27.00	3.89	1.00	6.00	05,08	1.00	0.96
Maxillaria grandiflora	(Kunth) Lindl.	36.00	29.00	4.27	1.00	7.00	3,10	0.98	0.90
Maxillaria kegelii	Rchb.f.	30.00	24.00	1.85	0.97	6.00	0.89	0.90	0.80
Maxillaria leucaimata	Barb.Rodr.	36.00	29.00	3.78	0.99	7.00	04,02	0.98	0.90
Maxillaria ochroleuca	Lodd. ex Lindl.	68.00	55.00	2.56	0.98	13.00	3.32	0.99	0.83
Maxillaria parkeri	Hook.	45.00	36.00	02,07	0.97	9.00	2,12	0.95	0.86
Maxillaria setigera	Lindl.	16.00	13.00	1.80	0.98	3.00	2.68	0.91	0.81
***Maxillariella* Blanco & Carnevali (46 species)**									
Maxillariella alba	(Hook.) M.A.Blanco & Carnevali	73.00	59.00	1.93	0.95	14.00	1.31	0.87	0.72
Maxillariella ponerantha	(Rchb.f.) M.A.Blanco & Carnevali	19.00	16.00	2.50	0.99	3.00	2.94	0.99	0.88
Maxillariella procurrens	(Lindl.) M.A.Blanco & Carnevali	22.00	18.00	2.96	0.99	4.00	02,04	0.92	0.80
Maxillariella robusta	(Barb.Rodr.) M.A.Blanco & Carnevali	25.00	20.00	3.94	1.00	5.00	4.50	1.00	0.98
Maxillariella tenuifolia	(Lindl.) M.A.Blanco & Carnevali	137.00	110.00	3.13	0.99	27.00	3.18	0.98	0.92
Maxillariella variabilis	(Bateman ex Lindl.) M.A.Blanco & Carnevali	461.00	369.00	2.82	0.98	92.00	2.96	0.98	0.95
***Mormolyca* Fenzl (25 species)**									
Mormolyca ringens	(Lindl.) Gentil	62.00	50.00	3.44	0.99	12.00	3.75	0.98	0.94
Mormolyca rufescens	(Lindl.) M.A.Blanco	94.00	76.00	1.89	0.96	18.00	2.24	0.95	0.75
***Nitidobulbon* Ojeda, Carnevali & Romero (three species)**									
Nitidobulbon nasutum	(Rchb.f.) Ojeda & Carnevali	64.00	52.00	2.32	0.97	12.00	2.92	0.95	0.83
***Ornithidium* Salisb. ex R. Br. (ca. 55 species)**									
Ornithidium aureum	Poepp. & Endl.	166.00	133.00	03,08	0.99	33.00	3.29	0.99	0.93
Ornithidium pendens	(Pabst) Senghas	54.00	44.00	2.61	0.99	10.00	1,12	0.87	0.85
Ornithidium pendulum	(Poepp. & Endl.) Cogn.	27.00	22.00	3.27	0.99	5.00	3.20	0.97	0.91
Ornithidium semiscabrum	Lindl.	9.00	8.00	03,04	1.00	1.00	3.54	1.00	0.88

