An exploration of the complex biogeographical history of the Neotropical banner-wing damselflies (Odonata: Polythoridae)

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Abstract

**Background:** The New World Tropics has experienced a dynamic landscape across evolutionary history and harbors a high diversity of flora and fauna. While there are some studies addressing diversification in Neotropical vertebrates and plants, there is still a lack of knowledge in arthropods. Here we examine temporal and spatial diversification patterns in the damselfly family Polythoridae; which comprises seven genera with a total of 58 species distributed across much of Central and South America.

**Results:** Our time-calibrated phylogeny for 48 species suggests that this family radiated during the early Eocene (~56 Ma), diversifying during the middle Eocene. As with other neotropical groups, the Most Recent Common Ancestor (MRCA) of most of the Polythoridae genera has a primary origin in the Northern Andes though the MRCA of at least one genus may have appeared in the Amazon Basin. Diversification rates have been uniform in all genera except one—*Polythore*—where a significant increase in the late Pliocene (~3 mya) correlates with mountain uplift. While our molecular clock suggests correlations with some major geographical events, our biogeographical modeling (with BioGeoBEARS and RASP) found little influence of the formation of the Pebas and Acre systems or Andean mountain building, possibly due to the short branch lengths in our time-dated phylogeny, or perhaps to climatic variability during the period in question.

**Conclusion:** The biogeographical models implemented here were unable to explain the effect of South American major geological events (i.e Pebas and Acre systems, Andes uplift) on the diversification of this damselfly family; while diversification in the tree shows some correlation with mountain building events, it is possible that
other abiotic and biotic changes during our study period have influenced
diversification as well. The high diversification rate observed in *Polythore* could be
explained by the late uplift of the Northern Andes. However, it is possible that other
intrinsic factors like sexual and natural selection acting on color patterns could be
involved in the diversification of this genus.

**BACKGROUND**

The New World tropics is a region of amazingly high diversity in a variety of plants
and animals, and has a complex history[1]. Shifting continents, multiple instances
of mountain building, rivers that change their course, and the expansion and retreat
of both freshwater and marine habitats all come together to make a complicated
and dynamic foundation on which biological diversity has developed over the last
50 million years (Ma). These include the uplift of the different regions of the Andes,
as well as the Venezuelan Highlands, the formation of the extensive Amazonian
floodbasin in the Miocene (Pre-Pebas, Pebas and Acre), the further development of
the Amazon and Orinoco drainages, the dry/wet climate cycles of the
Pliocene/Pleistocene, and the formation of the Panamanian Land Bridge between the
Tumbes-Chocó-Magdalena regions and Central America[2–4].
In a number of groups (tropical frogs[5, 6], butterflies[7, 8], lupines[9], birds[10])
this changing geography has driven substantial—sometimes rapid—diversification,
but not always in the same way. The diversification driven by these geographic
changes often promotes niche diversification, with species taking advantage of the
distinct ecological niches found in each local environment. This leads to adaptive
speciation [11], further increasing diversity beyond the form of the landscape. Here
we investigate the origins of diversity in the banner-winged damselflies
(Odonata:Zygoptera:Polythoridae), a group that is distributed across much of Central and South America.

Polythoridae comprises 58 species across seven genera (Chalcopteryx Selys, Chalcothore De Marmels, Cora Selys, Euthore Selys, Miocora Calvert, Polythore Calvert and Stenocora Kennedy) that differ markedly in their distribution: some are found widely across the continent, while others are limited to a single region. While damselflies as a group are generalist predators in both their larval and adult forms, the types of aquatic habitats in which their odonate larvae are found vary, with some species using lakes and ponds, while others inhabit streams and rivers. The larvae of Polythoridae prefer moving water; most species are found in small, fast-flowing streams in mountain regions, though some additionally exist within slow streams in the Amazon Basin[12]. These species display a diverse range of colors and patterns on both the wing and body within and among genera; previous work by Sánchez Herrera et al. [13, 14] has shown that for some genera, wing colors may be polymorphic, raising the question of what factors drive this color diversity.

We tested hypotheses concerning the diversity within Polythoridae, investigating the influence of geological events in shaping the distribution of the members of this family. Using relaxed-clock molecular methods we estimate divergence times within and among the genera of this family. We also investigate their biogeographical patterns of diversification in relation to the mountain building events of the Andes, as well as the extent of marine and freshwater environment incursions in different periods that might have influenced distribution and speciation in these genera.

