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Molecular phylogenetics of Atlantic Shield *Platynectes* diving beetles (Coleoptera: Dytiscidae): a first glance at the evolution of the genus in the Amazon Basin

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**Summary.** Neotropical diving beetles of the genus *Platynectes* are distributed across Central America, the Andes and different Precambrian shields in the Amazon Basin. Species from the northern Guiana Shield form a monophyletic clade, yet the phylogenetic relationships of the eastern Atlantic Shield species remain unknown. Here, we augmented an existing molecular dataset with a species from the Atlantic Shield that was not previously sampled. We reconstructed the phylogenetic relationships and estimated divergence times to understand the evolution of lineages dwelling in this region. The newly sampled specimens from the Atlantic Shield are recovered as sister taxa to Guiana Shield species. The dating analyses suggest a split between these two lineages in the late Oligocene to mid-Miocene, contemporary with the Miocene geological remodeling of the Amazon Basin. Additional sampling in the Atlantic and Central Brazilian Shields will be determinant to test the monophyly of *Platynectes* species distributed in these ancient shields, and to fully understand the biogeographical history of diving beetles in the Amazon Basin.

**Keywords:** Agabinae; Guiana Shield; Neotropical biogeography; Platynectini; Purus Arch

The recently defined tribe Platynectini Toussaint & Balke (Coleoptera, Dytiscidae, Agabinae) is comprised of only the diving beetle genus *Platynectes* Régimbart, 1879 (Toussaint et al. 2016). This genus is moderately diverse (76 described species), and occurs on both sides of the Pacific Ocean with a disjunct distribution (Nilsson 2015; Gustafson et al. 2016; Toussaint et al. 2016). *Platynectes* is found in tropical Southeast Asia, from Japan to Australia, and in the Neotropics, from Costa Rica to the southern Andes but also in Precambrian geological shields (Nilsson 2015; CReAC database available at [http://creac.kubiodiversityinstitute.org/collections](http://creac.kubiodiversityinstitute.org/collections)). The genus is currently described from the Guiana and Atlantic Shields, but is not known from the Central Brazilian Shield (Figure 1). There are currently 23 described species occurring in the Neotropics (Nilsson 2015; Gustafson et al. 2016). The subgenus *Agametrus* Sharp, 1882, comprises 12 species distributed in Central America and the Andes. The other Neotropical *Platynectes* species are restricted to the Brazilian Shield, a geological entity comprising the Atlantic Shield in the eastern coastal region, Central Brazilian Shield in the Amazonian region, and Guiana Shield in the North (De Alkmim 2015). Gustafson et al. (2016) recently revised this Neotropical clade (clade C2 in Toussaint et al. 2016), in which they recognized 11 species, seven of which occur in the Guiana Shield and four others in the Atlantic Shield. A recent comprehensive molecular phylogeny of the subfamily Agabinae recovered the Central American/Andean and Guiana Shield species in two separate and distant clades within *Platynectes* (Toussaint et al. 2016). Although the exact evolutionary
mechanisms responsible for this pattern are somewhat elusive, it is possible that the Central American/Andean clade resulted from late Miocene dispersal out of Australia, whereas the Guiana Shield clade might have originated from Gondwanian stock (Toussaint et al. 2016). Unfortunately, species from the Atlantic Shield could not be included in this previous study, although they might be of crucial importance to understand the evolution of Neotropical Platynectes lineages with respect to the geological evolution of the region in the Cenozoic.