***Rhetinantha* Blanco (15 species)**									
Rhetinantha cerifera	(Barb.Rodr.) M.A.Blanco	47.00	38.00	03,08	0.99	9.00	3.97	0.99	0.94
Rhetinantha friedrichsthalii	(Rchb.f.) M.A.Blanco	106.00	85.00	2.89	0.99	21.00	2.40	0.96	0.89
Rhetinantha notylioglossa	(Rchb.f.) M.A.Blanco	102.00	82.00	2.36	0.98	20.00	2.98	0.98	0.87
Rhetinantha scorpioidea	(Kraenzl.) M.A.Blanco	43.00	35.00	2.59	0.97	8.00	3.19	0.95	0.77
***Rudolfiella* Hoehne (six species)**									
Rudolfiella aurantiaca	(Lindl.) Hoehne	24.00	20.00	1.91	0.98	4.00	0.13	0.83	0.81
***Sauvetrea* Szlach. (13 species)**									
Sauvetrea laevilabris	(Lindl.) M.A.Blanco	11.00	9.00	1.28	0.99	2.00	0.31	0.87	0.87
***Scuticaria* Lindl. (nine species)**									
Scuticaria hadwenii	(Lindl.) Planch.	21.00	17.00	3.63	1.00	4.00	4.24	0.99	0.67
Scuticaria steelei	(Hook.) Lindl.	9.00	8.00	03,06	0.99	1.00	4.17	1.00	0.99
***Trigonidium* Lindl. (seven species)**									
Trigonidium acuminatum	Bateman ex Lindl.	79.00	64.00	0.87	0.88	15.00	1.46	0.89	0.57
Trigonidium egertonianum	Bateman ex Lindl.	249.00	200.00	2.80	0.99	49.00	2.82	0.96	0.93
Trigonidium obtusum	Lindl.	27.00	22.00	2.83	0.99	5.00	2.19	0.91	0.77
Trigonidium riopalenquense	Dodson	17.00	14.00	4,10	1.00	3.00	2.36	0.99	0.99
***Xylobium* Lindl. (30 species)**									
Xylobium foveatum	(Lindl.) G.Nicholson	66.00	53.00	2.25	0.98	13.00	1.99	0.90	0.78
Xylobium squalens	(Lindl.) Lindl.	21.00	17.00	2.70	0.99	4.00	2.76	0.98	0.88
Xylobium variegatum	(Ruiz & Pav.) Garay & Dunst.	80.00	64.00	2.64	0.98	16.00	1.96	0.89	0.77
Close related species									
Dichaea panamensis	Lindl.	121.00	97.00	2.89	0.98	24.00	2.75	0.98	0.89
Dichaea muricata	(Sw.) Lindl.	195.00	156.00	2.92	0.98	39.00	3.24	0.99	0.92
Eriopsis biloba	Lindl.	29.00	24.00	2.39	0.99	5.00	2.63	0.97	0.90
Koellensteinia graminea	(Lindl.) Schltr. K.	73.00	59.00	2.23	0.97	14.00	1.81	0.93	0.81
Peristeria elata	Hook.	19.00	16.00	3.97	1.00	3.00	5.18	1.00	0.99
Stanhopea ecornuta	Lem.	26.00	21.00	3.53	1.00	5.00	4.14	1.00	0.99
Stanhopea insigins	J.Frost ex Hook.	12.00	10.00	2.38	1.00	2.00	4.67	1.00	0.42
Vitekorchis excavata	(Lindl.) Romowicz & Szlach.	10.00	8.00	03,05	1.00	2.00	-1.24	0.74	0.50
Zygopetalum maculatum	(Kunth) Garay	188.00	151.00	2.99	0.99	37.00	2.82	0.98	0.88
Zygopetalum maculatum	(Kunth) Garay (2n=48)	8.00	7.00	2.23	1.00	1.00	4.38	1.00	0.86
Zygopetalum maculatum	(Kunth) Garay (2n=72)	6.00	5.00	1.93	1.00	1.00	3.31	0.99	0.66
Zygopetalum maculatum	(Kunth) Garay (2n=96)	14.00	12.00	3.56	1.00	2.00	3.42	0.99	0.78
Zygopetalum maxillare	Lodd.	88.00	71.00	2.99	0.99	17.00	2.96	0.98	0.93

* Low performance model according to Test AUC

Table S5. Evolution model test. For each trait, with and without polyploids, three evolutive models were tested, Brownian Motion (BM), Ornstein–Uhlenbeck (OU), and Early Burst (EB). The AICc value for each model is presented and the lowest value for each trait is presented in bold. Model parameters: "Pagel's Lambda"(for BM), "alpha" rate of adaptation (for OU), "a" rate change (for EB), rate of evolution (sigsq) and root state (z0)

