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Evolution and biogeography of acidocerine water scavenger beetles (Coleoptera: Hydrophilidae) shaped by Gondwanan vicariance and Cenozoic isolation of South America

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Abstract. The water scavenger beetle subfamily Acidocerinae is a cosmopolitan, ecologically diverse lineage with more than 500 described species whose morphology and classification are poorly understood. We present the first phylogenetic analyses of the subfamily inferred from five loci (18S, 28S, H3, CAD, COI). We used secondary calibrations to estimate divergence times and employ this phylogeny to revise the classification and examine the historical biogeography of this lineage. Most genera are resolved as reciprocally monophyletic, with several exceptions: *Horelophopsis* **syn. n.** is recovered as a derived lineage of and placed in synonymy with *Agraphydrus*. The large genus *Helochares*, as well as its primary constituent subgenera *Helochares* (s. str.) and *Hydrobaticus* are found to be polyphyletic. *Batochares* **stat. n.** and *Sindolus* **stat. rev.** are elevated from subgenera of *Helochares* to generic rank. *Crephelochares* **stat. rev.** is removed from synonymy with *Chasmogenus*. We found that the crown Acidocerinae date to the mid-Jurassic in South America + Africa (West Gondwana). South America and Africa remain important areas of endemism throughout the evolution of the lineage and are resolved either individually or in combination as the ancestral area for all but one clade that is older than 90 million years ago. Six of the seven lineages occurring in South America diverged more than 100 million years ago and are endemic to the region, suggesting the Neotropical acidocerine fauna became isolated following the breakup of West Gondwana. Conversely, lineages found on other Gondwanan fragments (India, Madagascar, Australia) are comparatively young and derived, with all being Cenozoic in age. The few taxa that occur in North America today are all the result of recent Cenozoic dispersal from South America, although North America may have played an important role as an ancestral area in the Mesozoic.

Introduction

Water scavenger beetles (Coleoptera: Hydrophiloidea) are an ancient lineage of aquatic insects originating in the late Triassic (Bloom *et al.*, 2014, Toussaint *et al.*, 2016b, Toussaint & Short, 2018). The largest constituent family of the lineage is Hydrophilidae, which contains six subfamilies and recently surpassed 3000 described species (Short & Fikáček, 2013; Short, 2018). Although untangling the phylogenetic structure of the Hydrophilidae has been a highly active research area in the last fifteen years, the majority of studies have focused on specific tribes within the Hydrophilinae (e.g., Berosini: Archangelsky, 2008, Hydrophilini: Toussaint *et al.*, 2017, Laccobiini: Toussaint *et al.*, 2016a, Hydrobiusini: Short *et al.*, 2017) and

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Sphaeridiinae (e.g., Protosternini: Fikáček *et al.*, 2015). The only subfamily to be broadly sampled for phylogenetic reconstruction is the Cylominae (Seidel *et al.*, 2020). Of the remaining three subfamilies, the Acidocerinae is the most diverse, with more than 500 described species (and many more undescribed), and has long been viewed as a taxonomic mess.

The modern concept of the Acidocerinae was first proposed by Hansen (1991), with subsequent modification by Short & Fikáček (2013) to remove several genera that now form the bulk of the closely related subfamily Enochrinae. The internal classification of the Acidocerinae, particularly the delimitation of genera and subgenera, has been challenging and no phylogeny of the group has previously been attempted. Relative to most water beetle clades, the subfamily is unusually varied in ecology, occurring in a variety of habitats from pond and river margins to rock seepages to terrestrial niches including rotting fruits and sap flows (Girón & Short, 2017; Girón & Short, 2019). The lineage also includes the only known blind, cave dwelling water scavenger beetle (Spangler, 1981). Additionally, many taxa within the subfamily exhibit maternal care, with the females carrying an egg case affixed to their abdomen (Archangelsky, 1997). This broad range of ecologies and behaviours may have led to repeated morphological convergence as lineages shifted back and forth between habitats, however, this cannot be tested until a robust phylogeny is in place.

