On the origin of the New World Pyrgomorphidae (Insecta: Orthoptera)

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ABSTRACT

The gaudy grasshopper family Pyrgomorphidae (Orthoptera: Caelifera) shows a peculiar geographical distribution. Of the 487 described species, less than 10% of the diversity is found in the New World, while the rest occur throughout Africa, Asia, and Australia. Only 41 species belonging to four tribes are found in Central and South America and the Caribbean. The biogeographic analysis using BioGeoBEARS showed that after diversifying in the Old World, the New World Pyrgomorphidae diverged (Algete + Jaragua) diverged 96 mya (Late Cretaceous, Cenomanian) and that their current distribution in the New World is explained by two possible events, a transatlantic colonization from Africa to Northern South America or a vicariance event between these two landmasses, followed by a subsequent dispersal to the Caribbean. The second wave of colonization occurred about 69 mya towards the end of the Late Cretaceous (Maastrichtian) with dispersal from Africa to South America and then to North America with a subsequent diversification in Mexico including Baja California.

1. Introduction

Pyrgomorphidae (Orthoptera: Caelifera) are one of the most charismatic grasshopper families, well known for their vibrant body color and conspicuous sculpting patterns on pronotum, often featured in display collections of large and showy insects (Mariño-Pérez and Song, 2018). The family currently includes 487 valid species, most of which occur in the Old World, with a great majority (384 species) distributed in Africa and Asia. While some of the most colorful members of the family are familiar to the general public and well studied, the majority of pyrgomorphs are actually cryptic and less known. Among these insect tribes of Sphenariini are disjunctly distributed in Madagascar, East Africa, and China, respectively. Because most pyrgomorphs are found in the Old World, the presence of these insects in the New World has drawn attention of several taxonomists. There have been three main biogeographical hypotheses proposed to explain the origin and diversification of the NWP. The first one was by Kevan and Akbar (1964) who hypothesized that the ancestral pyrgomorphs could have colonized...
are agriculturally important pests of crops such as corn and beans (Núñez-Farfán, 1999). In some areas in Mexico, males can spend up to 22 days (half of its adult life) after copulation (Santos, 2005; Sanabria-Urbán et al., 2015, 2017), and phylogeographic variation in terms of their mating biology (Cueva del Castillo, 2003; Cueva del Castillo and Núñez-Farfán, 1999), size and color (Alves dos Reis, 2018). Ecologically, the NWP occupy diverse habitats from sea level up to 2700 masl. They can be found in deciduous or semideciduous tropical forest, rainforest, cloud forest, pine forest, grasslands, xeric scrub and thorny scrub. They prefer open spaces with sunlight, usually found on the ground or perching on grasses, shrubs, trees and cacti (Table 1). Of the NWP, the genus Sphenarium has been studied in depth in terms of their mating biology (Cueva del Castillo, 2003; Cueva del Castillo and Núñez-Farfán, 1999), variation in size and color (Alves dos Santos, 2005; Sanabria-Urbán et al., 2015, 2017), and phylogeographic patterns (Sanabria-Urbán et al., 2015, 2017). For example, Sphenarium species show some of the longest mate guarding behavior in which males can spend up to 22 days (half of its adult life) after copulation mounted on the females (Cueva del Castillo, 2003; Cueva del Castillo and Núñez-Farfán, 1999). In some areas in Mexico, Sphenarium species are agriculturally important pests of crops such as corn and beans (COPR, 1982), while in Oaxaca they have been used as food (known as chapulines) for centuries (Cerritos and Cano-Santana, 2008).

In this study, we have investigated the biogeography of the NWP based on a molecular phylogeny generated using complete mitochondrial genomes and four nuclear genes. We have included representatives of all four lineages known from the New World, as well as a number of the Old World representatives of the family. We specifically test the three biogeographical hypotheses regarding the origin of the NWP, and infer a biogeographical scenario based on a divergent time estimate and a biogeographical analysis. We show that the current distribution of the NWP is a result of dynamic vicariance and dispersal events and propose a novel biogeographical hypothesis regarding the origin and diversification of the NWP.

