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Forest giants on different evolutionary branches: Ecomorphological convergence in helicopter damselflies

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The convergent evolution of analogous features is an evolutionary process occurring independently across the tree of life. From the evolution of echolocation, prehensile tail, viviparity, or winged flight, environmental factors often drive this astonishing phenomenon. However, convergent evolution is not always conspicuous or easily identified. Giant damselflies count among the largest flying insects on Earth, and have astonishing ecologies including orb-web spider plucking and oviposition in phytotelmata. One species occurs in the Afrotropics and 18 species are found in the Neotropics. Convergent evolution was historically hypothesized based on the ecological and morphological affinities of these two geographically distant lineages but was not supported by earlier phylogenetic inferences supporting their monophyly. Using a molecular supermatrix approach and a large selection of outgroups, we revisit and reject the monophyly of Afrotropical and Neotropical giant damselflies that is otherwise supported by a morphological phylogeny. Molecular divergence time estimation suggests an origin of Afrotropical giant damselflies in the late Paleogene, and of Neotropical ones at the Cretaceous/Paleogene boundary, thereby rejecting a long-standing West Gondwana vicariance hypothesis. The strong ecological and morphological resemblances between these two independent lineages represents an astonishing case of Amphi-Atlantic tropical convergent evolution.

KEY WORDS: Convergent evolution, *Coryphagrion*, Pseudostigmatinae, West Gondwana vicariance, Zygoptera.

Behavioral, ecological, and morphological convergence in insects has been illustrated in some remarkable empirical cases. Distantly related butterfly lineages rely on mimicry to reinforce (Müllerian) and copy (Batesian) aposematic toxic species and thereby gain protection against predators (Bybee et al. 2012; Reed et al. 2011). Praying mantises (Mantodea) showcase a remarkable case of rampant ecomorphological convergence, by independently evolving morphological features that mimic grass, bark, twigs or flowers (Svenson and Whiting 2009; Svenson et al. 2015). Morphologically derived ground-dwelling stick-insects (Phasmatodea) constitute at least three independent lineages descending from arboreal ancestors (Buckley et al. 2009). A review of studies focusing on empirical examples of insect convergence other than mimicry and cryptic convergence demonstrates that insects are a key model to investigate and understand evolutionary convergence. For example, eusociality evolved independently in termites, ants, bees, wasps, and ambrosia beetles (Rust and Wappler 2016). A recent phylogenetic study of rove beetles (Staphylinidae) showed that myrmecophily in army ant colonies has evolved at least 12 times (Maruyama and Parker 2017). Among myrmecophilous lineages, different ant-nest beetle lineages (Coleoptera, Carabidae, Paussinae) evolved convergent phenotypes across the Afrotropics in response to high selective pressure from ant-host lineages (Moore and Robertson 2014). In Lepidoptera, hawkmoths (Sphingidae), owlet moths (Noctuidae), and pyralid moths (Pyralidae) have repeatedly and independently evolved tympanic organs possibly to allow the detection of bat echolocation (Conner and Corcoran 2012). Stick insects from the subfamily Korinninae are the only lineage that evolved egg-laying in ootheca, as observed across dictyopterans (cockroaches, mantises, termites) as well as other insect orders (Goldberg et al. 2015).

It is clear that convergence across insects is likely common if not rampant, but studies focused on testing for ecomorphological convergence driven by pressures such as behavior (*e.g.*, hunting strategy, oviposition) are lacking. Here, we outline a system for studying convergence in an insect group where ecomorphological convergence has yet to be demonstrated and is likely driven by both hunting and oviposition behavior.

The largest damselflies on Earth are referred to as forest giants or helicopter damselflies (Odonata, Zygoptera, Coenagrionidae, Pseudostigmatinae, Fig. 1). This clade contains 19 species of long-lived damselflies, placed in six extant genera (Garrison and von Ellenrieder 2016). All species occur in Neotropical forested regions with the exception of a unique African species *Coryphagrion grandis*. Sexually mature adults of Neotropical Pseudostigmatinae lay their eggs in phytotelmata such as tree holes (Fincke 1992; Corbet 2004), and more rarely in bromeliad tanks (Calvert 1911), a behavior also found in several other genera across Zygoptera and in particular within Coenagrionidae (*Bromeliagrion*, *Diceratobasis*, *Leptagrion*, and *Papuagrion*, Frank and Lounibos 2009). Adults are unique among odonates by actively hunting and plucking orb-weaver spiders and/or gleaning the wrapped prey of spiders from their webs (Fincke 1992; Corbet 2004). Adults of Pseudostigmatinae set up territories where orb-weaver spiders are present and will defend these territories against intruders (Corbet 2004). *Coryphagrion grandis* is the only member of Pseudostigmatinae that occurs outside of the Neotropics and is found in coastal rainforests of Kenya and Tanzania. Adult *C. grandis* have similar habits to Neotropical Pseudostigmatinae, with documented oviposition in tree holes and occasionally in coconut husks, and a diet of spiders and other arthropods plucked from spider webs (Clausnitzer and Lindeboom 2002). The Afrotropical and Neotropical Pseudostigmatinae also share a number of morphological similarities (*e.g.*, larval caudal appendages, abdominal and wing extreme modifications) that led some authors to suggest them as a candidate case of ecomorphological convergent evolution (Fincke 1992; Clausnitzer and Lindeboom 2002).