The South American Plate is among the most ancient geological entities of the continent. This assemblage of Precambrian rocks and more recent Phanerozoic terrains constitutes most of present day South America except for the Andean and Patagonian regions (De Alkmim 2015). The geological history of South America is particularly complex, and consists of early tectonic events and more recent orogenies such as the rise of the Andes (Hoorn & Wesselingh 2010). While the different entities constituting the Brazilian Shield are very old, the connectivity between these has been likely shaped by the Cenozoic orogeny of the Andes, and subsequent paleodrainage system remodeling (Hoorn & Wesselingh 2010). Briefly, the recent Cenozoic evolution of paleodrainage systems in the Amazon Basin can be summarized in four major phases (Hoorn & Wesselingh 2010):

![Geological map of South America with the geographical range of Platynectes Régimbart diving beetles in the Brazilian Shield. Map of South America showing the collecting localities of Platynectes specimens used in this study as well as all known localities of the genus published in faunistic and taxonomic papers. The map also depicts the different geological regions of the continent based on the reconstruction of De Alkmim (2015). Pictures of Platynectes agallithoplotes (Gustafson et al. 2016) (left) and the Brazilian Platynectes species sampled in this study (right) were respectively taken by Andrew Short and Jennifer Girón (University of Kansas).]
– a precursor phase when the orogeny of the Andes triggered the transition from craton system to Andean fluvio-lacustrine system ~24–16 million years ago (Ma);
– the Pebas phase when an extensive lacustrine network, the Pebas wetlands, originated as a result of increased rainfall and occasional marine incursions west of the Amazon Basin ~16–11.3 Ma;
– the Acre phase when paleodrainages breached through the Purus Arch connecting at the time the Guiana and Central Brazilian Shields ~11.3–7 Ma;
– the contemporary Amazon phase when paleodrainages gave rise to the actual fluvial system composing the Amazon River ~7 Ma–present.

Considering that the crown of Guiana Shield Platynectes has been dated from the mid-Miocene (c.17 Ma; Toussaint et al. 2016), it is possible that the remodeling of the Amazon Basin had an impact on the biogeographical history of the group. In order to understand the evolution of the genus in the different shields of the basin, we sampled two Platynectes specimens of a species from the Atlantic Shield and inferred their phylogenetic relationships with respect to the rest of the genus. The main aims of the study were: (1) to test the hypothesis that the Atlantic and Guiana Shield faunas are more closely related to each other than to the Central American/Andean fauna, and (2) to test the competing hypotheses of vicariance versus dispersal to explain the present-day distribution of Atlantic Shield lineages with respect to the Cenozoic tectonic events in the Neotropical region.

Material and methods

**Taxon sampling and molecular biology**

Specimens of the genus Platynectes were collected and loaned to us by members of the Laboratório de Entomologia, Departamento de Zoologia, Universidade Federal do Rio de Janeiro (UFRJ) in the vicinity of Rio de Janeiro in Brazil during the spring of 2016 (Figure 1). The specimens were preserved in 96% ethanol and kept frozen at −20°C. Total genomic DNA was extracted from entire beetles with blood and tissue kits (Qiagen Inc., Alameda, CA, USA) according to the manufacturer instructions and frozen at −20°C. We used the same PCR protocols as in Toussaint et al. (2016) to amplify and sequence a fragment of the nuclear gene histone 3 (H3, 276 bp). The DNA sequences were edited in Geneious R8.0.5 (Biomatters, http://www.geneious.com/), and then aligned with the molecular matrix from Toussaint et al. (2016) using Muscle (Edgar 2004). The original dataset consisted of 90 species, some undescribed, sampled across the subfamily Agabinae, and sequenced for six gene fragments: cytochrome oxidase subunit 1 (Co1, 750 bp), cytochrome b (Cytb, 339 bp), arginine kinase (Ark, 687 bp), histone 3 (H3, 276 bp), histone 4 (H4, 153 bp) and RNA polymerase 2 (Rnp2, 669 bp). The final alignment comprised 92 specimens (73 Platynectes) for a total of 2874 bp. New sequences were deposited in GenBank (accession numbers KY046249-KY046250).

**Phylogenetic relationship inference**

We reconstructed the phylogenetic relationships among Platynectes using IQ-TREE (Nguyen et al. 2015) as implemented on the IQ-TREE web server (http://iqtree.cibiv.univie.ac.at/). The best partitioning scheme for the concatenated dataset was selected under PartitionFinder 1.1.1 (Lanfear et al. 2012) using the greedy algorithm, the all set of models and the BIC. The best-fit models of substitution for each partition were searched using the Auto function on the IQ-TREE web server based on the Bayesian information criterion (BIC). We performed 1000 ultrafast bootstrap replicates (Minh et al. 2013) to investigate nodal support across the topology.