Specifically, we made the following predictions: (1) We predict that the Andes uplift was a major driver of speciation in the family Polythoridae. (2) We predict there were multiple interchanges among Neotropical regions, particularly between
Amazon and Andean regions and the Andes and Central America, due to the geological events happening during the last 50 Ma in South America. (3) We predict that the family Polythoridae or genera within has experienced higher rates of diversification through recent evolutionary time. Overall, this work represents a continental-scale biogeographical analysis of a neotropical family of damselflies. With their reliance on aquatic habitats, damselflies are likely to respond very differently to the landscape than plants, vertebrates, or terrestrial insects; our analyses allow us to consider the formation of diversity in the modern Neotropics through an unexplored perspective.

RESULTS

Phylogeny and Time Divergence estimation

Our BEAST time calibrated phylogenetic reconstruction was generally consistent with the clades previously recovered by Sánchez Herrera et al. [13] (Fig 1), though our current dataset includes twelve additional species. The family Polythoridae separated from our Outgroups ~56 Ma (70 - 48 Ma 95% HPD [High Probability Density intervals], pp [posterior probability] = 1). Our time divergence reconstruction suggests that the MRCA (Most Recent Common Ancestor) of the family Polythoridae diverged ~ 42 Ma (48 - 35.11 Ma 95% HPD, pp = 0.992) during the Eocene epoch. The Chalcopteryx – Chalcothore crown clade itself began to diversify later during the late Oligocene ~24 Ma (35.7-12.52 Ma 95% HPD, pp = 0.999), while the crown group of all of the other Polythoridae genera (Cora s.s., Miocora, Euthore and Polythore clades) began to diversify ~33.14 Ma (41.52 - 25.52 Ma 95% HPD, pp = 1). Chalcothore separated from Chalcopteryx ~ 23.49 Ma (35.73-12.51 Ma 95% HPD, pp = 0.999) around the late Oligocene and early Miocene.
boundary, however the crown group of *Chalcopteryx* only diversified during the late Miocene ~ 9.62 Ma (16.97 - 4.325 Ma 95% HPD, pp = 0.997). The MRCA of the *Cora* s.s clade separates from all the other taxa ~33.14 Ma, however its crown group of *Cora* s.s. appears ~17 Ma (24.24 - 9.06 Ma 95% HPD, pp = 1) around the end of the early-Miocene (Fig 1). The crown group of the *Miocora* clade appears ~ 14.5 Ma (22.5 - 6.78 Ma 95% HPD, pp = 1), a few million years after *Cora* s.s during the mid-Miocene, although the MRCA of *Miocora* (separate from the *Euthore* and *Polythore* clade) appears ~ 24.75 Ma (32.18 - 17.28 Ma 95% HPD, pp = 1) during the late-Oligocene or early Miocene epochs (Fig 1). The two sister clades of *Euthore* and *Polythore* diverged from each other around the same epoch as *Miocora*, ~22.28 Ma (29.37 - 15.70 Ma 95% HPD, pp = 1), but both form crown groups concurrently around the early Miocene, though *Euthore* estimates are slightly older than those for *Polythore* (Fig 1). *Euthore* crown group diversified ~18.39 Ma (25.06 - 12.17 Ma 95% HPD, pp = 1) while *Polythore* seems to be ~16.68 Ma (22.80 - 10.46 Ma 95% HPD, pp = 1). Within the *Euthore* clade we recovered two sister clades: *Euthore sensu stricto* (e.i. *E. fasciata fasciata*, *E. fasciata fastigiata*, *E. fasciata plagiata*, *E. fassli*, *E. sp. nov*), and what used to be part of *Cora* (i.e. *E. lugubris* and *E. klenei*). Within the *Euthore* clade the divergence times recovered suggest that *Euthore sensu stricto*, ~6.62 Ma (10.72 - 3.09 Ma, 95% HPD; pp = 1), is significantly younger that the previous classified *Cora* species within this clade, ~12.8 Ma (19.04 - 6.13 Ma, 95% HPD; pp = 0.978).

In the highly speciose *Polythore clade* we recovered the divergence times for all the previous geographical clades reported in Sanchez Herrera et al[13] (see Fig 1). The crown group of the Amazonian clade appeared ~8.72 Ma (14.36 - 3.24 Ma 95% HPD, pp = 1), while the Andean clade was ~10.57 Ma (15.43 - 6.41 Ma 95% HPD, pp = 1).
Within the Andean clade, the crown groups of the West and East Andean clades appeared ~ 2.98 Ma (5.34 - 1.19 Ma 95% HPD, pp = 1) and ~7.16 Ma (10.78 - 4.18 Ma 95% HPD, pp = 1), respectively. Moreover, estimates for the North (~4.21 Ma, 6.35 - 2.35 Ma 95% HPD, pp = 1) and South Eastern (~3.25 Ma, 5.57 - 1.64 Ma 95% HPD, pp = 1) crown groups suggest they appeared around the Pliocene - Pleistocene epochs (Fig 1). Our lineage through time plot suggest that the major accumulation of lineages for Polythoridae occurred during the Miocene epoch (Fig 1).