Several studies have investigated the phylogenetic relationships among Pseudostigmatinae (Rehn 2003; Groeneveld et al. 2007; Ingley et al. 2012; Feindt et al. 2014). All these studies supported a similar pattern with *C. grandis* recovered as sister to Neotropical Pseudostigmatinae, thereby supporting the hypothesis of single evolution of their unique hunting and oviposition behavior (Ingley et al. 2012). Nonetheless, these studies were possibly biased either by homoplastic morphological characters and/or a reduced taxon sampling, specifically among the selection of outgroups. Based on the hypothesized monophyly of Pseudostigmatinae, several authors (Groeneveld et al. 2007; Ingley et al. 2012) further suggested that the group originated from the vicariance between Africa and South America some 100–120 million years ago (Ma) (Seton et al. 2012). However, such patterns of West Gondwana vicariance are rare and could in fact be the outcome of long-distance dispersal or ancient extinctions (see Toussaint et al. 2017). This could be the case in Pseudostigmatinae, since *C. grandis* is distributed along the eastern coast of the Afrotropics, ~3000 kilometers from the western border of the African continent that was once connected by land to South America.

In this study, we use a phylogenetic approach based on both molecular and morphological datasets, and encompassing a greater taxon sampling than in previous studies, to address the following questions:

- Does the Afrotropical helicopter damselfly *C. grandis* share a common ancestor with the Neotropical Pseudostigmatinae (H1) or did it originate in a different clade, thereby supporting convergent evolution (H2)?
- In the case of convergent evolution (H2), what ecological and/or morphological features are of particular interest in this remarkable system?
- What is the age of the different Pseudostigmatinae lineages and can it be reconciled with West Gondwana vicariance?

Material and Methods

MORPHOLOGICAL PHYLOGENETICS

In order to test for morphological convergence between (*i.e.*, monophyly of) *C. grandis* and New World Pseudostigmatinae, we coded 153 morphological characters from Bybee et al. (2008) that were largely based on Rehn (2003), and for which 47 had the potential to be phylogenetically informative. The characters were coded for a set of genera representing the major lineages within the core Coenagrionidae, which had nearly identical overlap with the molecular dataset and included most Pseudostigmatinae. Characters were derived from both wing venation and skeletal structures. We used maximum likelihood (ML) and maximum parsimony (MP) methods to infer phylogenetic relationships using the morphological data. ML analyses were run using IQ-TREE 1.6.1 (Nguyen et al. 2015) and the MK+F model (Lewis 2001). Several additional MK+F models (MK+Γ4+F, MK+I+Γ4+F) were used to model the morphological data during phylogenetic reconstructions. All produced congruent topologies and only the results for MK+F analyses are shown (see Results). Bootstrap support was calculated from 1000 ultrafast bootstraps (Minh et al. 2013). MP analyses were run using TNT 1.1 (Goloboff et al. 2008) on the morphological matrix using the default settings. All analyses were run with gaps treated as missing data. Bootstrap and