2. Material and methods

2.1. Taxon and character sampling

We sampled a total of 32 taxa, including 7 outgroup taxa representing 7 families of Acridomorpha (Acrididae, Lentulidae, Pyrgacrididae, Pamphagidae, Pneumoridae, Trigonopterygidae, and Tanaoceridae) and 25 ingroup taxa representing the Pyrgomorphidae. Particularly, we included representatives of all four tribes present in the New World: Sphenariini, Ichthiacridini, Ichthyotettigini, and Omurini. For all terminals, we included partial or complete mitochondrial genome (mtgenome) data, 21 of which were newly sequenced for this study. The remaining mtgenomes were previously generated by us (Song et al., 2015) or obtained from GenBank (Table 2). We used Tanaocerus koebelii (Tanaoceridae) as a root, which has been consistently shown to be the earliest diverging lineage within Acridomorpha. For nuclear genes, we generated complete 18S and 28S ribosomal RNA genes and Histone 3 (H3) gene for all except Mekongiella kingdoni, Mekongiana xiangchengensis, and Yunanites coreaicae (due to lack of specimen). For Atractomorpha sinensis, we did not include H3 gene because it was not available, but obtained 18S and 28S from GenBank. For the 21 newly generated taxa we were also able to generate Histone H2B gene. DNA-grade tissue samples used for this study were collected by us. They were preserved in 100% ethanol and vouchered at −80 °C freezer in the Insect Genomic Collection at Texas A&M University Insect Collection (TAMU-IGC). To generate mtgenome sequences for the 21 newly generated taxa, we performed shotgun sequencing of genomic DNA using the Illumina platform. To extract high molecular weight DNA required for Illumina sequencing, we used Gentra Puregene Tissue Kit (Qiagen) following the manufacturer’s guideline. The quality and concentration of DNA extracts were initially measured using either Qubit Fluorometer (Thermo Fisher) or DeNovix Spectrophotometer, and more thoroughly analyzed using Fragment Analyzer. We used Nextera XT DNA Library Prep Kit for library preparation and performed either 150 bp paired-end (PE) sequencing using NextSeq500 or 125 bp PE sequencing using HiSeq2500. Library preparation and next generation sequencing (NGS) were conducted at either Georgia Genomic Facility (NextSeq500) or Texas A&M Genomics and Bioinformatics Service (HiSeq2500). The resulting raw reads were quality-trimmed in CLC Genomics Workbench 8 (Qiagen). We used the MITObim pipeline to assemble mtgenomes de novo from the NGS reads (Hahn et al., 2013). All newly assembled mtgenomes were first uploaded as raw fasta files to MITOS (Bernt et al., 2013) to identify open reading frames (ORFs) and tRNAs. The initial MITOS annotation was used as a guideline to delimit gene boundaries and start and stop codons of each protein-coding gene was manually identified in Geneious 10.0.9 (Biomatters), following the recommendation by Cameron (2014). We also extracted 18S, 28S, H3, and H2B genes from the shotgun sequence data by using ‘Map to Reference’ tool in Geneious. Using the pyrgomorph Atractomorpha sp. 18S and 28S sequences (KMB53228 and KMB53462), a pyrgomorph Sphenarium totonacum H3 sequence (KU147119) and a spider H2B sequence (XM_016058247) downloaded from GenBank as references, we used the Geneious mapper with low sensitivity to search for short reads that mapped to the reference sequences. This approach was very effective in extracting these four genes from the 21 newly generated taxa. The rest of nuclear genes used were obtained from a previous publication by Song et al. (2015) (Table 2). DNA sequence data generated for this study have been deposited to GenBank (Table 2).

2.2. Phylogenetic analyses

For mitochondrial and nuclear protein-coding genes, we aligned based on the conservation of reading frames by first translating into
amino acids and aligning individually in MUSCLE (Edgar, 2004) using default parameters in Geneious. tRNAs were individually aligned in MUSCLE using default parameters, also in Geneious. 12S, 16S, 18S and 28S were aligned in MAFFT using the E-INS-i setting, also in Geneious. All these individual alignments were concatenated into a single matrix using SequenceMatrix (Vaidya et al., 2011). We divided the data into a total of 71 data blocks (13 mitochondrial and 2 nuclear protein-coding genes divided into individual codon positions, 22 tRNAs, 2 mitochondrial rRNAs, and 2 nuclear rRNAs). We then used PartitionFinder 2 (Lanfear et al., 2012) using the “greedy” algorithm (heuristic search) with branch lengths estimated as “unlinked” to search for the best-fit scheme as well as to estimate the model of nucleotide evolution for each partition.

We performed a maximum likelihood (ML) analysis and a Bayesian analysis on the total evidence dataset (21,853 aligned bp and 32 taxa). Because we included partial mtgenomes, our matrix included missing
Five taxa had between 14,000 and 17,000 bp (data for some taxa). Of the 32 taxa, 22 had more than 18,000 aligned bp.

### Table 2

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Data for some taxa. Of the 32 taxa, 22 had more than 18,000 aligned bp. Five taxa had between 14,000 and 17,000 bp (Caprothinus sp., Colemania sphenarioides, Ichthyotettiginae mexicanus, Mekongiana xiangchengensis and Mekongiana kingdoni) and two taxa had around 11,000 bp (Desmoterra sp. and Ichthyotettiginae rehni). Only three had a significant amount of missing data (Jaragua oviedenis 9726 bp; Pyrgoteitsis pueblensis 6579 bp and Yunnanites coriacea 5113 bp). For the ML analysis, we used the best-fit partitioning scheme (17 partitions) recommended by PartitionFinder with the GTRCAT model applied to each partition and analyzed using RAxML 7.2.8 (Stamatakis et al., 2008) on CIPRES (Extreme Science and Engineering Discovery Environment, https://www.cipres.org) through CIPRES Science Gateway (Miller et al., 2011). Nodal support was evaluated using 1000 replications of rapid bootstrapping implemented in RAxML. For the Bayesian analysis, we used default priors and applied the GTRCAT model applied to each partition and analyzed using MrBayes 3.2.6 (Ronquist et al., 2012). We plotted the likelihood trace for each run to assess convergence in Tracer (Rambaut and Drummond, 2003–2009), and discarded an average of 25% of each run as burn-in. This analysis was also run on XSEDE through the CIPRES Science Gateway. For both ML and Bayesian analyses, the resulting trees were visualized in Gen- eious.