The Acidocerinae is an attractive lineage to examine ancient biogeographic patterns of diversification. The subfamily occurs in all major biogeographic regions and subregions. The Afrotropical, Neotropical, and Indo-Malayan regions are the most diverse, each of which contains well over 100 described species. The Australian and Palearctic regions have very modest diversity with between ca. 25 and 40 species each, whereas the Nearctic region is very species poor with only four species. Most hydrophiloid families and subfamilies had diverged and were well established by 150 million years ago (Ma), when the continents Gondwana and Laurasia were still largely intact and closely situated (Seton *et al.*, 2012). Consequently, water scavenger beetles are an ideal group to study the dynamics of Gondwanan biogeography in a comparative framework. Signatures of both East Gondwanan (India/Madagascar) and West Gondwanan (South America/Africa) vicariance have already been documented in the subfamily Hydrophilinae (Toussaint *et al.*, 2016a, 2017; Toussaint & Short, 2018), suggesting similar patterns are likely in other parts of the hydrophilid family tree. In addition to Acidocerinae being of sufficient age to be affected by the breakup of Gondwana (Bloom *et al.*, 2014), it putatively exhibits high lineage endemism (e.g., endemic genera and subgenera) in the West Gondwanan fragments of South America and Africa. Therefore, it has the potential to contribute to our understanding of Cretaceous and Cenozoic biogeography.

To advance our understanding of acidocerine evolution and its historical biogeography, we here use DNA sequence data from five loci and comprehensive taxonomic and geographic coverage to infer the first detailed phylogenetic estimate of the Acidocerinae. We employ this phylogeny to (i) review and update the genus-level classification of the Acidocerinae and (ii) reconstruct the biogeographic history of the lineage with a particular emphasis on the role South America played in the subfamily's diversification.

Methods

Taxon sampling

We sampled 200 putative species of Acidocerinae (a few species were represented by multiple genetically distinct terminals) for a total in-group matrix of 206 terminals (see Table S1). We included 15 of the 19 currently recognized genera within the Acidocerinae, and four of the five subgenera of the extremely large and diverse genus *Helochares* Mulsant. We sought to include species across the geographical range and morphological diversity of each genus, and targeted unusual taxa for inclusion (e.g., *Helochares ellipticus* d'Orchymont, *Helochares discus* Hebauer, Hendrich, and Balke, *Chasmogenus cremnobates* (Spangler)). No material suitable for DNA extraction was available for the monotypic genera *Acidocerus* Klug, *Helopeltarium* d'Orchymont, *Peltochares* Régimbart, and *Troglochares* Spangler (all but *Peltochares* are known only from type material) or the *Helochares* subgenus *Helocharimorphus* Kuwert. We examined the type material of all four above mentioned genera that were missing from the analysis, enabling us to discuss their potential placement in our phylogeny, as well as nontype specimens of *Helocharimorphus*. For outgroups, we included three species of Cylominae and six species of Sphaeridiinae, which together form the sister group to Acidocerinae (Short & Fikáček, 2013). We rooted the tree with *Notionotus liparus* Spangler (Enochrinae). All unidentified taxa in the genera *Tobochares* Short and García, *Nanosaphes* Girón and Short, and *Radicitus* Short and García and the majority of unidentified terminals of *Chasmogenus* Sharp represent undescribed species. *Helochares* Mulsant and *Agraphydrus* Régimbart are substantially harder to identify and given the apparent undescribed diversity in these two large genera, we (AEZS and JCG) made species identifications only for specimens that we had high confidence in the ID; as such, unidentified terminals in these genera do not necessarily indicate new species.

Molecular biology

We extracted total genomic DNA from whole beetle specimens that had been preserved in 95% ethanol and frozen, using a DNeasy kit (Qiagen, Alameda, CA). In a few cases, museum DNA was extracted from dry, pinned specimens when no other material was available. Voucher specimens are deposited at the University of Kansas (Lawrence, USA) unless otherwise indicated (Table S1). We amplified and sequenced five gene fragments following the PCR protocols from Short & Fikáček (2013), Baca *et al.* (2017), and Wild & Maddison (2008): ribosomal 18S (18S, 1870 bp), ribosomal 28S (1052 bp), cytochrome oxidase subunit 1 (CO1, 798 bp), carbamoyl-phosphate synthetase 2 (CAD, 654 bp), and a fragment of histone 3 (H3, 339 bp). All DNA sequences were assembled and edited in Geneious R 8.1.9 (Biomatters, http://www.geneious.com/). The protein-coding gene fragments were aligned with MUSCLE (Edgar, 2004) whereas the ribosomal gene fragments were aligned with MAFFT using the E-INS-I algorithm. The final concatenated alignment consisted of 4713 bp (Appendix S2). A complete list of included taxa and sequences, including GenBank accession numbers is given in Table S1.