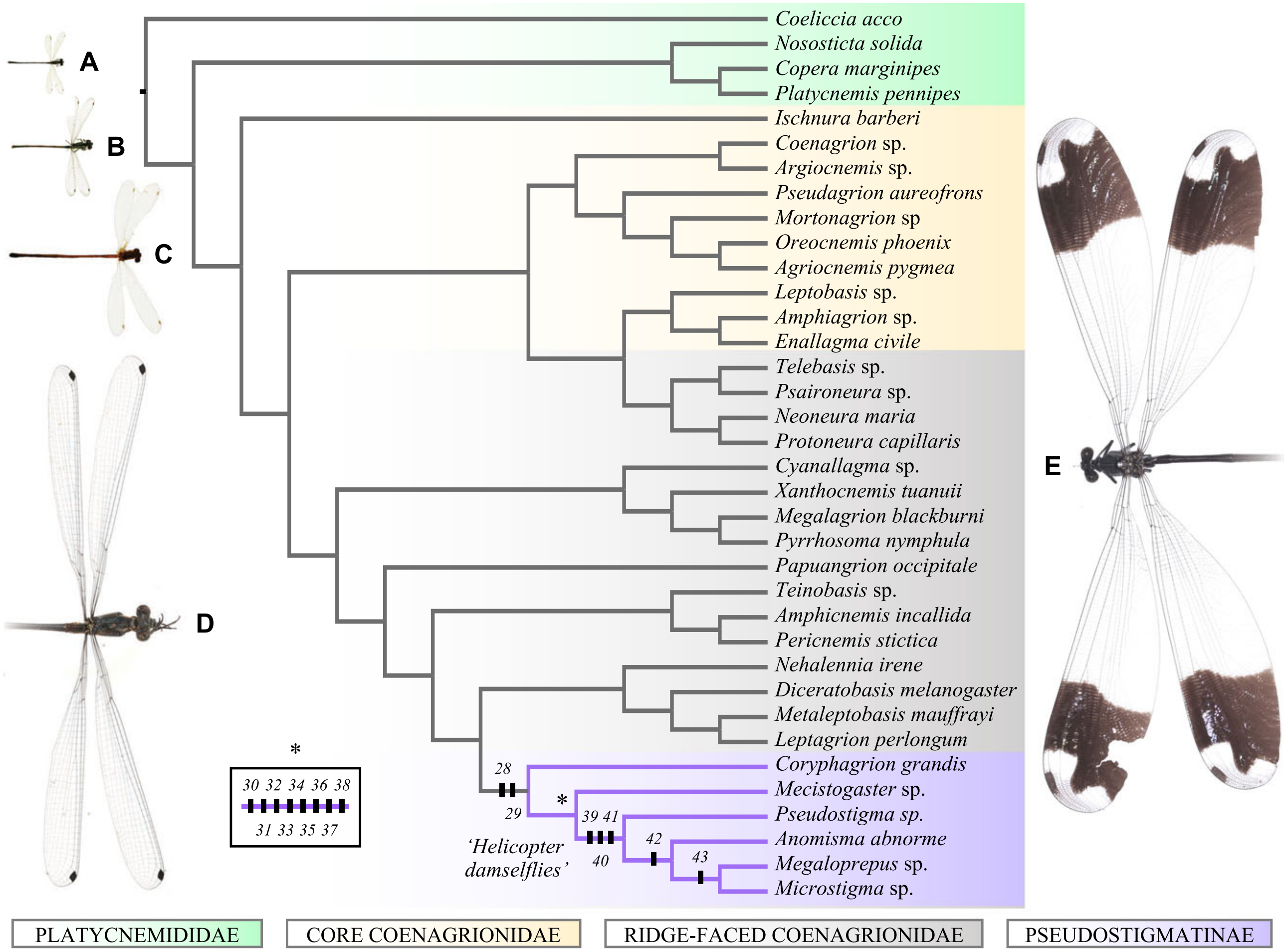

Figure 1. Phylogeny of Pseudostigmatinae inferred with maximum likelihood and 153 morphological characters. Black squares indicate synapomorphies within Pseudostigmatinae and as treated in TNT (*n.b.*, synapomorphies in the rest of the tree are not presented, see File S1 and Supplementary Information for more details). Photographs of habitus illustrate size discrepancies across Coenagrionidae (from top to bottom and left to right): (A) *Agriocnemis femina* (credit: National Taiwan University), (B) *Ischnura senegalensis* (credit: National Taiwan University), (C) *Ceriagrion latericium* (credit: National Taiwan University), (D) *Coryphagrion grandis* (credit: Ben Price, Natural History Museum in London), (E) *Megaloprepus caerulatus* (credit: Ben Price, Natural History Museum in London).

jackknifing values were generated in TNT using 1000 iterations. Jackknifing was performed using 80, 60, 40, and 20 percent of the morphological matrix. The input file containing the final matrix is given in File S1 and the details of morphological characters used to build the matrix are given in Supporting Information.

MOLECULAR PHYLOGENETICS

Since there is no described fossil of Pseudostigmatinae, we relied on alternative fossil calibrations to date the tree (see below). We carefully selected representatives among Coenagrionidae and its sister family Platycnemididae following the most recent phylogenetic studies of the suborder Zygoptera (Bybee et al. 2008; Dumont et al. 2010; Dijkstra et al. 2014) (see Table S1 for detailed information on taxon sampling). We included the three families of Coenagrionoidea recognized by Dijkstra et al. (2014): Isostictidae, Coenagrionidae, and Platycnemididae. The tree was rooted with *Labidiosticta vallisi* (Isostictidae) following the comprehensive phylogenies of Zygoptera cited above. We sampled representatives of the subfamilies Allocnemidinae, Calicnemiinae, Disparoneurinae, and Platycnemidinae, belonging to the two large platycnemidid clades found in Dijkstra et al. (2014). In total, we sampled 33 coenagrionid genera, following the studies of Bybee et al. (2008), Dumont et al. (2010), and Dijkstra et al. (2014). In particular, we included representatives of the two major taxonomic groups recognized by Dijkstra et al. (2014), the core Coenagrionidae (12 genera included), and the ridge-faced complex (21 genera included) to which Pseudostigmatinae belong. In total, we sampled 13 species of Pseudostigmatinae out of 19 described species (*ca.* 70% of the clade diversity).

We obtained the DNA sequence data from GenBank, and imported it in Geneious R8 (Biomatters, USA) for cleaning and assembly. We selected the nine following gene fragments;

ribosomal 12S (353 base pairs, bp), 16S (528 bp), 18S (1,816 bp), 28S (2,127 bp), cytochrome c oxidase subunit 1 (CO1, 657 bp), cytochrome c oxidase subunit 2 (CO2, 567 bp), elongation factor 1 alpha (EF1a, 411 bp), histone 3 (H3, 324 bp), and NADH dehydrogenase subunit 1 (ND1, 330 bp). Considering possible cryptic speciation in *Coryphagrion* (Bergmann et al. 2013), we sampled one specimen of each *C. grandis* population (Kenyan and Tanzanian). The final matrix contained a total of 85 taxa. The protein-coding gene fragments were aligned using MUSCLE (Edgar 2004), and the ribosomal gene fragments were aligned using MAFFT 7 (Katoh and Standley 2013), with the Q-INS-I algorithm to account for RNA secondary structures. Individual alignments were then checked manually for coding frames and obvious errors. The final concatenated alignment comprised 7113 bp with 40.8% of missing data (including gaps that are not taken into account in the phylogenetic methods used below) with no obvious bias in GC content (48.9% of GC).