Biogeographical Reconstruction

We designed and tested three different scenarios; Control, Pebas & Acre Systems (P&AS), and ANDES, to reconstruct the biogeographical history of Polythoridae. For the control scenario, our hypothesis was that geological events including the Andes uplift and Marine incursions of South America did not play an important role in the dispersal and diversification of the family Polythoridae. While for scenarios P&AS and ANDES, the latter geological events played a role in the dispersal and diversification of this family. In particular, scenario P&AS tested only the geological events in South America including marine incursion and Pebas/Acre systems; while the scenario ANDES accounts for the gradual uplift of the Andes Cordillera (see Supplementary Material S8). For the three scenarios (Control, P&AS, and ANDES) we evaluated, the best biogeographical model selected by BioGeoBEARS was DEC+J and the least fitted was the BayArea model (Supplementary Table 1). However, following the Ree and Sanmartín [15] critique of the DEC+J model, we also reconstructed the next best fitted biogeographic model based on the AICc, using both BioGeoBEARS and RASP, for all the scenarios (Table 1). Comparing across the different models in both the BioGeoBEARS and RASP reconstructions, there were no major differences among our three scenarios. Although, we found disparities among the ancestral area
estimation performed by BioGeoBEARS and RASP within each scenario tested (first row in Table 1). RASP models’ reconstructions for all scenarios show a more conservative and consistent ancestral areas pattern than BioGeoBEARS. To simplify, here we will describe the ancestral areas estimated for the ANDES scenario (Fig 2). The MRCA (node 81) for the family Polythoridae has an uncertain ancestral area; the MRCA (node 80) for the clade that includes Cora s.s, Miocora, Euthore and Polythore is also uncertain (Fig 2). For the MRCA (node 79) of the Miocora, Euthore and Polythore clade the Northwest Andes (C) is the most commonly predicted ancestral area (Fig 2); the same is true for the MRCA (node 78) of Euthore and Polythore. The MRCA (node 56) for the crown group of Euthore is consistently found in the Northwest Andes (C). The MRCA (node 47) for the crown group of Cora s.s. has an ancestral area in the Northwest Andes (C), Northeast Andes (D) and the Central Andes (F). The MRCA (node 77) of the Polythore crown group has ancestral areas in the Northwest Andes (C), Northeast Andes (D) and the Amazon Basin (G). The MRCA (node 49) for the Miocora crown group is found in the Tumbes-Chocó-Magdalena Valley (B) and the Northwest Andes (C). Finally, the MRCA (node 43) of the Chalcopteryx-Chalcothore crown group showed as more plausible areas the Amazon Basin (G), the Venezuelan Highlands (E) and the Brazilian Shield (I) (Fig. 2, Table 1).

It is interesting to note that the different scenarios (Control, P&AS, ANDES) within our BioGeoBEARS and RASP analyses, which accounted for many of the major geographical events in South America over the last 50 million years, did not produce fundamentally different results; inclusion of the formation of the Pebas and Acre wetlands systems, as well as mountain building events, did not alter our results in comparison with a simple control scenario that allowed free dispersal between adjoining regions at different periods of geological times (see
Supplementary material S8). This may be due to the relatively short branch lengths within the tree for most of the species groups, especially within *Polythore*. Conversely, any long branch lengths within the tree may also suggest the potential for undetected extinction events early in the formation of some of these genera.

Our best selected S-DEC model implemented in RASP for the Andes scenario suggest several dispersals (29), vicariance (10) and extinction (3) events within the different genera of Polythoridae (Supplementary Figure S2); many of these involve movement within and between the different ranges of the Andes, as well as movement into the Amazon, Guiana Shield, Venezuela Highlands, the Tumbes-Chocó-Magdalena Valley, and Central America.

**Diversification Analyses**

To investigate the patterns of diversification and extinction rate variation through time and across lineages, we chose RevBayes[16, 17] to test the best diversification models and rate shifts, due to the high uncertainty associated with phylogenetic tree estimation. The Episodic Birth-Death with multiple time periods was the best model explaining the diversification pattern in this group of damselflies (Table 2). The estimated net diversification ($\lambda - \mu$) and speciation ($\lambda$) rates show an increase, however the relative extinction ($\lambda/\mu$) and extinction ($\mu$) rates pattern seem to behave constantly through time (See Supplementary Figure S3). The Branch specific model detected a shift in diversification ($\lambda - \mu$) rate corresponding to the Andean Clade in *Polythore* with the highest shift in the Eastern Clade (Fig 3). When we observed the relative extinction ($\lambda/\mu$) rate across the tree, we observed that this rate also decreases for this clade (Fig 3). Our estimates show that the other clades within this family have a more constant diversification ($\lambda - \mu$) and relative extinction ($\lambda/\mu$) rates across the branches of the estimated tree.
DISCUSSION