**Divergence time estimation**

We inferred divergence times using the same approach developed in Toussaint et al. (2016) with BEAST 1.8.2 (Drummond et al. 2012). Only one Atlantic Shield specimen was kept in the dataset in order to infer divergence times at the species rank. Therefore, we pruned one specimen from the MrBayes consensus topology and used the latter as a fixed starting tree for the BEAST analysis. The dataset was partitioned by gene fragment with all substitution models unlabeled. The models of nucleotide substitution for each partition were selected under PartitionFinder 1.1.1 (Lanfear et al. 2012) using the greedy algorithm, the beast set of models and the BIC. The clock models were unlabeled except for the mitochondrial gene fragments (CO1 and CytB). The molecular clock test was performed in MEGA6 (Tamura et al. 2013) by comparing the maximum likelihood value of the IQ-TREE topology with and without the molecular clock constraints under the Tamura-Nei model. The null hypothesis of equal evolutionary rate throughout the tree was rejected at a 5% significance level (p < 0.001). Therefore, we used a Bayesian relaxed clock approach as implemented in BEAST 1.8.2. We assigned a lognormal relaxed clock with uncorrelated rates to each clock model. The Tree Model was set to Speciation: Birth-Death Process.

As in Toussaint et al. (2016), two fossils of the subfamily Agabinae were used to calibrate the IQ-TREE topology; †Agabus florissantiensis (Wickham 1913) from the Florissant Formation (~33.9 Ma) and †Hydrotrupes prometheus (Gómez & Damgaard 2014) from Baltic amber (~44 Ma: Engel 2001). These fossils were respectively used to constrain the stem of the extant genera Agabus Leach, 1817, and Hydrotrupes Sharp, 1882. We enforced the two minimum constraints with exponential distributions going from the age of the fossil to 150 million years (Myr), an age equivalent to the oldest dytiscid fossil known, †Palaeodytus gutta (Ponomarenko 1987). The exponential prior parameters for the Agabus and Hydrotrupes fossils were respectively as follows: offset = 33.1/43.27 and mean = 31.7/28.92. The runs consisted of 30 million generations sampled every 3000 generations. The convergence of the runs was investigated using ESS, a burn-in of 10% applied after checking the log-likelihood curves and the different runs merged using LogCombiner 1.8.2 (Drummond et al. 2012). The maximum credibility tree, median ages and their 95% highest posterior density (HPD) were generated afterwards under TreeAnnotator 1.8.2 (Drummond et al. 2012).

**Results**

**Phylogenetic relationships**

The phylogeny inferred using IQ-TREE is presented in Figure 2. We recover Hydrotrupini and Agabini as sister
Figure 2. Maximum likelihood phylogenetic tree of the subfamily Agabinae based on the concatenated molecular matrix. Phylogenetic hypothesis for the subfamily Agabinae with a focus on Platynectes. Nodal support from the IQTREE analysis is given according to the caption inserted on the left of the topology. Major clades within Platynectes Régimbart are given and correspond to the ones recognized in Toussaint et al. (2016). The South American clades are highlighted with shaded gray rectangles and a map of the Neotropical region. The regional distribution of Platynectes main clades is given on the right side of the figure.
tribes with strong bootstrap support. *Platynectes* is found sister to these two tribes also with strong bootstrap support. Within *Platynectes*, we recover eight major clades including two restricted to the Neotropics (clades C2 and C5; clade names follow Toussaint et al. 2016). The first clade C2 comprises the Atlantic and Guiana Shield endemics. In this clade, the two specimens of the Atlantic Shield sampled in this study are found sister to the other specimens endemic to the Guiana Shield with strong bootstrap support (BS = 98).