We used MrBayes 3.2.6 (Ronquist et al. 2012) to infer phylogenetic relationships. The partitions and corresponding models of substitution were selected in PartitionFinder 1.1.1 with the greedy algorithm (Lanfear et al. 2012). The ribosomal gene fragments were left unpartitioned, and the protein-coding gene fragments were partitioned by codon position (one partition for each codon position, *i.e.*, three partitions per gene fragment). The corrected Akaike Information Criterion was used to select the best-fit model for each partition. We performed two independent runs consisting of eight Markov Chain Monte Carlo (MCMC) of 30 million generations sampled every 3000 generations. The convergence of the different runs was assessed by checking the potential scale reduction factor (PSRF) and effective sample size (ESS) of all parameters, as well as the standard deviation of split frequencies. We assumed convergence when PRSF and ESS of each parameter was close to 1.0 and above 200, respectively, and when the standard deviation of split frequencies was close or below 0.01. As a result, the first 2500 posterior samples that predated convergence of the runs were discarded as burn-in (*i.e.*, 25%) and the remaining samples were used to generate a 50% majority-rule consensus phylogenetic tree. The MrBayes input file containing the final matrix is given in File S2.

TOPOLOGICAL CONSTRAINT ON THE MONOPHYLY OF PSEUDOSTIGMATINAE

To test the monophyly of Pseudostigmatinae in a statistical framework using the molecular matrix, we performed different analyses, with or without enforcing the monophyly of the subfamily. The MrBayes analyses were run with the same priors and parameters as described above. The marginal likelihood of each analysis was computed using stepping-stone sampling (Xie et al. 2011), with 100 steps of 100,000 generations each. The marginal likelihood estimates (MLE) were then compared using Bayes factors (BF) following Kass and Raftery (1995). We considered $2\text{x}\log_e(\text{BF})$ values >10 as significantly favoring a model over another (Kass and Raftery 1995). The MrBayes input file with the topological constraint enforced is given in File S3.

DIVERGENCE TIME ESTIMATION

The dating analyses were performed in BEAST 1.8.4 (Drummond et al. 2012). The best partitioning scheme and corresponding models of nucleotide substitution were selected in PartitionFinder for the models included in BEAST.

Based on an extensive review of the fossil record in Coenagrionidae and Platycnemididae, we selected three fossil calibrations to constrain internal nodes of the phylogeny (see Supplementary Information for more details). †*Diceratobasis worki* (Dominican amber, *ca*. 15 Ma) was used to calibrate the stem of the genus *Diceratobasis*, †*Ischnura velteni* (Dominican amber, *ca*. 15 Ma) was used to calibrate the stem of the genus *Ischnura*, and †*Palaeodisparoneura burmanica* (Burmese amber, *ca*. 99 Ma) was used to calibrate the stem of the family Platycnemididae. All fossil calibration priors were set with an upper bound equal to the most recent estimated age of the crown Zygoptera (*ca.* 215 Ma, Thomas et al. 2013). We used both soft exponential and hard uniform prior distributions to enforce fossil information in different analyses as recommended by Ho and Phillips (2009). These prior distributions permit to take into account the taphonomic bias (incomplete fossil record) in two different ways (equal probability between hard bounds vs diminishing tail of probability between soft bounds). Note that the exponential priors, unlike the uniform ones, allow an additional diminishing tail of probability beyond the two soft bounds (minimum and maximum ages). The exponential prior distributions used to implement Dominican amber fossil information were set with a Mean = 22.7 and an Offset = 14.43. The one used to implement the Burmese amber fossil information was set with a Mean = 31.89 and an Offset = 97.36. These settings allowed 95% of the prior distribution to be comprised between the minimum age derived from fossil information and the maximum age derived from Thomas et al. (2013).

In addition to testing different fossil prior distributions (exponential/uniform), we tested three different clock partitioning schemes: (1) a unique clock for all partitions; (2) a clock for the mitochondrial gene fragments and another one for the nuclear gene fragments (2 clocks); and (3) a clock for each partition selected in PartitionFinder (10 clocks). We also tested two different tree models, Yule or birth-death. The partitions were assigned Bayesian uncorrelated lognormal relaxed clock models to take into account rate heterogeneity across the phylogeny. We performed independent MCMC runs with 200 million generations, with a sampling frequency every 10,000 generations. We estimated MLE for each analysis using path-sampling and stepping-stone sampling (Baele et al. 2012; Xie et al. 2011), with 1000 path steps, and chains running for one million generation with a log-likelihood sampling every 1000 cycles. After checking for convergence of the Bayesian runs with Tracer 1.7 (Rambaut et al. 2018), we discarded the first 10% of generations as the burn-in period. We computed the maximum clade credibility tree with median ages and the 95% credibility interval (CI) at each node using TreeAnnotator 1.8.4.

Results and Discussion

ARE PSEUDOSTIGMATINAE GIANT DAMSELFLIES MONOPHYLETIC?

We inferred a phylogeny of damselflies from morphological data alone (153 characters) to test if *Coryphagrion* and Neotropical Pseudostigmatinae are monophyletic, suggesting a single origin of gigantism and associated characters, (H1), or if they do not form a clade, suggesting evolutionary convergence (H2). The results of our morphological phylogenetic reconstructions were congruent in both the ML and MP topologies (Fig. 1 and Fig. S1). Both the ML and MP topologies recovered Neotropical Pseudostigmatinae as monophyletic (BS = 100 and 98, respectively). *Coryphagrion* was always recovered as sister group to the Neotropical Pseudostigmatinae, with moderate bootstrap support (ML = 92, MP = 65). These results support H1 (monophyly of Pseudostigmatinae), where *Coryphagrion* and Neotropical Pseudostigmatinae form a clade, therefore providing evidence for the unique evolution of a complex suite of morphological and ecological features.