Phylogenetic methods

We used the maximum likelihood (ML) program IQ-TREE 1.6.7 (Nguyen *et al.*, 2015) to infer phylogenetic relationships of Acidocerinae using the concatenated matrix (Appendix S2). The dataset was initially divided by codon position for each protein coding gene fragment whereas ribosomal gene fragments were left unpartitioned resulting in 11 initial partitions. The best partitioning scheme and models of substitution for each resulting partition were simultaneously selected in IQ-TREE using ModelFinder (Kalyaanamoorthy *et al.*, 2017), with the greedy algorithm and based on the Akaike information criterion corrected (AICc). The optimal models of nucleotide substitution were determined across all available models in IQ-TREE including the FreeRate model (+R, Soubrier *et al.*, 2012), that relaxes the assumption of gamma distributed rates. We conducted 300 tree searches starting from random parsimony topologies to avoid local optima and selected the resulting best ML tree by comparing log-likelihood scores. To assess nodal support, we performed 1000 ultrafast bootstrap replicates (UFBoot, Minh *et al.*, 2013; Hoang *et al.*, 2018), and SH-aLRT tests (Guindon *et al.*, 2010) with 1000 replicates. To reduce the risk of overestimating branch support values with UFBoot due to severe model violations, we used hill-climbing nearest neighbor interchange (NNI) to optimize each bootstrap tree.

Divergence time estimation

Divergence times were inferred in a Bayesian framework with BEAST 1.8.4 (Drummond *et al.*, 2012). As for the phylogenetic analyses, the best partitioning scheme and models of substitution were selected in PartitionFinder2 (Lanfear *et al.*, 2017) using the greedy algorithm and the Bayesian Information Criterion across all models included in BEAST (option models = beast). We tested different clock partitioning schemes by assigning either (i) a unique uncorrelated lognormal relaxed clock for all partitions; (ii) two uncorrelated lognormal relaxed clocks, one for all mitochondrial partitions and one for all nuclear partitions; or (iii) nine uncorrelated lognormal relaxed clocks, one for each partition selected in PartitionFinder. We also tested different tree models by using a Yule (pure birth) or a birth-death model in different analyses. The rates of the uncorrelated lognormal relaxed clocks were set with an approximate continuous time Markov chain rate reference prior (Ferreira & Suchard, 2008). The analyses consisted of 100 million generations with a parameter and tree sampling every 5000 generations. We estimated marginal likelihood estimates (MLE) for each analysis using path-sampling and stepping–stone sampling (Xie *et al.*, 2011; Baele *et al.*, 2012, 2013), with 1000 path steps, and chains running for one million generations with a log likelihood sampling every 1000 cycles. The MLE were then calculated using a .xml script (Appendix S5). Since the fossil record of Acidocerinae is very scarce, we relied on the most recent and comprehensive dated phylogeny of Hydrophilidae published by Toussaint & Short (2018) who relied on 10 carefully chosen fossils within Hydrophilidae to estimate divergence times (Appendix S7). As a result, we constrained seven nodes along the backbone of the phylogeny as inferred in Toussaint & Short (2018). The secondary calibrations were implemented with uniform priors encompassing the credibility intervals estimated in the best BEAST run of Toussaint & Short (2018) (i.e., run set with the maximum age of the stem Hydrophiloidea at 273 Ma, five partitions and an exponential prior density for all fossil calibrations). The use of secondary calibrations is not optimal but is sometimes necessary when too few fossil calibrations can be applied. In such cases, the recovered 95% uncertainty from the primary study needs to be transferred to the new analyses. To do so, the use of normal distributions, even though appealing, is problematic because it assumes that posterior distributions from the primary study are normally distributed which is rarely the case depending on how priors were set up in the analyses. For this reason, a more ‘conservative’ approach using more relaxed priors such as uniforms is preferable. This issue is explored for instance in Schenk (2016) where normal distributions are shown to be suboptimal compared to uniform ones when applying secondary calibrations. Future explorations of divergence times within Hydrophilidae should ideally be performed using fossil calibrations when possible to refine the age of Acidocerinae.