Overall, our results suggest that the Polythoridae MRCA first appeared around the Eocene, however most of the diversification within the family appeared during the Miocene, Pliocene and Pleistocene epochs (Fig. 1). During these periods there were a few major geographical events occurring, including the uplift of different regions of the Andes, as well as the formation of the Pebas and Acre Systems[1, 18]. While the ancestral area for the MRCA of Polythoridae is uncertain, our biogeographical analyses suggest an origin—or at least early diversification—in the Northwestern and Northeastern Andes for most genera in Polythoridae. Chalcopteryx-Chalcothore are the only two genera with a MRCA that may have had an Amazonian origin (Fig. 2, Supplementary Figure S4).

Our comparison of the three different biogeographical scenarios (Control, P&AS, and ANDES) found no significant effect of these major geographical events on the speciation in the family Polythoridae. We found this result surprising, as a number of polythorid genera are associated exclusively with the Andes and show patterns of diversification that align with mountain building events (Fig. 2). For example, the genus Polythore contains an Andean clade which shows a concordant pattern with the North Andes uplift. Likewise, Cora s.s., also shows a similar pattern separating two clades from the Northwest and Northeast Andes (Fig. 2). [11]

Moreover, our results suggest that most of the diversification within Polythoridae is due to dispersal events rather than vicariance (Supplementary Fig. 2). However, the distribution of those vicariance events (Supplementary Fig. 2.) in the time calibrated phylogeny is mainly within groups distributed along the Andes or that have representatives in Central America, reflecting the influence of these major
geographical events on diversification.

We don’t see a significant influence of major South American geological events (e.g. Pebas and Acre Systems and Andes uplift) on the diversification of this family. Changes in the climatic conditions over that period of geological time may have a stronger influence on the biogeography of the family, which our current analyses focused on geological events will not capture. However, these events may cause significant changes in other biotic and abiotic conditions of different regions that may promoted Polythoridae damselflies dispersion to new suitable habitats. Despite the fact that damselflies are generalist predators in both terrestrial and aquatic ecosystems, their distribution patterns might be affected by the vegetation and climatic barriers over evolutionary time. A recent definition of the Neotropical forest relies in the combination of the following abiotic and biotic parameters: climate, floristic composition, vegetation structure and plant physiognomy [19]. Jaramillo [18] suggests that the formation of Neotropical terrestrial communities can be divided in two major phases, Cretaceous and Cenozoic, based on the defining parameters Neotropical forests in these two geological eras. In our case the Cenozoic phase is more relevant, and it was characterized by the dominance of the flowering plants [20–23]. Particularly, during the Paleogene, fossil and palynological evidence suggests that Neotropical forests were multi-stratified, like our current forests but with two major differences[18, 22]. First, the mean annual temperature was 1.5-2 °C higher [24–27] and the CO₂ level was almost double than in extant forests[28]. Second, there was a significantly lower diversity of plants in comparison with actual forest diversity. Moreover, during the Paleogene, the Paleocene-Eocene Thermal Maximum (hereafter PETM)[29] was a warming event produced by a significant addition of carbon due to volcanism in the North Sea [28]. The PETM was
a major climate change event, and fossil evidence suggests that vegetation during this period was around 30% more diverse, and that extinction rates did not change while origination rates doubled [18]. Other DNA-based phylogenies of plants and herbivorous insects show radiations around those times [30-32]. Besides the PETM there was great variation in the global temperature during the Eocene (e.g. Early Eocene Thermal Maxima) and Oligocene epochs [18, 33, 34]. During the Neogene, new biomes within the Neotropical forest flourish, like savannas, dry forest, xerophytic forest, deserts, montane forest and paramos [18]. These biomes are determined by the precipitation regimes rather than temperature [19]. However, questions in regards how climatic and vegetation can influence the diversification of these damselfly family remains untested.

We found multiple interchanges among the Amazon and Andean regions; many of them involved movement within and between the different ranges of the Andes, as well as movement into the Amazon, Guiana Shield, Venezuela Highlands, the Tumbes-Chocó-Magdalena Valley, and Central America (Supplementary Fig. 2). The different genera within Polythoridae showed an array of biogeographical patterns. The genus Polythore shows a pattern of dispersal from the Andean regions to the Amazon in some clades, as well as movement from the Northern to Central Andes (Fig. 2). On the other hand, Euthore moved from the Northwestern to Northeastern Andes and then to the Venezuelan highlands (Fig. 2). This genus is currently found at locations from 1000–2000 meters in elevation; it is plausible that they colonized new habitats that became available during mountain building events in the Andes and Venezuelan highlands. Cora s.s and Miocora diversification was more likely driven by dispersal, as they cover a range of landscape types, and have moved across the Isthmus of Panama, the only groups in Polythoridae to do so.
Interestingly, the age at which both of these genera diversified matches the age suggested by recent geological studies of the Central American Seaway closure during the middle of the Miocene [2, 35]. This closure may also influence the ITCZ (Inter Tropical Convergence Zone)[36] that will have affected precipitation regimes in the Neotropical Region [37].