Figure 3. Bayesian divergence time estimates and biogeography of Brazilian Shield *Platynectes* Régimbart diving beetles. Chronogram presenting the median age estimates as inferred in BEAST for clade 2 where the rest of the topology has been pruned for the sake of clarity. The 95% credibility intervals estimated in BEAST for each node are given with horizontal blue bars. The asterisks indicate a maximum posterior probability (pp = 1.0) as inferred in the MrBayes analysis. Major tectonic and paleo-hydrologic events are schematized above the geological time scale. A reconstruction of the Guiana and Central Brazilian Shield paleogeography following Hoorn and Wesselingh (2010) is given below the chronogram spanning three different periods of time as indicated by the legend below the maps.
**Divergence times**

The BEAST analyses converged well as indicated by ESS > 200 for all parameters. A chronogram highlighting the median age estimates within clade C2 and respective 95% credibility intervals (CI), is given in Figure 3. The full chronogram is presented in Figure S1, online. Overall, we find a crown age of Platynectes in the early Eocene about 52 Ma (95% CI: 39–71 Ma). We recover a crown age of clade C2 in the early Miocene about 20 Ma (95% CI: 12–30 Ma). The daughter node in clade C2 comprising only Guiana Shield endemics is recovered in the mid-Miocene about 17 Ma (95% CI: 11–26 Ma).

**Discussion**

As expected from the reduced amount of additional data, our results are in full agreement with Toussaint et al. (2016), both for the inferred phylogenetic relationships and divergence time estimates. Although we only sequenced the H3 fragment for this study, we recover with strong support a sister relationship between the sampled Atlantic Shield Platynectes and Guiana Shield species. This result is important in the context of the biogeographical history of Platynectes diving beetles in the Neotropics. Indeed, Toussaint et al. (2016) were not able to include Atlantic Shield species in their dated phylogeny of the genus. Here, we show that Atlantic Shield lineages are more closely related to Guiana Shield species than to Central American/Andean species. Therefore, the hypothesis of Gondwanian origin of clade C2 formulated in Toussaint et al. (2016) is further supported by the results of this study.

Unsurprisingly, our age estimates of the divergence between Atlantic and Guiana Shield species reject any potential vicariant scenario involving the ancient positioning of the different shields. However, our estimates are contemporary with the existence of a ridge connecting the Central Brazilian and Guiana Shields called the Purus Arch (Figure 3). The lower bound of this credibility interval at 30 Ma largely predates the breach of this ridge (~11.3 Ma; Hoorn & Wesselingh 2010), which could support a vicariant hypothesis. On the other hand, the higher bound of this interval in the late Miocene (11 Ma) corresponds to the period of time when the Purus Arch is supposed to have been breached by the Amazon river as a result of the orogeny of the Andes (Hoorn & Wesselingh 2010). Hence, it is not possible to rule out a vicariant hypothesis. However, a potentially major piece of this jigsaw puzzle could be missing: a Central Brazilian fauna. A vicariant hypothesis invoking a role of the Purus Arch would require the genus to have existed in this region at some point in its evolution. Our reconstruction focuses on the divergence between Atlantic and Guiana Shield Platynectes, but these two shields are not geographically the most closely related. The Central Brazilian Shield laying in the middle of these two geological entities has not been sampled thoroughly and it is possible that Platynectes diving beetles occur or occurred at some point in this part of the Amazon Basin. Therefore, it is impossible to predict if the genus ever existed in this region without additional sampling effort. Likewise, it is not currently possible to know if the Atlantic and Guiana shield lineages form reciprocally monophyletic clades without a broader sampling of the other existing species found in the Atlantic Shield (Nilsson 2015; Gustafson et al. 2016). This could have implications for the biogeographical pattern inferred here as well as on the larger-scale evolutionary pattern of Platynectes diving beetles in the Brazilian Shield. Future targeted taxon sampling in the Atlantic and Central Brazilian Shields will hopefully help shedding some light on this intricate pattern. However, if Platynectes diving beetles are absent from the Central Brazilian Shield, it may be difficult to understand if what we observe today as a disjunct distribution across the Amazonian Basin is the result of allopatric vicariance following recent extinction, or dispersal.

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**Supplemental data**

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