Ancestral range estimation

We inferred the biogeographical history of Acidocerinae using the R-package BioGeoBEARS 1.1.1 (Matzke, 2018). We conducted the analyses under the Dispersal Extinction Cladogenesis (DEC) model (Ree & Smith, 2008). We used the BEAST Maximum Clade Credibility (MCC) tree from the best analysis (see Results) with outgroups pruned. The distribution of taxa was extrapolated from the literature and our field notes. We used the following regions for the analysis: P, Eastern Palearctic region and Oriental region up to the Wallacea (i.e., Sulawesi), I, Indian region excluding northernmost ranges that are more closely related geographically to the Oriental region, U, Australian region up to the Wallacea, M, Madagascar, A, Africa, T, Neotropics, N, Nearctic region, and W, Western Palearctic. To take into account plate tectonics and the widespread landmass rearrangements that occurred during the Cenozoic and Mesozoic (Seton *et al.*, 2012; Hall, 2013), we designed five time slices: TS1, between 180 and 150 Ma corresponding to the Pangean stage with connectivity between most landmasses, TS2, between 150 and 130 Ma corresponding to the progressive split between Eastern and Western Gondwana and the separation of West Gondwana and Laurentia in the north, TS3, between 130 and 90 Ma, corresponding to the breakup of West Gondwana with India and Madagascar forming an island detached from Africa, Antarctica and Australia, but also the split between Africa and South America, TS4, between 90 and 50 Ma corresponding to the breakup of Eastern Gondwana with India drifting northward, but also the final stage of the Gondwana breakup in the south with Australia detaching from Antarctica, and TS5, between 50 Ma and the present, corresponding to the collision of India with Eurasia and the final stages of the connection between the Australian and Oriental regions through the Wallacea. The dispersal rate scaler values were selected according to terrain and water body positions throughout the timeframe of the group evolution. Dispersal between adjacent areas was not penalized when dispersal between areas separated by water barriers or another area was penalized using a dispersal rate scaler of dr = 0.5 (i.e., a penalty of 0.5 was applied). Penalties were summed as areas were progressively more distant from each other or separated by other barriers with a dr = 0.1 being the minimum value, therefore allowing long-distance dispersal to occur. The adjacency matrices were also constrained to avoid implausible area combinations (Appendix S6).