These damselflies are restricted to fast flowing forested streams and waterfalls in montane forests; these habitat requirements might be the limiting factor of their distribution [38-40]. However, as general predators they are not limited by the distribution of food sources such as the host plant for other phytophagous insects (e.g. Lepidoptera). They are likely good dispersers in their immature stage, moving with the flow of creeks, but distribution between watersheds is likely to be limited, which could explain the vicariance patterns observed in our results.

Our analyses suggest that Polythoridae has been diversifying through an episodic pattern (Table 2). Speciation (\(\lambda\)) and net diversification (\(\lambda-\mu\)) show an overall increase through time, while relative extinction (\(\lambda/\mu\)) and extinction (\(\mu\)) rates seem to remain somewhat constant through their evolutionary history. However, our branch-specific model of diversification shows a significant shift for the Andean Clade within the genus Polythore which might suggest that at least for this genus the intensified Andean uplift during the Pliocene has been promoting speciation (Fig. 3). The Andean uplift produced great modifications to the landscape; one of the major changes was in the flux of the hydrographic system towards the east, producing the actual Amazon and Orinoco basins. Furthermore, when the Andes reached their modern elevation by the end of the Miocene (i.e. 5–6 Ma) [1, 41–44], it generates two new biomes: cloud (montane) forest and paramo[18, 45]. The montane forest is the key habitat for the genus Polythore, and it has been
suggested that the slopes of the Andes are an engine for speciation as the increase in topographic complexity generates diversity of microenvironments [1]. While all these species have relatively similar habitat requirements, they have generally disjunct distributions within the Andes, such that any local stream normally hosts only a single Polythore species, sometimes two species. The wing color diversity characteristic of Polythore is highest within the Polythoridae—the central Andean Polythore have wings that include bands of black, orange and yellow patterns, while those of the Northeastern Andes have intense black and white patterning (except for P. concinna, which is orange). This color diversity also appears to be polymorphic within some of these clades[13, 14, 39]. Some of the color diversity could be explained by sexual selection, with local mate choices driving diverse color patterns in different regions[46, 47]. Likewise, wing color may be under other constraints, such as thermal tolerance or selection by predators [48]. Having robust phylogenetic hypotheses will allow for further exploration of the reasons behind this radiation in Polythore, where population-level analysis will be a likely next step to disentangling the complex history of these striking creatures.

CONCLUSIONS

In conclusion, our data did not support our prediction that the Andes uplift and other major geographical events were major drivers of speciation of the family Polythoridae. However, we were able to detect multiple interchanges across the Andean, Amazon, Guiana Shield and Central America mostly driven by dispersal events during the last 50 Ma. Finally, the genus Polythore, was the only genus who might be experiencing a high rate of diversification. Further exploration of the climatic and vegetation history of South and Central America in conjunction with
population-level analyses may provide a better understanding of the Polythoridae family members distribution than geological and geographical conditions alone.

METHODS

Taxon Sampling

A total of forty-eight of the 58 species of Polythoridae were included for all reconstructions presented here. All the taxa from Sanchez et al. 2018, including outgroups of other related Calopterygoid taxa (Philogangidae, Euphaeidae, and Pseudolestidae), are included within the analyses, with an additional 12 polythorid species new to these analyses. Geographic origin, collector details, and Genbank Accession Numbers for all specimens are summarized in Supplementary Table S6.

DNA amplification, sequencing, and alignment

For the 12 species new to this analysis we extracted DNA from either the legs or ¼ of the pterothorax using a DNeasy Tissue Kit (QIAGEN) from each specimen following the manufacturer’s protocol. We amplified three mitochondrial and three nuclear fragments: Cytochrome Oxidase I (~799bp), NADH subunit I dehydrogenase (~548 bp), 16S, (~340bp), Elongation Factor (~900 bp), 28S (~340bp) and 18S (~600bp) (see Supplementary Table S7 for a list of primers used). All gene fragments were amplified using PCR conditions as described in the associated publications for each pair of primers (Supplementary Table S7) Macrogen USA Inc. laboratories (NY) performed the purification protocol for the PCR products (15μl final volume for each primer) and the Sanger DNA sequencing. Primer contig assembly, peak chromatogram verification and the generation of per-individual consensus sequences were done using Geneious v 8[49]. All fragments were aligned using MAFFT [50] and then manually aligned in Mesquite[51]. Ribosomal genes were
aligned manually with reference to secondary structure using the methods described in Kjer [52] and Kjer et al. [53]. Finally, all genes were concatenated using Mesquite for the overall analyses.