### 2.3. Divergence time estimation

In order to estimate timing and rates of divergence in the Pygromorphidae, we performed a divergence time estimate analysis using BEAST v.1.8 (Drummond et al., 2012). There is only one fossil pygromorph known. Kevan (1965) reviewed a fossil from Miocene (originally described as an acridid subfamily Oedipodinae), and placed it as Pygromorphidae (Miopyrgomorpha fisheri). The estimated age of this fossil (11.6 to 5.3 mya) is very recent and thus it is not useful for calibrating the age of the group, which Song et al. (2015) estimated to be the early Cretaceous. Therefore, we decided to use two estimates that Song et al. (2015) calculated by using nine fossil calibration points. The first calibration point was 152.79 mya, which was estimated for the clade consisting of Pyrgomorphoidea and Acridoidea. Because
these calibrations were based on literature data, we conducted a sensitivity analysis, in which the lower and upper bounds of the highest posterior density (HPD) of these two calibration points estimated in Song et al. (2015) were used in separate BEAST analyses to compare the resulting divergent time estimates. For our divergence time estimate analysis, we used the best-fit partitioning scheme and the models of nucleotide evolution recommended by PartitionFinder. We created an xml file in BEAUti (Drummond et al., 2012), specifying the fossil priors, monophyly constraints, and parameters for molecular clock models. We used the relaxed clock log normal model for the clock model, the birth-death model with a uniform distribution as a tree prior, and a log normal distribution as a distribution prior for calibration points. To assess convergence across independent runs, we conducted ten separate analyses each for 100 million generations, sampling every 2500 generations. We inspected the results using Tracer (Rambaut and Drummond, 2003–2009) and discarded 25% of each run as burn-in, and combined the two best trees that converged using LogCombiner (Rambaut and Drummond, 2002–2013a). A maximum clade credibility tree was summarized in TreeAnnotator (Rambaut and Drummond, 2002–2013b), and visualized in FigTree.

2.4. Ancestral range estimation

We used the R package BioGeoBEARS [Biogeography with Bayesian (and Likelihood) Evolutionary Analysis in R Scripts] (Matzke, 2013) in R 3.3.2 (R Core Team, 2017) to infer the biogeographical history of the New World Pyrgomorphidae. BioGeoBEARS performs different models of ancestral range estimation because different ancestral-area reconstructions have different assumptions and are likely to produce conflicting outputs. The input files were: (1) a dated phylogeny, and (2) a file of geographical ranges indicating presence/absence of each species in each discrete area in the analysis. We compared six models implemented in the program: (1) DEC (dispersal-extinction-cladogenesis) (Ree et al., 2005); (2) DEC + J (including founder-event speciation); (3) DIVALIKE, a likelihood version of DIVA (dispersal-vicariance) (Ronquist, 1997); (4) DIVALIKE + J (including founder-event speciation); (5) BAYAREALIKE, a likelihood version of the Bayesian inference of historical biogeography for discrete areas (BayArea; Landis et al., 2013); and, (6) BAYAREALIKE + J (including founder-event speciation). The six models included two free parameters (d = dispersal and e = extinction). We defined nine areas; North America (from USA to Panama), Caribbean, South America, Africa (Sub-Saharan) including Madagascar, West Paleartic (Europe and Northern Africa), India, Template Asia (China), Tropical Asia and Australia. The reason on the selection of these areas is based on the distribution of the species, species richness, endemic biotas and historical geomorphology of the areas. The distribution of the taxa was obtained from Kevan (1978) and Cigliano et al. (2019). Likelihood values of these models were compared using Likelihood Ratio Test. We used Akaike Information Criterion (AIC) to directly compare how well the different models fit the data and to select the most likely biogeographical scenario (Matzke, 2013, 2014).

3. Results

3.1. Phylogeny of Pyrgomorphidae and the position of the New World genera

We recovered monophyletic Pyrgomorphidae with strong nodal support in both ML and Bayesian analyses (Fig. 2). The tree topology was highly congruent between the two analyses and only three taxa with low support value in the ML analysis (Atractomorpha sinensis, Mekangsiella kingdoni and Chromogonus sp.) were incongruent in both topologies but their placements did not affect our discussion and conclusion about the origin of the NWP. The NWP did not form a monophyletic group, but instead resulted in three separate clades spread throughout Pyrgomorphidae (Fig. 2): (i) The clade comprised of the South American Algea brunneri (Omurini) and the Caribbean Jaragua oviedensis (Sphenarini: Sphenarina) that diverged earlier than other NWP clades; (ii) The clade comprised of the Mexico and Central American Sphenarium purpurascens and Prosphena scudder (Sphenarini, recovered as sister to the African Ochrophlegma sp. and Tanita sp. (Pyrgomorphini: Pyrgomorphina); and (iii) The clade comprised of species currently placed in the Mexican tribes Ichthiacridini (Sphenacris crassicornis and Ichthiacris rehmi) and Ichthyotettigini (Pygottetix pueblensis, Sphenotettix nobilis, Piscacris robertsi and Ichthyotettix mexicanus). In terms of four lineages that include the NWP, we found that Ichthiacridini and Ichthytettigini were monophyletic, but Sphenarini (Sphenarina) was paraphyletic because Jaragua oviedensis grouped with Algea brunneri, rather than Sphenarium purpurascens and Prosphena scudder. Because we only included one representative of Omurini (Algea brunneri), we could not test the monophyly of this lineage.