On the other hand, we inferred a molecular phylogeny of helicopter damselflies and closely related lineages based on nine gene fragments. The results of Bayesian inferences recovered a well-resolved phylogeny with moderate to strong support for most nodes (Fig. 2). We did not recover Pseudostigmatinae as monophyletic, with *Coryphagrion grandis* being inferred as sister to the monotypic African genus *Oreocnemis* with strong nodal support (PP = 1.0). The Neotropical Pseudostigmatinae were recovered as monophyletic with moderate support (PP = 0.94), in a derived position of the tree and as sister to the Neotropical genus *Metaleptobasis* with low support (PP **=** 0.55). We also ran topological tests to confirm the non-monophyly of Pseudostigmatinae using MLE in a Bayesian framework. We recovered a MLE = –50,802.87 for the unconstrained topology, and a MLE = –50,814.33 for the constrained topology, providing strong evidence for the non-monophyly of Pseudostigmatinae (2xlog(BF) = 22.92). Based on the results of our analyses, it is clear that these lineages do not form a monophyletic group and that the astonishing morphology and ecology of helicopter damselflies are the result of convergent evolution (Fig. 2).

ORIGIN AND EVOLUTIONARY CONVERGENCE OF GIANT DAMSELFLIES

The results of the BEAST analyses and the age estimates of focal clades including the ones comprising the fossil calibrations are summarized in Table 1. The dating analyses (exponential/uniform, Yule/birth-death, different clock partitioning schemes) are mostly congruent with close median ages and 95% CI broadly overlapping. The choice of prior distribution and number of clocks resulted in marginally contrasting results with tree model choice having little impact on the inferred divergence times (Table 1). Based on MLE comparison, the best BEAST analysis included two clocks (mitochondrial/nuclear), a birth-death tree model and uniform fossil prior distributions (Table 1). The median divergence time estimates of this analysis are given in Figure 3, and we discuss these below.

We inferred an origin of Coenagrionidae in the Lower Cretaceous about 118.0 Ma with a 95% CI of 85.0–173.2 Ma. These ages are substantially older than the most ancient fossils described and unambiguously assigned to the family from Dominican amber (see above). The divergence between *Coryphagrion* and *Oreocnemis* is recovered around the Eocene-Oligocene boundary (median = 35.3 Ma, CI = 14.8–62.9 Ma), a period marking the transition from warm to cold climates (Zachos et al. 2008). The divergence between the Kenyan and Tanzanian populations of *Coryphagrion grandis* is estimated in the Pleistocene (median = 2.0 Ma, CI = 0.6–4.1 Ma). This age estimate supports the view of *Coryphagrion* as potentially representing a cryptic species complex (Bergmann et al. 2013). We find an ancient origin for the diversification of Neotropical Pseudostigmatinae, which likely originated around the Cretaceous-Paleogene boundary (median = 65.4, CI = 43.1–97.7 Ma), shortly after the divergence from *Metaleptobasis*. Our results suggest a 65 million-year evolutionary history for the Neotropical giant damselflies that gave rise to only 18 extant species. This indicates that “Pseudostigmatinae” on both sides of the Atlantic Ocean are truly relictual lineages within Zygoptera, especially considering the diversity in closely related genera (*e.g.*, *Metaleptobasis ca.* 30 species, *Teinobasis ca.* 70 species).

Our phylogenetic and dating analyses clearly reject the monophyly of Pseudostigmatinae and highlight two important results. First, and despite the moderate support for the new phylogenetic hypothesis, our divergence time estimation recovers a split between the Malawian endemic *Oreocnemis* and the Kenyan and Tanzanian populations of *Coryphagrion grandis* in the Eocene, and an origin of Neotropical Pseudostigmatinae in the Paleocene. Although the origin of these clades is ancient, these age estimates rule out the hypothesis of a West Gondwanan vicariant origin because the latter implies an origin of African and Neotropical clades around 100–120 Ma (Seton et al. 2012; Toussaint et al. 2017). Second, and more interestingly, the non-monophyly of giant damselflies represents a remarkable case of ecomorphological convergence on each side of the Atlantic Ocean. Given our phylogenetic hypothesis, this evolutionary convergence involves at least five features:

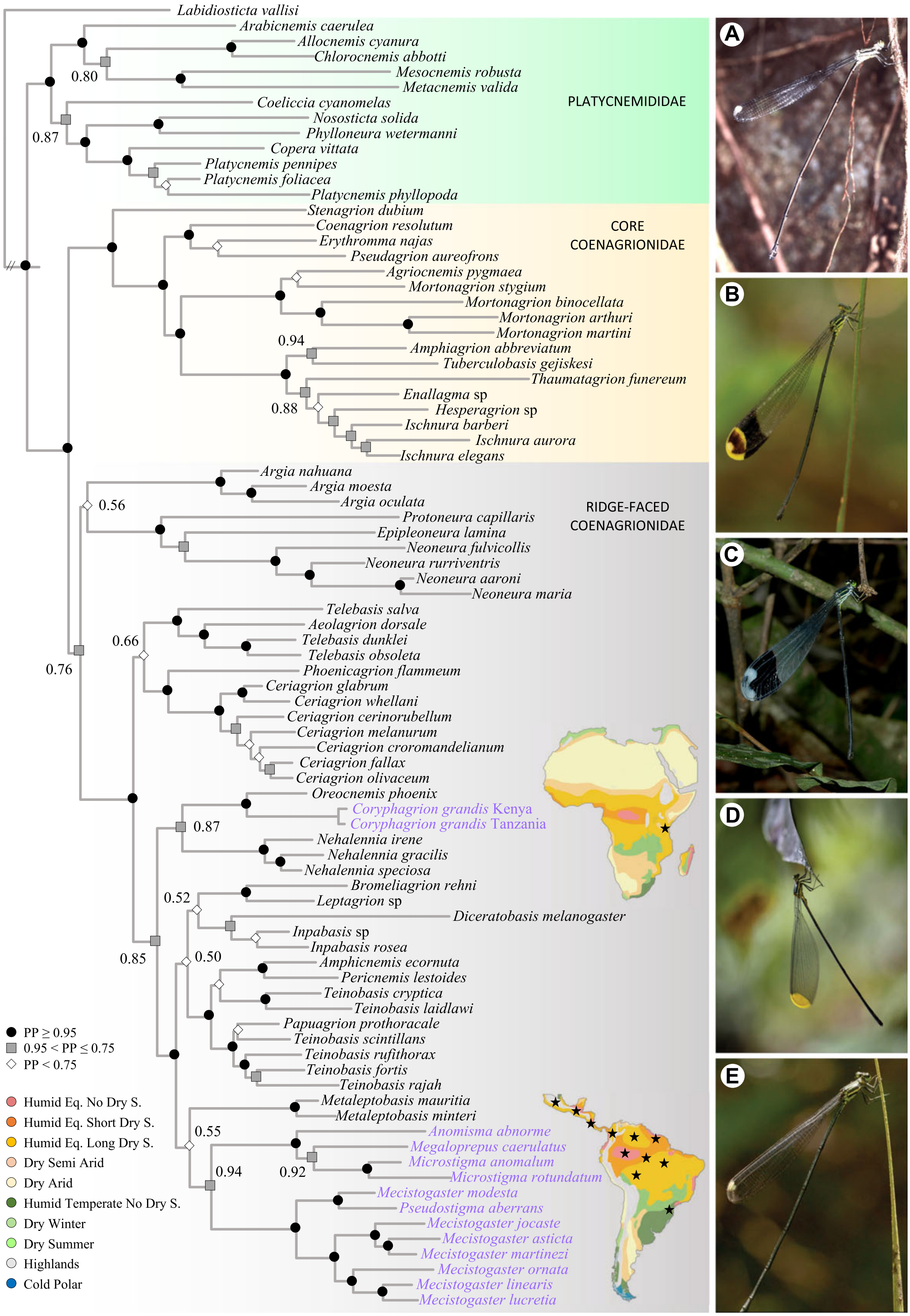

Figure 2. Molecular phylogeny of Pseudostigmatinae inferred with Bayesian inference and nine gene fragments.The approximate distribution of Pseudostigmatinae in Afrotropics and Neotropics is indicated with black stars. Pictures: (A), *Coryphagrion grandis* (Credit: Viola Clausnitzer); (B), *Anomisma abnorme* (Credit: Karl Kroeker); (C), *Megaloprepus caerulatus* (Credit: Thomas Schultz); (D), *Microstigma* sp. (Credit: Almir Cândido de Almeida); (E), *Pseudostigma accedens* (Credit: Karl Kroeker).