Trait	Model	AICc	Model parameters		
			Parameters	Rate of evolution (sigsq)	Root state (z0)
Chromosome number (without polyploids)	BM	**328.8184**	lambda = 0.905051	sigsq = 156.229349	z0 = 40.877737
	OU	765.3284	alpha = 2.718282	sigsq = 3.36E+10	z0 = 40.973178
	EB	768.1426	a = -0.000003	sigsq = 3.35E+10	z0 = 40.887036
Chromosome number (with polyploids)	BM	**693.3014**	lambda = 0.218484	sigsq = 2.75E+09	z0 = 42.805172
	OU	2570.7096	alpha = 2.718149	sigsq = 6.01E+18	z0 = 41.435888
	EB	2534.3337	a = -0.000032	sigsq = 6.05E+18	z0 = 40.936697
Genome size (without polyploids)	BM	**137.479**	lambda = 0.999647	sigsq = 56.868331	z0 = 4.257916
	OU	139.346	alpha = 0.000001	sigsq = 60.812684	z0 = 4.257914
	EB	139.346	a = -0.000001	sigsq = 60.816166	z0 = 4.257914
Genome size (with polyploids)	BM	**245.541**	lambda = 0.592295	sigsq = 110.037679	z0 = 4.572707
	OU	1507.420	alpha = 2.718046	sigsq = 1.74E+17	z0 = 4.324063
	EB	1506.345	a = -0.000211	sigsq = 1.77E+17	z0 = 4.278202

Table S6. Regressions summary between the genomic traits with and without polyploids in the sample.

Branch length transformations

without polyploids		**with polyploids**	
kappa [Fix] :	1.0	kappa [Fix] :	1.0
lambda [ML] :	1.0	lambda [ML] :	0.0
Lower bound:	0.000, p = < 2.22e-16	Lower bound:	0.000, p = 1
Upper bound:	1.000, p = 1	Upper bound:	1.000, p = < 2.22e-16
95.0% CI:	0.996, NA	95.0% CI:	NA, 0.577
delta [Fix]:	1.0	delta [Fix]:	1.0

Coefficients

	Estimate Std.	**Error**	**t value**	**Pr(>|t|)**	
(Intercept)	3.867.695	91.258	423.822	<2e-16	***
Genome Size	42.255	16.629	2.5411	0.0138	*

Signif. codes:	0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1				
Residual standard error:	9.513 on 57 degrees of freedom				
Multiple R-squared:	0.1018				
Adjusted R-squared	0.086	p-value:	0.0138		
F-statistic:	6.457 on 1 and 57 DF				

	Estimate Std.	**Error**	**t value**	**Pr(>|t|)**	
(Intercept)	2.545.840	157.869	16.126	<2e-16	***
Genome Size	424.089	36.748	11.540	<2e-16	***

Signif. codes:	0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1				
Residual standard error:	33.26 on 60 degrees of freedom				
Multiple R-squared:	0.6894				
Adjusted R-squared	0.6842	p-value	<2e-16		
F-statistic:	133.2 on 1 and 60 DF				

GENERAL CONCLUSIONS

We used an integrative approach combining systematics, phylogenetics, cytogenetics and ecology to understand the putative associations of chromosome number and genome size changes with the responses to environmental factors that could determine species occurrence in Maxillariinae orchids. That was achieved through macroevolution analysis with a phylogenetic background, and allowed interesting conclusions not only on the drivers of species occurrence, but also on the evolution of the subtribe.

A Bayesian Inference for Maxillariinae species with basis in DNA sequences from nuclear and plastid markers provided a mostly well resolved phylogenetic hypothesis, revealing relationships among genera within the subtribe and supporting the splitting of the core Maxillariinae into smaller clades, ranked as genera. These gerena are highly supported by molecular data both in our results and in literature, but there is an ongoing discussion on whether to keep then divided or to lump then into a single megagenus *Maxillaria*, on the grounds of avoiding nomenclatural disruption, serving taxonomy learning and teaching, and mainly, of the lack of unambiguous morphological traits to diagnose some of these genera. Despite disagreement among taxonomists about the Maxillariinae genera classification, it is a consensus that the subtribe needs more molecular and morphologic studies to support genera circumscriptions, and our results, by revealing non monophyletic taxa and presenting minor divergences with the literature, point towards the main clades to be further investigated to achieve better resolution in the relationships within both the basal and the core Maxillariinae.