**Time Divergence Analysis**

A relaxed-clock molecular dating analysis on the partitioned dataset was run using BEAST v 1.8.4 [54]. Specifically, we partitioned the gene fragments as follows: (i) We linked the sites and clock models for all mtDNA fragments and (ii) unlinked all nuclear ones from their clock and site models. We implemented the appropriate model selection for each partition: HKY + G4 for all mtDNA, JC for 18S and PMRT, 28S and for EF1 we set the model to GTR + G, models obtained using the model selection tool of IQTree [55]. We used lognormal relaxed clock models for all partitions, under a Yule speciation model tree prior. We generated a best ML phylogram with proportional branch lengths using the appropriate partition models using IQTree [55] as the starting tree for the analysis. Most of the fossil calibrations prior distributions were uniform considering the oldest fossil age as the maximum and the youngest fossil age as the minimum bound. For the Euphaedidae outgroup calibration we created a Lognormal distribution of all the fossil ages that accounted for all the age variation. Table 3 shows the calibrated nodes (4), stem fossils (10) and prior distributions selected for the analyses. We ran four independent analyses to ensure convergence of the MCMC; convergence was checked using Tracer 1.7 [56]. Finally, the independent runs for each treatment were combined using LogCombiner v 1.8.3 [54]. The dated ultrametric tree was obtained using TreeAnnotator v 1.8.3 [54] and visualized using Figtree v 1.4 (http://tree.bio.ed.ac.uk/software/figtree/) and the R package ggtree [57]. With the best ultrametric tree we obtain a lineage through time plot using the R package APE.
Biogeographical Reconstructions

The ancestral range estimation was performed with the following software: R package Biogeobears v.0.2.1\cite{59} and RASP (Reconstruct Ancestral State in Phylogenies)\cite{60}. Both software packages allowed us to customize dispersal rates matrices and time stratification events, as well as infer areas among the following historical biogeography frameworks: Dispersal-Vicariance Analysis (DIVA\cite{61}), Statistical Dispersal-Vicariance (S-DIVA\cite{62}), Dispersal-Extinction Cladogenesis (DEC\cite{63}), and Bayesian inference of historical biogeography for discrete areas (BayArea\cite{64}). In addition, Matzke\cite{65} included a new parameter in all the models accounting for what he calls the founder-event speciation. These are described as “jumping dispersal events (J)”, which are rare events that occur when a new population colonizes a new area \cite{65}. In particular, BioGeoBEARS has implemented model selection among six different historical biogeographic scenarios (DEC, DEC+J, DIVALIKE, DIVALIKE + J, BayArea, BayArea + J; \cite{59}). Although, Ree and Sanmartín \cite{15} recently highlighted there are conceptual and statistical issues with the DEC + J model implemented in BioGeoBEARS, which can sometimes favour unparsimonious numbers for “jumping dispersal events”; and as a result it will not reflect a more close approximation of the “true” model of range evolution. For our analyses, we designated nine distinct geological areas based on the geological literature \cite{1, 41, 42}, which include; (A) Central America, (B) Tumbes-Choco-Magdalena, (C) North Western Andes, (D) North Eastern Andes, (E) Venezuela Highlands, (F) Central Andes, (G) Amazon Basin, (H) Guiana Shield, and (I) Brazilian Shield (See Figure 2, S4). The extant species distributions for each of our species at the tips of our time calibrated tree (Fig 2) were compiled from our locality data, and specimen records
information from the following collections: Florida State Arthropod Collection (FSCA), U.S National Entomological Collection (USNM), Andes Museum of Natural History (ANDES), Entomological Collection of the Universidad de Antioquia, Colombia (CEUA), and the Rutgers Newark Entomological Collection (RUN_ODO). Ranges were restricted to be comprised of at most three different areas as there are no extant species that occupy a range made up of more than three of the determined areas. Impossible adjacency range combinations were manually removed from the ranges list used by BioGeoBEARS during the inference of ancestral states. Details on the model parameters, areas allowed, dispersal probabilities and time stratification schemes for each of the three scenarios (Control, P&AS and ANDES) are explained in the Supplementary Appendix S8. Each of these scenarios were subject to the six available historical biogeographic models of BioGeoBEARS, from the best-fit model based on the corrected Akaike Information Criterion (AICc) weights. However, following the recommendations of Ree and Sanmartín[15] we assessed model consistency by comparing among the selected BioGeoBEARS models and carefully examined the reconstructions performed for those models in both BioGeoBEARS and RASP. Afterwards, we favored the model based on best fit for the empirical information on Polythoridae and method consistency. The best selected reconstructed areas models, for each scenario were mapped over the best time calibrated phylogeny and the directionality of the dispersal and vicariance events was represented using maps for each major clade [59, 65].