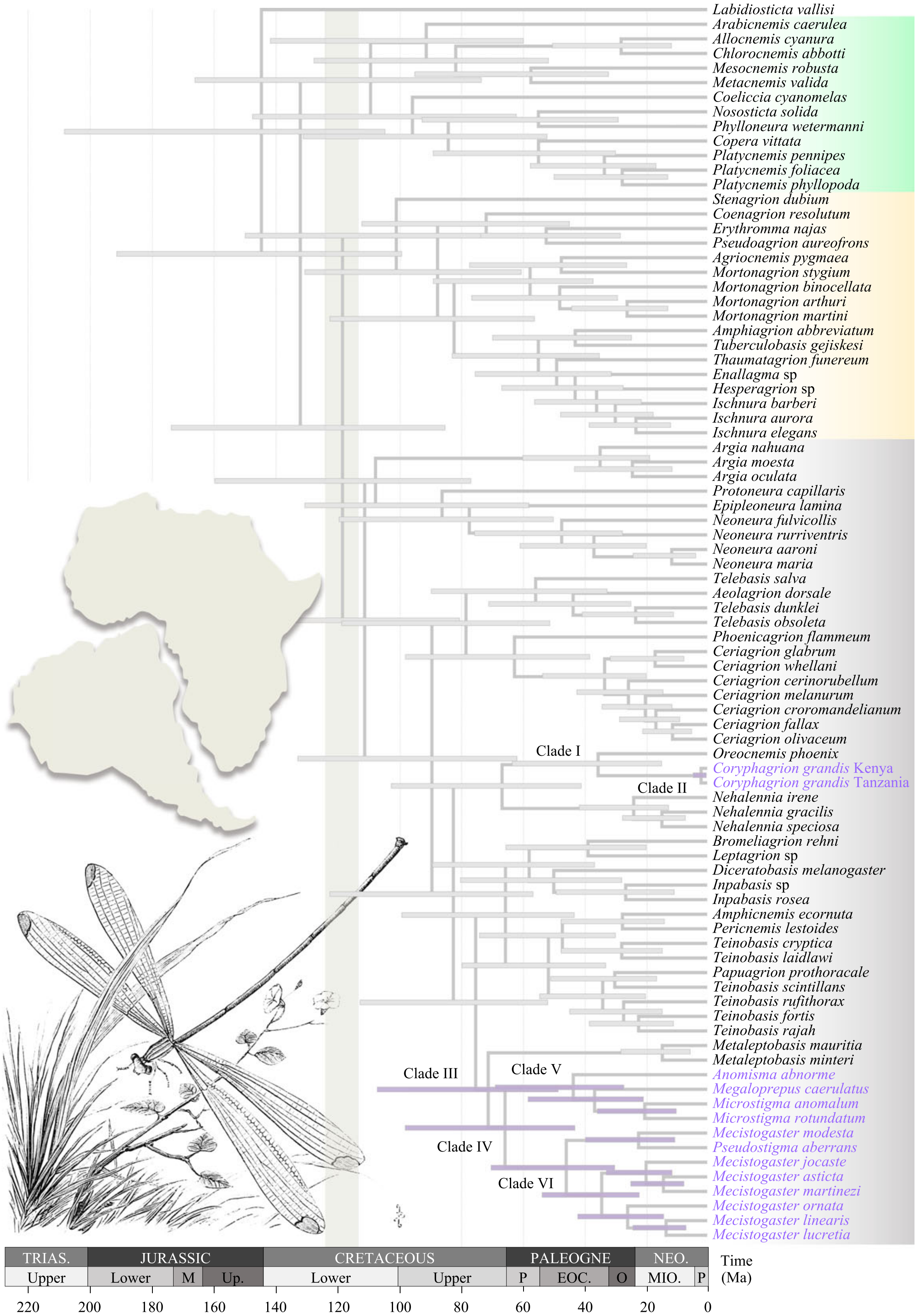

Figure 3. Bayesian divergence time estimates within Coenagrionidae and Platycnemididae. Maximum clade credibility tree with median ages derived from the best BEAST analysis using uniform fossil prior distributions, a birth death tree model and a separate clock for mitochondrial and nuclear gene fragments. The 95% credibility interval for each node is given with a horizontal gray bar. The split between Africa and South America is highlighted with a beige bar.

Table 1. Bayesian median estimates of major clade crown divergence times inferred in different BEAST analyses.

Clocks	Model	Prior	PS MLE	SS MLE	Root	Platycnemididae	*Ischnura*	*Diceratobasis**	Neo. Pseudo.	*Coryphagrion*
1 UCL	BD	Exp.	–52281.514	–52192.523	118.215	107.149	27.160	38.200	50.991	1.377
1 UCL	Yule	Exp.	–52243.296	–52194.132	117.890	107.046	27.252	38.310	51.128	1.364
1 UCL	BD	Unif.	–52357.345	–52356.505	145.960	132.282	34.122	48.603	62.864	1.672
1 UCL	Yule	Unif.	–52355.833	–52354.699	147.891	132.540	34.281	49.251	63.291	1.694
2 UCL	BD	Exp.	–52121.061	–52119.856	120.366	106.864	28.453	39.114	53.558	1.605
2 UCL	Yule	Exp.	–52121.545	–52120.122	120.293	107.062	28.348	38.746	53.430	1.619
2 UCL	**BD**	**Unif.**	**–52045.027**	**–52025.088**	**146.306**	**131.613**	**35.759**	**49.619**	**65.389**	**1.963**
2 UCL	Yule	Unif.	–52120.151	–52118.845	146.303	132.066	35.760	49.945	65.877	1.979
10 UCL	BD	Exp.	–52174.492	–52173.997	117.647	107.306	31.007	35.677	58.532	2.956
10 UCL	Yule	Exp.	–52175.471	–52175.034	117.632	107.254	31.037	35.356	58.865	2.966
10 UCL	BD	Unif.	–52175.210	–52175.019	145.726	135.990	39.239	47.300	73.601	3.671
10 UCL	Yule	Unif.	–52175.675	–52176.455	145.725	135.859	39.659	48.284	73.650	3.639

ULC, uncorrelated lognormal clock; BD, birth-death; Exp., exponential fossil prior distribution; Unif., uniform fossil prior ditribution; PS, path sampling; MLE, marginal likelihood estimate; SS, stepping-stone sampling; *Diceratobasis*,* stem age of *Diceratobasis*; Neo. Pseudo., Neotropical Pseudostigmatinae.

(i) morphological similarity, (ii) gigantism (Neotropical giant damselflies have a wingspan up to 19 cm and *Coryphagrion* up to 15 cm), (iii) similar climatic niche (both are restricted to tropical rainforests), (iv) oviposition in phytotelmata, and (v) exceptional convergence of feeding behavior with both lineages hunting orb-web spiders and their prey (Corbet 2004).