Results

Phylogeny

Sequencing resulted in a final matrix that was 93% complete (Table S1). The BI and ML analyses resulted in trees with highly similar topologies with few conflicting relationships. The BEAST topology is shown in Figs 1–4, with support from both analyses (PP and UFBS) mapped at all nodes (also see Appendices S3 and S4 for resulting .tre files). The monophyly of the Acidocerinae was affirmed in both analyses with strong (PP = 0.98) to weak (UFBS = 67) support. Within the Acidocerinae, the recently described Neotropical genus *Primocerus* Girón and Short is resolved as monophyletic (PP = 1.0/ UFBS = 100) and sister to the rest of the subfamily. The remaining Acidocerinae is split between two large clades, the first of which we here refer to as the *Helochares*-group (Figs 1, 2). The *Helochares*-group is recovered with strong to weak support (0.99/60) and includes the New World genera *Aulonochares* Girón and Short, *Helobata* Bergroth, and *Radicitus* (each of which is strongly supported as monophyletic: 1.0/100) and the strikingly polyphyletic *Helochares*. The *Helochares*-group itself is split into several large clades, although there is some disagreement between the arrangement of these lineages between BI and ML topologies. Both analyses strongly agree that most *Helochares* species fall within *Helochares* clade A (1.0/100) which contains all included species currently classified in the subgenus *Hydrobaticus* and a few species included in *Helochares* (s.str.), including the type species for both *Hydrobaticus* and *Helochares* (s.str). The remaining species of *Helochares* (s.str.) are distributed among *Helochares* clade B (monotypic; *Helochares ellipticus*), *Helochares* clade C (1.0/100), and *Helochares* clade D (0.97/−−), although the latter clade is paraphyletic with respect to the subgenus *Sindolus* Sharp in the ML analysis. The *Helochares* subgenera *Sindolus* (1.0/100) and *Batochares* Hansen (with a single included species) also are resolved as distinct lineages within the *Helochares*-group. Sister to the *Helochares*-group is a lineage consisting of three large clades which we here refer to as the *Agraphydrus*-group, *Chasmogenus*-group, and *Tobochares*-group respectively. All three genus-groups are recovered with strong support (1.0/100). The *Agraphydrus*-group contains the large and diverse Old World genus *Agraphydrus* in which the enigmatic genus *Horelophopsis* Hansen was recovered as deeply nested with strong support (1.0/100). The *Chasmogenus*-group consists of the cosmopolitan genus *Chasmogenus* as it is currently circumscribed. The group is composed of two strongly supported clades (both 1.0/100) that reflect the historical division between the subgenera *Chasmogenus* (s.str.) for the Neotropical species and *Crephelochares* Kuwert for the Old World taxa. The *Tobochares*-group is an exclusively Neotropical clade comprising seven recently described genera (*Crucisternum* Girón and Short, *Ephydrolithus* Girón and Short, *Globulosis* García, *Katasophistes* Girón and Short, *Nanosaphes*, *Quadriops* Hansen, and *Tobochares*), all of which are resolved as monophyletic in both ML and BI analyses. However, support for the relationships among these seven genera was less clear. Although the BI tree favours a topology with *Crucisternum* as sister with *Tobochares* (PP: 0.98), the ML analysis favours a topology in which *Crucisternum* flips to being sister to the remaining *Tobochares*-group genera but essentially without support (UFBS: 31). In addition, BI tree resolves *Katasophistes* + *Ephydrolithus* with modest support (PP: 0.87) with ML favouring a topology with *Katasophites* and *Ephydrolithus* branching sequentially, also essentially without support (UFBS: 63 and 7 respectively).

Divergence time estimation and ancestral range estimation

All BEAST analyses converged well and resulted in broadly overlapping age estimates (Tables 1 and S3). We present the results of the preferred BEAST analysis (9 clocks and a birth-death tree model) based on MLE comparisons in Figs 1–4. The ancestral range estimation performed in BioGeoBEARS under the DEC model is also presented in Figs 5, 6. We recover a crown age for Acidocerinae ca. 177 (95% credibility interval: 162–191) Ma with the first split between *Primocerus* and the remainder of the subfamily dated to ca. 167 (155–177) Ma. The origin of Acidocerinae is estimated to have been in a joint area encompassing Africa and South America with the daughter clades being a Neotropical *Primocerus* and a large clade in a conserved widespread range also including the Nearctic region. We estimate the colonization of the Oriental/Palearctic region in the *Helochares* group ca. 115 Ma, whereas the sister clade represents a vicariant event between Africa and South America ca. 150 Ma. In the second part of the tree, we recover