**Time Diversification Analyses**

To investigate the patterns of diversification and extinction rate variation through time and across lineages, we chose RevBayes[16, 17] to test the best diversification models and rate shifts, due to the high uncertainty associated with phylogenetic
tree estimation. We calculated the Bayes Factors[66] for each pair of candidate models estimating the marginal Likelihood (mlnL) using two sampling algorithms (stepping-stone sampling[67] and path-sampling[68]) among the following models: Yule Pure-Birth Model, Birth-Death Constant Model and Episodic Birth-Death Models with multiple time intervals (4, 10 and 100 [69]). Once the best model through time was selected we calculated the speciation ($\lambda$), extinction ($\mu$), net diversification ($\lambda-\mu$) and relative extinction ($\lambda/\mu$) for the best model; all plots were generated using the RevGadgets package in R[70]. In addition, we used the Branch Specific Diversification Model implemented in RevBayes[16, 17] to detect rate shifts across lineages. For all the models implemented in RevBayes[16] we assumed a uniform taxon sampling and an incomplete sampling fraction of 48/57. For the model selection, we ran a total of 5000 MCMC generations with a burnin of 1000 generations, all the parameter outputs were checked in Tracer[56] to assess the ESS, prior and posterior probability, and markov chain proper behavior. We estimated the mlnL of the models sampling the power posterior of each model for 1000 MCMC generations with a burnin of 10%. For the branch specific estimation, we assumed the heterogeneous model and we test several rate categories ($N=1$(constant), 4 and 10) across the lineages, for each rate we obtained the marginal probabilities and Bayes Factors as explained above. For the best selected model, we wrote a file with all the estimated parameters ($\lambda$ avg, $\mu$ avg, $\lambda-\mu$ avg, $\lambda/\mu$ avg and its 95% confidence ranges) for each branch that can be mapped over our BEAST time divergence tree and was visualized using FigTree (http://tree.bio.ed.ac.uk/software/figtree/).

Declarations
Ethics approval and consent to participate

Not Applicable

Consent for publication

All authors have read and approved the manuscript.

Author Contributions:

MSH performed field collection, lab work and analysis and drafted the manuscript.

CDB performed field collection and manuscript drafting. RN performed analysis and drafted components of the manuscript. CS and JW contributed to manuscript preparation.

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Competing interests

The author(s) declare no competing interests.

Availability of data and material.

Data are available in GenBank; see Supplementary Table S6 for accession numbers.

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Abbreviations

MCRA Most Common Recent Ancestor
RASP Reconstruct Ancestral State in Phylogenies
ANDES Andean uplift
P&AS Pebas and Acre Systems

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Tables

Table 1. Most likely estimated ancestral areas for the main nodes within the Polythoridae family. The best biogeographical models of the three scenarios (Control, Pebas and Acre Systems and Andes uplift), using both BioGeoBEARS (grey columns) and RASP (white columns) reconstruction methods, are shown. * indicates high uncertainty values for these nodes numbers correspond to Fig 2.
| Node-Clade/ Model                  | Control          | P&A Systems       |
|-----------------------------------|------------------|-------------------|
|                                   | DEC+J  | S-DEC  | DIVALIKE | S-DIVA  | DEC+J  | S-DEC  | DIVALIKE | S-DIVA  |
| Chalcopteryx - Chalcothore (node 43) | *CD     | EGI    | CD      | EG      | BE      | EG     | CD      | EG      |
| Cora sensu stricto (node 47)      | *C      | CDF    | *CDG    | CF      | DEG     | CDF    | CDG     | CF      |
| Miocora clade (node 49)           | C       | BC     | DFG     | BC      | C       | BC     | DFG     | BC      |
| Euthore sensu lato (node 56)      | C       | C      | FHI     | CD      | C       | C      | FHI     | CD      |
| Polythore (node 77)               | C       | CDG    | BD      | CDG     | DF      | CDG    | BD      | CDG     |
| Euthore s.l + Polythore (node 78) | C       | C      | C       | C       | C       | CDG    | C       | C       |
| (Euthore s.l + Polythore) + Miocora (node 79) | C | C | C | C | C | CDG | C | C |
| (Euthore s.l + Polythore) + Miocora + Cora s.s. (node 80) | C | CD | C | C | C | CDG | C | C |
| (((Euthore s.l + Polythore) + Miocora) + Cora s.s.) + Chalcopteryx (node 81) | *DGH | DFG | *DGH | CDG | *EGH | CDG | *BD | CDG | *I |

Table 2. Multiple comparisons of the Bayes Factors and marginal likelihoods for all the diversification models tested (Yule, Birth-Death (BD), Episodic Birth-Death (EBD); the latter with multiple episodes 4, 10 or 20).