3.2. Divergence time estimate and biogeographical history of the New World Pyrgomorphidae

Our divergence time estimate analysis suggested that Pyrgomorphidae diverged from other grasshopper families about 121 mya ± 18 mya (Early Cretaceous) and the lineage diversification took place until the Cenozoic (Fig. 3). The sensitivity analysis showed that using the lower and upper bounds of the two calibration points based on literature had no impact on the inferences regarding the divergence time estimates. Our BioGeoBEARS analysis recommended DIVALIKE + J (LnL = −89.09) as the best-fit model (Table 3 and Fig. 3). The origin of Pyrgomorphidae is estimated to be somewhere in an area comprised of Australia, Africa, India and Tropical Asia. We inferred that there were two independent events that resulted in the NWP. The first event could be colonization or vicariance, and occurred about 96 mya in the beginning of the Late Cretaceous (Cenomanian) from Temperate Asia to Africa and then South America. Taking the highest posterior density (HPD) into account, this event could have taken place either when the distance between Africa and South America was narrow (inferring dispersal) or when both landmasses were still together (inferring vicariance). This resulted in the clade consisting of Algea brunneri and Jaragua oviedensis, which represents the earliest lineage of Pyrgomorphidae to colonize and diversify in the New World. The second colonization occurred towards the end of the Late Cretaceous (Maastrichtian) from Africa to South America and then North America. We inferred that the common ancestor of Ichthiacridini, Ichthyotettigini, Sphenarium + Prosphena, and Ochrophlegma Bolivar + Tanita Bolivar colonized across the Atlantic Ocean. This clade diverged into two lineages around the Cretaceous–Paleogene (K–Pg) boundary, one that gave rise to Ichthiacridini and Ichthyotettigini, another to the rest. It is also possible to infer that the common ancestor of Ochrophlegma and Tanita (Pyrgomorphini: Pyrgomorphina) re-colonized Africa via eastward transatlantic route around 59 mya (Paleocene), but the ancestral range of the common ancestor of Ichthiacridini, Ichthyotettigini, Sphenarium + Prosphena, and Ochrophlegma + Tanita was estimated to have a higher probability of North America than Africa, which allowed us to favor two colonization events from Africa as a more likely scenario.

4. Discussion

4.1. Phylogeny of Pyrgomorphidae and the placement of the New World genera

This study represents the first formal test of the monophyly of New World Pyrgomorphidae based on molecular data. Previous molecular studies included some members of Pyrgomorphidae as part of their taxon sampling, but did not specifically set out to understand the
A. Maximum Likelihood

B. Bayesian

Fig. 2. (A) Phylogeny of Pyrgomorphidae based on Maximum Likelihood. The numbers on nodes are bootstrap support values. (B) Phylogeny of Pyrgomorphidae based on Bayesian analysis. The numbers on nodes are posterior probability values.

internal relationships of this family. For example, Flook and Rowell (1997) included three Pyrgomorphidae taxa (Prosphena scudderi, Atractomorpha acutipennis and Zonocerus elegans), Flook et al. (1999, 2000) included Prosphena scudderi and Pyrgomorpha conica, and Hong et al. (2003) used Mekongiella kingdoni, Atractomorpha acutipennis and A. sinensis. Zhang et al. (2013) and Leavitt et al. (2013) used Atractomorpha sinensis, Mekongiella xiaensis and Mekongiana xiangchenguensis. In these six studies, the taxon sampling was low (2–3) and did not allow to infer internal relationships for Pyrgomorphidae. Lu and Huang (2012) used COI gene of Mekongiana xiangchenguensis, Yunanites coriaeae, Mekongiella xiaensis, and Atractomorpha sinensis but found it to be paraphyletic because the only representative of Pneumoridae (Physemacris variolosa) appeared inside the Pyrgomorphidae. Song et al. (2015) inferring the phylogeny of Orthoptera using 258 taxa included eleven Pyrgomorphidae taxa and recovered monophyly as well as two clades that we have recovered in the present study, Colemania sphenarioides + Phymateus morbillosus and Monistria discrepans + Desmoptera sp. The strength of the present study is the inclusion of 25 Pyrgomorphidae taxa, which is by far the largest molecular phylogenetic analysis focusing on this family, with an emphasis in the NWP (10 out of 13 genera) with 12 non-NWP and 7 outgroups (Fig. 2).

Recently, Mariño-Pérez and Song (2018) proposed a morphological phylogeny of Pyrgomorphidae based on 119 characters, covering 28 out of 31 current recognized tribes, figured out the family to be monophyletic. The other subfamily, Pygmoorphophaeinae, was reconstructed as a paraphyletic grade. The present study is narrower in scope compared to the previous study because the aim here is to specify some notable discrepancies between the two. For instance, in the morphological study, Mariño-Pérez and Song (2018) included four sphenarini genera (Rabellia Stål, Mekongiella, Prosphena, and Sphenarium) and recovered the Sphenarini as monophyletic (as the clade C in Mariño-Pérez and Song, 2018). In this study, we did recover a sister relationship between Prosphena and Sphenarium, but Mekongiella did not cluster with the two former genera, thus making Sphenarini polyphyletic. A second clade recovered in the morphological analysis of Mariño-Pérez and Song (2018) was the clade B, which comprised among others the genera Monistria Stål, Dictyophorus Thunberg, Phymateus Thunberg, and Pseudocerus Serville. However, in this study, these genera did not form a monophyletic group, but scattered throughout the phylogeny. This type of incongruence in topologies between morphological and molecular phylogenies is often reported in literature (Baker et al., 1998; Friedrich et al., 2014; Kjer et al., 2016; Peters et al., 2014), but what is surprising in this particular case is how dissimilar the higher-level relationships are. This disparity can be largely attributable to the fact that Pyrgomorphidae is an ancient family (139–104 mya) and that morphological convergence is rampant in this lineage. This family currently has less than 500 extant species, but given the diversity of biology and morphology, it is conceivable that it could have contained many more species in the past. Throughout the long period since the divergence, a number of lineages that were intermediate between today’s highly divergent lineages could have gone extinct, leaving numerous morphologically disparate groups. In fact, the reason Kevan created so many tribes was because he could not find any intermediate lineages (Kevan et al., 1969). Morphological convergence is a well-known phenomenon in grasshoppers, as Uvarov (1966) specifically discussed about ecomorph convergence. In Pyrgomorphidae, it appears that similar selective pressures could have led to a lot of morphological convergence among divergence lineages, even in male genitalia, which has played a crucial role in creating confusion when coding morphological characters. For example, fusiform body has clearly evolved multiple times, and some of the morphological convergence is so convincing that the character coding reported in Mariño-Pérez and Song (2018) could have been affected by it. Another convergent character is the shape of the wings, in which brachypterous tegmina have evolved multiple times in different pyrgomorph lineages. Regarding the sculpting patterns of the body, we may have to re-evaluate different areas of head and pronotum. In relation to male genitalia, the shape of epiphallus has been demonstrated to be valuable for character coding, but geographically disjunctive taxa sometimes seem to converge on very similar epiphallus. However, there are some
Fig. 3. Dated phylogeny of Pyrgomorphidae based on BEAST analysis. The numbers next to nodes are estimated divergence time in million years. The light blue bars on nodes represent 95% confidence interval. Green and yellow dots on nodes represent 100% and 90–99% posterior probability values, respectively. The posterior probability values below 90 are not shown. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
BioGeoBEARS analysis results of the six models.