The combination of the five features outlined above is a compelling reason to invoke convergent evolution. Yet, there are several genera of damselflies that also oviposit in phytotelmata (Frank and Loubinos 2009, Kalkman and Orr 2016) and a few genera that have been observed consuming spiders (Pers. comm. K. Tennessen, Pers. observ. S. Bybee), although these spiders were not plucked from webs. Therefore, it is important to place the hypothesis of convergent evolution in a detailed morphological context. Beyond the convergence of characters from the phylogenetic analysis, both *C. grandis* and the members of the Neotropical Pseudostigmatinae have extended abdominal segments, sometimes to the extreme (*e.g*., *Mecistogaster*), to facilitate oviposition in many different types of phytotelmata such as deep tree holes, water containers between leaf axils (*e.g*., both epiphytic and terricolous bromeliads) and bamboo (Fincke 2006). They also present similar larval morphological features such as branched setae on tibiae and tarsi, a unique leaf-like shape of the caudal appendages and two end-teeth on the labial palpi (Clausnitzer and Lindeboom 2002). The wings of both groups are also modified to become longer. *Anomisma, Megaloprepus,* and *Microstigma* also have expanded wings due to intercalated wing vein networks between major radial veins and/or apical branching of radial veins, which is likely a derived condition (see Ingley et al. 2012, Fig. 1A–C). *Coryphagrion grandis, Pseudostigma,* and *Mecistogaster* exhibit comparatively little to no wing expansion although the wings are modified to become longer. Additional wing modification involves the pterostigma, the heavier weighted and colored cell in the apical portion of the odonate wing, which has been demonstrated to enhance the gliding speed of dragonflies up to 25% (Norberg 1972). Both *C. grandis* and the remaining Pseudostigmatinae demonstrate a modification of the pterostigma in several ways. The pterostigma among the New World genera is expanded and may appear as a heavy network of veins and/or multiple colored cells in at least the forewing called the pseudostigma. The pseudostigma may function to dampen vibration over the large wing during flight, similarly to the pterostigma (Nordberg 1972). It might also play a role in species discrimination (Córdoba-Aguilar et al. 2003; Contreras-Garduño et al. 2008; Svensson & Waller 2013). Members of *Microstigma* and *Mecistogaster* may have a simple pseudostigma or even a pterostigma in the hindwing. A pterostigma is present in both fore- and hindwing of *C. grandis*, but it is unusual in that it is inflated (presumably to hold more hemolymph during flight to dampen vibration) and thicker than the surrounding wing. The pterostigma of other zygopterans and even the supposed sister genus to *C. grandis*, *Oreocnemis*, is flattened and the thickness is more in line with the rest of the wing. The lengthening expanding of the wing presumably supports an efficient hovering style of flight required to pluck spiders and their prey from a web as well as oviposition in phytotelmata. Such lengthening and/or broadening must require modifications to support flight for such a large wing. Although currently untested, the pseudostigma, whether a heavy network of veins and/or multiple colored cells, very likely plays a role in the flight kinetics of helicopter damselflies and their ability to hover while conducting their highly specialized hunting strategy. Thus, it would follow that convergence would drive modification of wing length and wing structures to support convergent lifestyles. There are other morphological features that could be explored between *C. grandis* and the New World Pseudostigmatinae (*e.g.*, thoracic angle, genitalia, abdominal appendages, etc.), but such features are highly plastic across Zygoptera.

Without molecular data, when considering overall morphological similarity in combination with the ecological similarities between *C. grandis* and the New World Pseudostigmatinae, one could easily be "fooled" into placing them into the same taxonomic group (Fig. 1). Indeed, they exhibit a fantastic example of ecomorphological convergent evolution driven by similar hunting and oviposition behavior on two different and distant continents.

CONCLUDING REMARKS

Spider feeding among other coenagrionid genera has been observed and orb-weaver spiders have a worldwide distribution with a relatively high diversity, especially in the tropics (Cardoso et al. 2011). A cursory examination of the morphology of *C. grandis* and Neotropical Pseudostigmatinae demonstrates that occupying a niche of both spider feeding and oviposition in phytotelmata requires relatively extreme morphological change. Not only are there other genera of damselflies that occupy phytotelmata but other insect groups as well (*e.g.*, riffle bugs, Polhemus and Polhemus 1991; diving beetles, Balke et al. 2008; see Frank and Loubinos 2009 for a review), that likely compete directly with helicopter damselflies. Further, the size and seasonality of phytotelmata could be a constraint on the development of such large insects as food and both the amount and duration of water in phytotelmata is limited (Fincke 1992).

Finding an example of convergence among such conspicuous taxa highlights the need to assess similar patterns more deeply and broadly across invertebrates, notably within insects. Recently, a few other examples have been proposed (*e.g.*, Buckley et al. 2009; Conner and Corcoran 2012; Maruyama and Parker 2017), illustrating how intricate the evolution of derived ecological and morphological features can be. Based on our results, we propose giant damselflies as a complimentary, textbook case of ecomorphological convergence among invertebrates. Further fine-scale studies will be paramount to understand which factors act as evolutionary triggers to shape ecomorphological convergence across the tree of life.

AUTHORS' CONTRIBUTIONS

E.F.A.T. conceived the study, analyzed the data, designed the figures, and drafted the manuscript; S.M.B. reviewed the fossil record, and helped drafting the manuscript; R.E. generated the morphological data with help from S.M.B., analyzed it and helped drafting the paper; F.L.C. helped performing analyzes and drafting the manuscript.

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Figure S1.
Table S1.