| Model | mLikelihood | Bayes Factors |
|-------|-------------|---------------|
|       | Yule | BD | EBDN4 | EBDN10 |
| Yule  | -224 | 0  | 0     | 11     | 9     |
| BD    | -221 | 0  | 0     | 140    | 110   |
| EBDN4 | -226 | 11 | 140   | 0      | 1     |
| EBDN10| -226 | 9  | 110   | 1      | 0     |
| EBDN20| -226 | 12 | 153   | 1      | 1     |

Table 3. Fossils supporting each of the node calibrations and the prior distributions selected for the Bayesian time divergence analysis. Node numbers are depicted in Fig 1.
| Node/TAXA       | Fossil Classification and estimated ages                                                                 | Type Locality/PaleoDB                                  | Prior distribution |
|----------------|----------------------------------------------------------------------------------------------------------|-------------------------------------------------------|--------------------|
| Root           | Odonata, Zygoptera, Eosagriionidae, *Eosagriion risi* †, Early Jurassic, Toarcian, 183-182 Ma (Handlirsch, 1920) | Germany/ Dobbertin, Mecklenburg PaleodB 123987          | Uniform prior distrib max = 183 min = 183               |
| *Calopterygoidea* (node 52) | Odonata, Zygoptera, Calopterygoidea, Calopterygidae *Sinocalopteryx shanyongensis* †, Eocene, Ypresian (56 - 47.8 Ma) NIGP 151367 (Lin et al., 2010) | Yunnan, China PaleodB 113892                          |                    |
|                | Odonata, Zygoptera, Epallagidae, *Labandeiraia europae* †, Eocene, Ypresian (56 -47.8 Ma)                 | Island of Fur, Denmark PaleodB 123998, 127173          |                    |
|                | Odonata, Zygoptera, Epallagidae, *Ejerslevia haraldi* †, Eocene, Ypresian (56 -47.8 Ma) (Zessin, 2011)   | Ejerslev, Mors, Denmark PaleodB 157041                 |                    |
| Outgroup       | Odonata, Zygoptera, Epallagidae, *Labandeiraia americaborealis* †, Eocene, Bridgerian (50 -46.2 Ma) 31.665A-B (Petrulevicius et al, 2007) | Colorado, USA PaleodB = 107337                         | LogNormal; mean = 41; std = 0.15                       |
| *Euphaeidae* (node 99) | Odonata, Zygoptera, Epallagidae, *Litheuphaea coloradensis* †, Eocene, Bridgerian (50 -46.2 Ma) BMNH Pl I 562 (Petrulevicius et al, 2007) | Colorado, USA PaleodB = 107337                         |                    |
|                | Odonata, Zygoptera, Epallagidae, *Eodichroma mirifica* †, Late/Upper Eocene, (37.2 - 33.9) (Cockerell 1923) | Texas, USA PaleodB = 130390                           |                    |
|                | Odonata, Zygoptera, Epallagidae, *Litheuphaea ludwigi* †, Eocene, Priabonian (38 - 33.9 Ma) (Bechly,1990) |                                        |                    |
Odonata, Zygoptera, Epallagidae, *Elektroeuphaea flecki* †, Eocene, Priabonian (38 - 33.9 Ma) (Nel et al., 2013)  
Baltic Amber, Russian Federation  
PaleoDB = 123911

Odonata, Zygoptera, Epallagidae, *Parazacallites aquisextanea* †, Oligocene, Chattian (28.1-23.03 Ma)  
Baltic Amber, Poland  
PaleoDB = 123215

| Ingroup/ Polythoridae (node 53) | Odonata, Zygoptera, Polythoroidea, *Bolcathore colorata* †, Eocene, Lutetian (47.8 - 41.3 Ma) MCSNV I.G. 37582 (Gentilini, 2002) | Pesciara di Bolca, Italy |
|---|---|---|
| | Odonata, Zygoptera, Polythoroidea, *Bolcathore sp.* †, Eocene, Priabonian (38 - 33.9 Ma) MCSNV I.G. 37582 (Nel and Fleck, 2014) | Isle of Wight, United Kingdom |
| | Odonata, Zygoptera, *Protothore explicata* †, Eocene, Bartonian (41.3 - 38 Ma) (Cockrell, 1930) | California, USA |

**Figures**
Bayesian time-calibrated best tree obtained in BEAST v1.8.4 with median node ages

Time-Calibrated tree showing the ancestral area reconstruction of the S-DEC mod
Diversification and Extinction rate shifts within the Polythoridae family obtained using the RevBayes Branch... linear model. To the left, the net diversification rate is represented, while to the right is the relative extinction rate.

Supplementary Files

This is a list of supplementary files associated with the primary manuscript. Click to download.

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