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<th>e</th>
<th>j</th>
<th>AIC</th>
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Fig. 4. The result from the BioGeoBEARS analysis under DIVALIKE + J model (d = 6e-04; e = 0; j = 0.0345; LnL = −89.09). The colored circles on the nodes represent the probabilities of each possible geographical range just before and after each speciation event. Some of the colored circles do not match with one of the pre-defined colors for the regions, which show ambiguity in the ancestral distribution. Arrows indicated the two NWP lineages. A. Scenario explaining the origin of the younger NWP clade (60 ± 15 mya) based in BioGeoBEARS DIVA-LIKE + J model. An ancestral lineage in Africa (black) dispersed to South America. Around 60 mya, one of the South America lineages dispersed back to Africa giving rise to *Ochrophlegma* and *Tanita*. Within South America, two northward dispersal events took place giving rise to two separate lineages (black). Around 50 mya, both northerly dispersal continued (blue and red/orange) and an extinction (black cross) of the South American lineage occurred. Around 40 mya, a *Sphenarium/Prosphena* lineage (blue) diversified in Central America and Southern Mexico whereas the *Ichthyotettigini* (orange) and *Ichthyacridini* (red) lineages continued diversifying to Central and Northern Mexico respectively. B. Scenarios explaining the origin of the NWP older clade (95 ± 15 mya) based in BioGeoBEARS DIVA-LIKE + J model. If the oldest age is considered (∼110 mya), a vicariance event dividing South America and Africa is the most likely event to explain the cladogenesis. On the other hand, if both continents were already separated (∼95 mya), a dispersal from Africa to South America is the most likely explanation. Once in South America (∼90–80 mya), the clade diversified and expanded its range throughout the northern half of South America. Approximately 68 mya, a dispersal event from South America to the Caribbean gave rise to *Jaragua* lineage. Green shapes represent NWP lineages and black circle represents non-NWP ancestral pyrgomorphs. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
similarities between the two analyses as well. For instance, both analyses recovered the relationship between Spharanium and Prophena, and Poekilocerus and Phymateus. This suggests that at finer scales (such as between two closely related genera), morphological characters show congruent patterns with molecular data. We need to consider a careful inspection of homology statements in a taxon-expanded phylogeny based on morphology from the insights generated from this molecular evidence. This will lead to a better understanding of the evolution of this interesting family.

Our analysis did not find the NWP as a monophyletic group, but rather as consisting of three separate clades, spread throughout the phylogeny of Pygromorphidae (Fig. 2). Marínó-Pérez and Song (2018) also recovered a similar pattern in that the New World genera included in their study (Omura Walker, Prophena, Spharanium, Ichthyotettix, and Sphenacris) did not form a monophyletic group. In this study, the first clade of the NWP consists of the Caribbean genus Jaragua Perez-Gelabert, Dominici & Hierro and the South American genus Algete Bolivar (green in Fig. 2). Currently, Jaragua is classified as a member of the tribe Spharanini (Spharanini) and Algete is classified under Omurini. Jaragua is the only pygromorph known from the Caribbean, endemic to Dominican Republic, and described in 1995 (Perez-Gelabert et al., 1995). When the genus was described, it was placed in Spharanini because of the resemblance in general shape of the epiphallus of Jaragua with that of the Chinese genus Mekongia (Spharanini: Mekongiainina), and because of the intraspecific color variation patterns that are similar to Spharanium (Spharanini: Spharanini), it is possible to find various color morphs in a single population (Sanabria-Urbán et al., 2015). However, Perez-Gelabert et al. (1995) discussed that the relationship to the Mexican and Central American genera Spharanium and Prophena was not clear (based on general morphology alone). They commented that there were multiple morphological differences compared to Spharanium and Prophena such as size, pronotum form and fastigium of vertex length, and proposed that Jaragua could be a reticulate form that has changed little due to isolation. The size of adults (the smallest of all known NWP) was attributed to their island habitat (Losos and Ricklefs, 2009). They also discussed that the most distinct morphological character of Jaragua is a triangular projection of the lateral pronotal lobes, which is seen only in the South American genus Minorissa, which belongs to Omurini. However, Perez-Gelabert et al. (1995) did not place the genus within Omurini because they considered genitalia resemblance with Asian Spharanini was sufficient enough to assign Jaragua to Spharanini. Although we did not use Minorissa in this analysis, we did include another South American omurine genus, Algete that does not have the lateral pronotal lobe but share other characteristics such as the length of fastigium and the form of hind femur. Our phylogeny suggests that Jaragua is more closely related to Omurini, than to Spharanini, and thus future work is needed to reassess Jaragua to Omurini.