Besides contributing to the Maxillariinae taxonomy quarrel, our work has added DNA sequences for underrepresented genera of Maxillariinae, which will serve any researches aiming to better understand systematics and ecological components of the neotropical flora, and also our own ongoing projects. In fact, starting from these sequences, added to NCBI data, we could draw a second phylogeny, in which species sampling matched the available data on genomic traits of Maxillariinae species, to be used as a phylogenetic background for macroevolution analizes.

Regarding Maxillariinae macroevolution, our results provided interesting additions to the comprehension of changes in chromosome number and genome size in association with habit preferences and responses to environment. Species Distribution Models performed with MaxEnt maximum entropy algorithm revealed that the probability of environmental suitability for occurrence of Maxillariinae species increases in highly isothermic sites, with low temperature seasonality along the year and little daily temperature variations. Probability of suitability for occurrence is also increased by increasing precipitation and by an optimum mean temperature of 15-20°C in the coldest trimester. Such responses are indicative that species occurrence is probably favoured in stable, humid sites, which is in accordance with the fact that they are mostly epiphytic.

Our data suggest that chromosome number and genome size changes might be associated with these environmental preferences, given the correlations found in the phylogenetic regressions. For instance, chromosome number increased by polyploidy was correlated to the desertion of epiphytism as an obligatory habit and to dryer environments, while disregarding polyploidy, the same conditions were associated with decreasing genome sizes. That is due to the fact that once a genome gets too large, the nucleotypic effect makes unrealizable to organisms to be epiphytic, so polyploidy, which inheritably causes the genome to increase, hinders epiphytic habits in orchids, and as geophyte species can more easily access soil moisture, they can better thrive under dryer conditions. At the same time, polyploidy potentially confers species putative benefits allowing them to better upbeat disadvantageous conditions, but without the benefits of the heterogeneity provided by genome doubling, an increased genome may imply only greater coats to the development and maintenance of the organism. Hence, disregarding polyploidy the decrease, and not the increase of genome size would allow species to live in harsher scenarios.

Additionally, chromosome number and genome size relationships with environmental and ecological variables in Maxillariinae species were shown to present phylogenetic signals, hence the genomic traits variations would depend mostly of phylogeny, under Brownian Motion (i.e. relying on stochastic factors), but regression models fitting these traits against the environmental variables have outperformed models fitted against the phylogeny,

suggesting that these niche correlations, despite representing a small portion of the variation in traits, might offer a better explanation than solely the phylogeny.

In conclusion, by correlating with habit preferences and environmental variables, particularly with stressful conditions, chromosomal changes were demonstrated to be a central factor determining the occurrence of Maxillariinae.

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Anexo I – Declaração referente à Bioética e Biossegurança

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DECLARAÇÃO

Em observância ao **§5º do Artigo 1º da Informação CCPG-UNICAMP/001/15**, referente a Bioética e Biossegurança, declaro que o conteúdo de minha Tese de Doutorado, intitulada "***Macroevolutionary studies in Maxillariinae (Orchidaceae)***", desenvolvida no Programa de Pós-Graduação em Biologia Vegetal do Instituto de Biologia da Unicamp, não versa sobre pesquisa envolvendo seres humanos, animais ou temas afetos a Biossegurança.

Assinatura: _____
Nome do(a) aluno(a): Thaíssa Brogliato Junqueira Engel

Assinatura: _____
Nome do(a) orientador(a): Eliana Regina Forni Martins

Data: 25/06/2021

Anexo II – Declaração referente aos direitos autorais

Declaração

As cópias de artigos de minha autoria ou de minha co-autoria, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, que constam da minha Dissertação/Tese de Mestrado/Doutorado, intitulada **Macroevolutionary studies in Maxillariinae (Orchidaceae)**, não infringem os dispositivos da Lei n.° 9.610/98, nem o direito autoral de qualquer editora.

Campinas, 25 de junho de 2021

Assinatura : _____
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