The second NWP clade is composed of the Mexican and Central American genera Spharanium and Prophena (blue in Fig. 2). These two genera are assigned to the subtribe Spharanini of the tribe Spharanini, which includes four subtribes that have distinctive distribution patterns. Kevan and Akbar (1964) created these subtribes to unite eight genera that are morphologically similar: Spharanini to include Spharanium and Prophena, Rubellini to include a Madagascar genus Rubella, Sphexeniina to include two East African genera Sphexenexia Karsch and Xenephias Kevan, and Mekongiainina to include three Chinese genera Mekongiana, Mekongiain ina and Yun nanites. As mentioned above, Jaragua was added to Spharanini (Perez-Gelabert et al., 1995). In the phylogeny based on morphology, Marínó-Pérez and Song (2018) included Rubella, Mekongiainina, Prophena, and Spharanium, and found them to form a monophyletic group. However, this clade was supported by only two homoplasious characters: triangular male cerci and rectangular epiphallus. In the present phylogeny, the monophyly of Spharanini is not supported. Moreover, we found the subtribes Spharanini and Mekongiainina to be paraphyletic and recover in different clades. We did not include representatives from Rubellina and Sphexenexia, but it is clear from our topology that the molecular data do not agree with the morphology. This pattern suggests that Spharanini is possibly an artificial group at best, defined by convergent morphological traits with monophyletic relationships found only in geographically close taxa such as Spharanium and Prophena in Central America and Mekongiana and Yunnanites in China. The second NWP clade consisting of Spharanium and Prophena is recovered as sister to the African genera Ochroplegma and Tanita, both of which belong to the subtribe Pyrgomorphina of the tribe Pyrgomorphini. Kevan (1978) mentioned that some members of Pyrgomorphini, particularly the African genus Chirinæites Ramme in the subtribe Parasphenina, convergently evolved similarities in form, size, and variation in color and even exhibit sexual dimorphism where the males are often as large or even larger than females. He also mentioned that the type species of the African genus Parasphenia Bolivar in the subtribe Parasphenina was first described within Spharanium. Finally, he stated some larger specimens of the African genus Pygromorphella Bolivar (Pyrgomorphini: Pygromorphina) were reminiscent with the less robust Spharanini. These claims were proposed without any phylogenetic method and were based on his vast experience of working with Pygromorphidae worldwide.

In many cases Kevan’s claims are substantiated in our phylogenetic analysis, but in others they do not accurately reflect phylogeny.

The third NWP clade we recovered consists of the lineages Ichthiacridini (Ichthiacris Bolivar and Sphenacris) (red in Fig. 2) and Ichthyotettigini (Pygrotettix Kevan, Singh & Akbar, Sphenotettix Kevan & Akbar, Piscarcis Kevan, Singh & Akbar and Ichthyotettix) (orange in Fig. 2), each of which is found to be monophyletic. Both share cylindrical bodies, presence of columbiae (small, paired, sclerotized structures on the floor of the genital chamber, situated near the base of the egg guide. It vertically connects the postvaginal sclerite with the upper surface of the subgenital plate), and the extreme reduction of tegmina. They can be distinguished from each other by the rugose integument with small tubercles and a longer fastigium in Ichthiacridini and the smooth integument and short fastigium in Ichthyotettigini (Kevan et al., 1971 and Fontana et al., 2011), Sanabria-Urbán et al. (2015, 2017) used Sphenacris, Pygrotettix and Sphenotettix as outgroups for an analysis focusing on the species-level relationships within Spharanium and recovered them as a monophyletic group. As Kevan and Akbar (1964) and Kevan (1978) stated, Ichthiacridini is found in Northwestern and Central Mexico, in lower and more arid regions whereas Ichthyotettigini is found in Central to Southern Mexico at higher elevations and/or less arid conditions. These two tribes overlap very little in Central Mexico.

4.2. Dated phylogeny and biogeography

We estimate that the ancestral Pygromorphoidea diverged from the ancestral Acridoidea between 141 ± 18 mya, from the Late Jurassic to the Early Cretaceous, and it began to diversify in the Early Cretaceous (between 139 and 104 mya) according to the results of the BEAST analysis (Fig. 3). During the Late Jurassic, South America and Africa were still connected; Madagascar, India, Australia and Antarctica although connected were recently separated (Blakey, 2008). The earliest diverging lineage within Pygromorphidae is the genus Psedna Key, which includes stick-like grasshoppers endemic to Australia. The next lineage that diverged near the base of the phylogeny is a monophyletic group consisting of Dictyophorus (Africa), Monistria (Australia) and Desmoptera Bolivar (India, Tropical Asia and Australia). Because of the distribution patterns of these genera, the most likely model selected using BioGeoBEARS (DIVALIKE + J) inferred that the ancestral range of the entire family was Australia (Fig. 4). However, because our taxon sampling is not very broad, this inference should not be accepted at its face value. A more reasonable inference would be that the ancestral range of the early pygromorphs was somewhere in Gondwana. Later, during the Cretaceous, there was a major range expansion northwards towards Temperate Asia, according to the BioGeoBEARS analysis.
(Fig. 4), but at this time, there was no direct route of colonization. Again, because our taxon sampling is lacking several African representatives, a more reasonable explanation would be northerly expansion and diversification in Africa, followed by the range expansion to Arabian Peninsula and then Temperate Asia.

There were two colonization events that gave rise to the New World Pyrgomorphidae. The first colonization of the New World was by the common ancestor of *Jaragua* and *Alge*, or the ancestral *Umurini*, which took place in the Cretaceous (Fig. 4). Our dated phylogeny estimated the divergence of this clade to be between 112 and 81 mya (green in Fig. 3). Depending on the divergence time, it is possible to invoke either vicariance or dispersal. Specifically, at this period, South American continent and African continent were either together (early date) or recently split (later date). Thus, it is difficult to determine which process was responsible for the patterns we observe today (Fig. 4). There are other organisms with similar patterns and explanations based in vicariance or dispersal scenarios between Africa and South America. Vicente et al. (2017) found evidence of multiple scenarios involving vicariance and colonization events with Eneeopterine crickets from the Old World to South America. Qin et al. (1998) found that wax scale insects (Hemiptera: Coccidae) have the majority of species in either Africa or South America. They hypothesized an origin in the combined African-South American area at least 97 mya and considered vicariance as the preferred explanation. For Colletidae bees, Almeida et al. (2012) also found vicariance and dispersion events between South America and Africa. For frogs, Feller and Hedges (1998) suggested that families Hylidea (South America) and Ranoidae (Africa) diverged when South America separated from Africa in the mid Cretaceous (~105 mya). In the case of the turtle family Pelomedusoidae, Noonan (2000) tested the hypothesis that their speciation was due to vicariance by the separation of South America and Africa. He found evidence to suggest that the present-day distribution of these turtles together with their phylogenetic relationships could be explained with extinctions and the extant taxa are relics of an originally widespread group.

After this initial colonization, this lineage probably diversified giving rise to several groups, one of which colonized the Caribbean (Hispiania Island) about 69 mya (Campanian/Maastrichtian in Late Cretaceous) to give rise to the present day *Jaragua* (Fig. 4). Regarding the dispersal from northern South America to the Caribbean, Rosen (1975) postulated that in the Late Mesozoic – the Early Cenozoic (70–60 mya), the proto-Antilles (situated where Costa Rica and Panama are currently located) moved to the east and originated the Antillean archipelago. MacPhee and Vincent (2005) argued that terrestrial vertebrates were able to disperse to islands in the Caribbean at any time, and the actual islands of Greater Antilles (Hispiania Island among them) are younger than ~ 40 mya (Middle Eocene). Earlier islands must have existed but probably are now submerged. Morrone (2017) argued that during the Cretaceous, there were three uplift events with potential of creating land bridges between North America/South America with the Cretaceous Antillean island arc. The most likely is the Late Campanian/Early Maastrichtian uplift event (75–66 mya). Graham (2003) considered the existence of a Cretaceous volcanic island arc with an extension from Ecuador in the south to Mexico/Chortis block in the north that was gradually moving through the area between North and South America towards the Bahamas platform in the Middle Eocene. During this 40 my period (110–70 mya), there is evidence of complex patterns of land separation and collision as well as emergence and submergence. Graham (2003) concluded that although both vicariance and dispersal could have happened, the latter was the key driver for diversification of Antillean Lytraeaceae. Meanwhile, there were continued diversifications of pyrgomorph lineages in Africa, giving rise to *Caprohirus* Sausseur, *Colemania* Bolivar, *Phymateus* and *Poekilocerus*.

According to the BioGeoBEARS analysis, there was a second dispersal event from Africa to the New World about 69 mya (Fig. 4). The analysis suggests that the common ancestor of Ichthiacridini, Ichtthytettigini, and a clade consisting of *Sphenarium*, *Prophena*, *Ochrophlegma*, and *Tanita*, colonized North and Central America from Africa. However, this pattern requires more assumptions than the first dispersal event that gave rise to Omurini because of two main issues. By the end of the Cretaceous when this dispersal event took place, there was no direct connection between Africa and North America, which means that the dispersal must have taken place through the westward transatlantic colonization from Africa to South America. This lineage must have colonized northward to give rise to the present-day genera in North and Central America. Thus, the inference made by this biogeographical analysis makes an implicit assumption of connection by South America. This, however, raises another problem because there is currently no pyrgomorph species in South America that has taxonomic affinities to Ichthiacridini, Ichtthytettigini, *Sphenarium* and *Parasphena*. This means that we need to invoke extinction for this ancestral lineage that crossed the Atlantic Ocean and gave rise to the North and Central American taxa (Fig. 4). While the westward transatlantic colonization from Africa to South America could have been a rare event, the South America faunal connection has been well documented. For example, there are different groups whose current distribution is explained by long-distance dispersal from Africa to South America, such as Caviomorph rodents through waif dispersal (~40 mya) (Antoine et al., 2011; Poux et al., 2006), monkeys (~36 mya) (Lynch Alfaro, 2017; Bond et al., 2015), amphibians and gekkotan lizards (Gamble et al., 2011) and the iconic Neotropical bird *Opisthocomus hoazin* (Mayr et al., 2011). In plants, Christenhuz and Chase (2012) found that the flora of the Neotropics has several shared relationships with the Paleotropics, and the dispersion across oceans was the key driver. For Pyrgomorphidae, the common ancestor that colonized the New World for the second time gave rise to two lineages. The first lineage comprised the Mexican tribes Ichthiacridini and Ichtthytettigini, which diversified in situ, the first one in Northern Mexico and the second one in Southern Mexico. The second lineage comprised a clade consisting of *Sphenarium* and *Prophena*, and another clade consisting of *Ochrophlegma* and *Tanita*. Because both *Ochrophlegma* and *Tanita* are African, the observed pattern suggest that the common ancestor of these two African genera recolonized Africa from South America. There is another equally parsimonious explanation, which suggests that there were two separate westward colonization events from Africa, first by the common ancestor of Ichthiacridini and Ichtthytettigini, and another by the common ancestor of *Sphenarium* and *Prophena*, but this scenario is less likely under the best-fit model selected by the BioGeoBEARS analysis.

For the genus *Sphenarium* (central Mexico to northwest Guatemala), Sanabria-Urbán et al. (2015, 2017) discussed that in most recent geological times there are drivers for diversification such as the Neogene formation of the Mexican Transvolcanic Belt (19–3 mya) which caused an increase in topographical complexity and later the Quaternary climatic shifts (2.6–0.01 mya) provoked shifts in distribution ranges in highlands and lowlands of Mexico. Certainly these events could have shaped distribution and diversification of the NWP genera other than *Sphenarium*, such as the ones belonging to tribes Ichthiacridini and Ichtthytettigini. We infer that the common ancestor of *Ochrophlegma* and *Tanita* recolonized the Old World from the New World, based on the results of the BioGeoBEARS analysis. While both *Sphenarium* and *Prophena* are wingless, *Tanita* and *Ochrophlegma* include fully winged species. While the eastward transatlantic recolonization from South America to Africa seems far-fetched, it has been reported from many taxa. For example, SanMartin and Ronquist (2004) conducted an analysis to test the role of vicariance and dispersal in the composition of Southern Hemisphere biotas using 19 plant and 54 animal phylogenies. They found that dispersal could be more important than previously assumed and showed that for the dispersal event from northern South America to Africa in the late Cretaceous-early Tertiary (70–60 mya), there were 4.09 dispersal events versus 1.54 events from Africa to northern South America.

The tectonic history and biota origin of South America and the
Caribbean is old and complex. However, the amount of geological and phylogenetic evidence is overwhelming. For example, Bacon et al. (2015) found an earlier connection of North and South America than commonly assumed, with waves of dispersal of terrestrial taxa around 20 and 6 mya. We found our scenarios to be plausible and we consider that dispersal has played a significant role in the distribution and diversification of the NWP.

5. Conclusions and future directions

Despite the low number of species of Pyrgomorphidae in the New World, its origin is very old and complex. We have provided evidence and rationale about the biogeography of the NWP based on a molecular phylogeny. We infer that the first wave of colonization was probably due to a vicariance event (split of Africa and South America) or dispersal from West Africa to northern South America with a subsequent dispersal from South America to the Caribbean. It is probable that the first fauna of NWP was diverse at some point and we are now seeing the presence of only the relic lineages due to the antiquity of the group and probable extinction events. The second wave of colonization came by dispersal from West Africa to northern South America and then North America much later when the continents were already separated. The fauna found in North America (from Mexico to Costa Rica) consists of the most speciose NWP in genera such as Sphenarium (17 spp.) and Ichthiactis (8 spp.) Both genera harbor 60% of the current species diversity of the New World Pyrgomorphidae.

As a result of this analysis, we reject the hypothesis by Kevan and Akbar (1964), which suggested that the NWP originated from Temperate Asia twice and subsequently dispersed to South America. Their claim of a relationship between the Chinese genera Yununnatix and Mekongiana with the Central American genera Prophsna and Sphenarium is not supported with molecular evidence. The morphological similarities among these genera are probably due to convergent evolution. Regarding the Orthacridini – Ichthiactini/Ichthytettigini relationship, we were not able to include genus Orthacris in the analysis and these two tribes were recovered in a clade with Sphenarium and Prophsena and two African Pyrgomorphini (Ochrhoplegma and Tanta). Concerning the hypothesis by Amédégnato (1993), which suggested that the NWP originated from Africa, we found general support although her original formulation was vague in terms of specific Pyrgomorphidae taxa involved. Finally, although the hypothesis by Marínó-Pérez and Song (2018) did not explicitly state the origin of the NWP, it could imply that the origin was from Asia and Madagascar based on their topology. In this sense, we reject their hypothesis as well. However, they recovered the NWP in three separate clades as in this study although a different taxon sampling was used with only a small number of the NWP taxa. Thus, in terms of the paralogy of the NWP, we concur with their conclusion.

In the future, an increased taxon sampling of the South American Omurini (Omura and Minorissa) will allow us to test the monophyly of Omurini. We need to include African and Malagasy Sphenancrii (Sphenexina and Rubelliina) to infer their placement in the phylogeny. Finally, we need to conduct a phylogeny based on morphology for the NWP with emphasis in the genus Jaragua and all the members of the South American tribe Omurini to update the current classification.

Declaration of Competing Interest

Author declares that there is no conflict of interest.

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