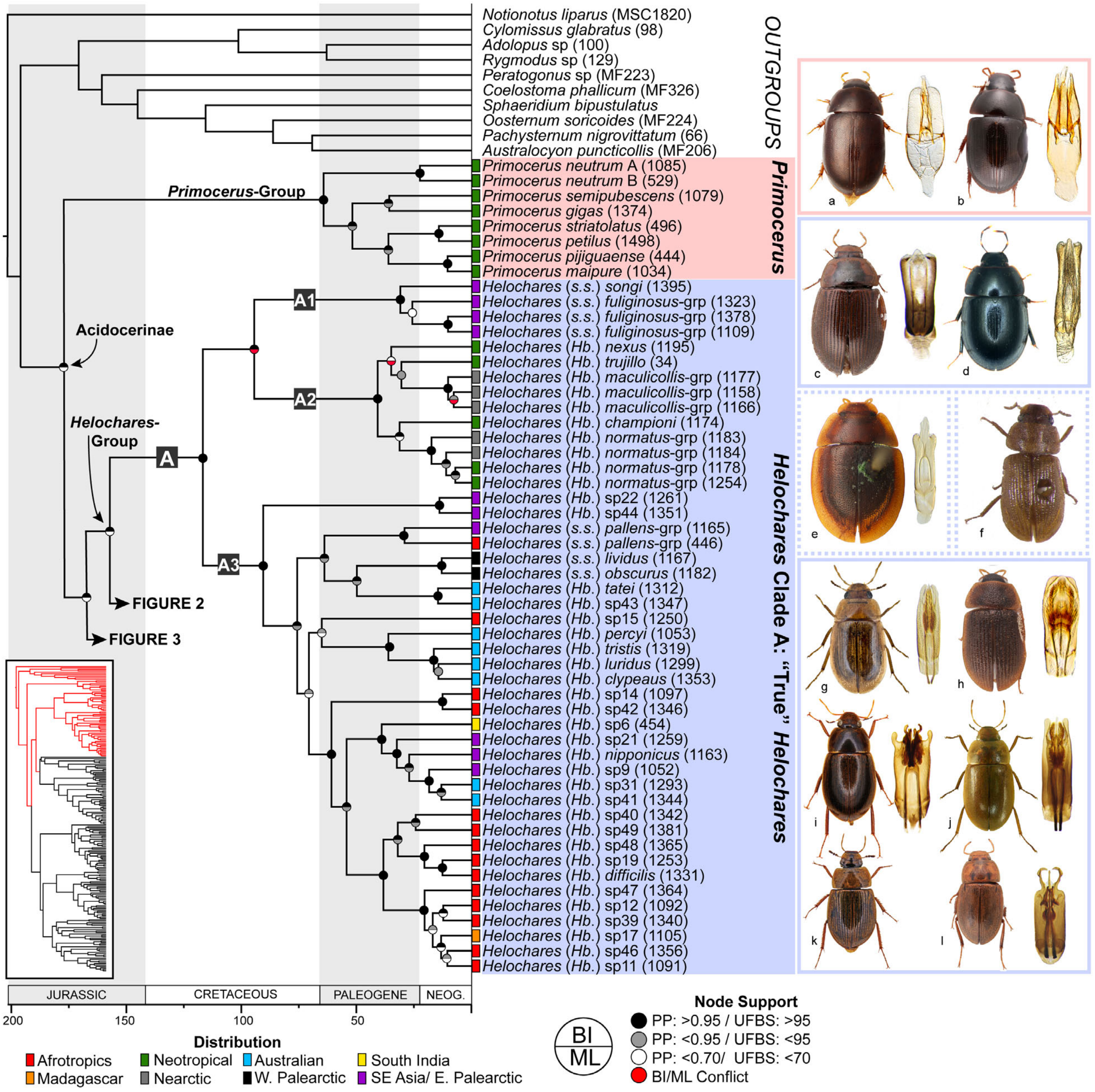

Fig. 1. Phylogeny of the Acidocerinae based on a Bayesian analysis of five genes. Part 1 – *Primocerus*-group and *Helochares*-group (in part). Right panel illustrates dorsal views and genitalia of representative species of major clades: (a) *Primocerus pijigueanse*, (b) *Primocerus petilus*, (c) *Helochares maculicollis*, (d) *Helochares songi* (image from Jia & Tang, 2018), (e) *Helopeltarium ferrugineum*, (f) *Acidocerus aphodiodes*, (g) *Helochares pallens* (ex. Udo Schmidt), (h) *Helochares* sp. (i) *Helocahres dilutus* (image from Bird *et al.*, 2017), (j) *Helocahres lividus*, (k) *Helochares* sp. (image from Bird *et al.*, 2017), (l) *Helochares tristis*. Bottom panel includes key for distribution and node support. The abbreviation '*Hb.*' refers to the *Helochares* subgenus *Hydrobaticus*. Dashed boxes around (e) and (f) indicate that these genera were not included in the analysis but are likely members of this clade (see discussion). [Colour figure can be viewed at wileyonlinelibrary.com].

yet another ancestral range encompassing Africa and South America, with two daughter clades having their ancestors in Oriental/Palearctic and in South America. Colonization of Australia/New Guinea, India, Madagascar, and West Palearctic are much more recent events, that mostly occurred in the past ca. 60 Ma.

Discussion

Classification of the acidocerinae

Given the large number of lineages and genera within the subfamily, it would be helpful to establish tribes to facilitate

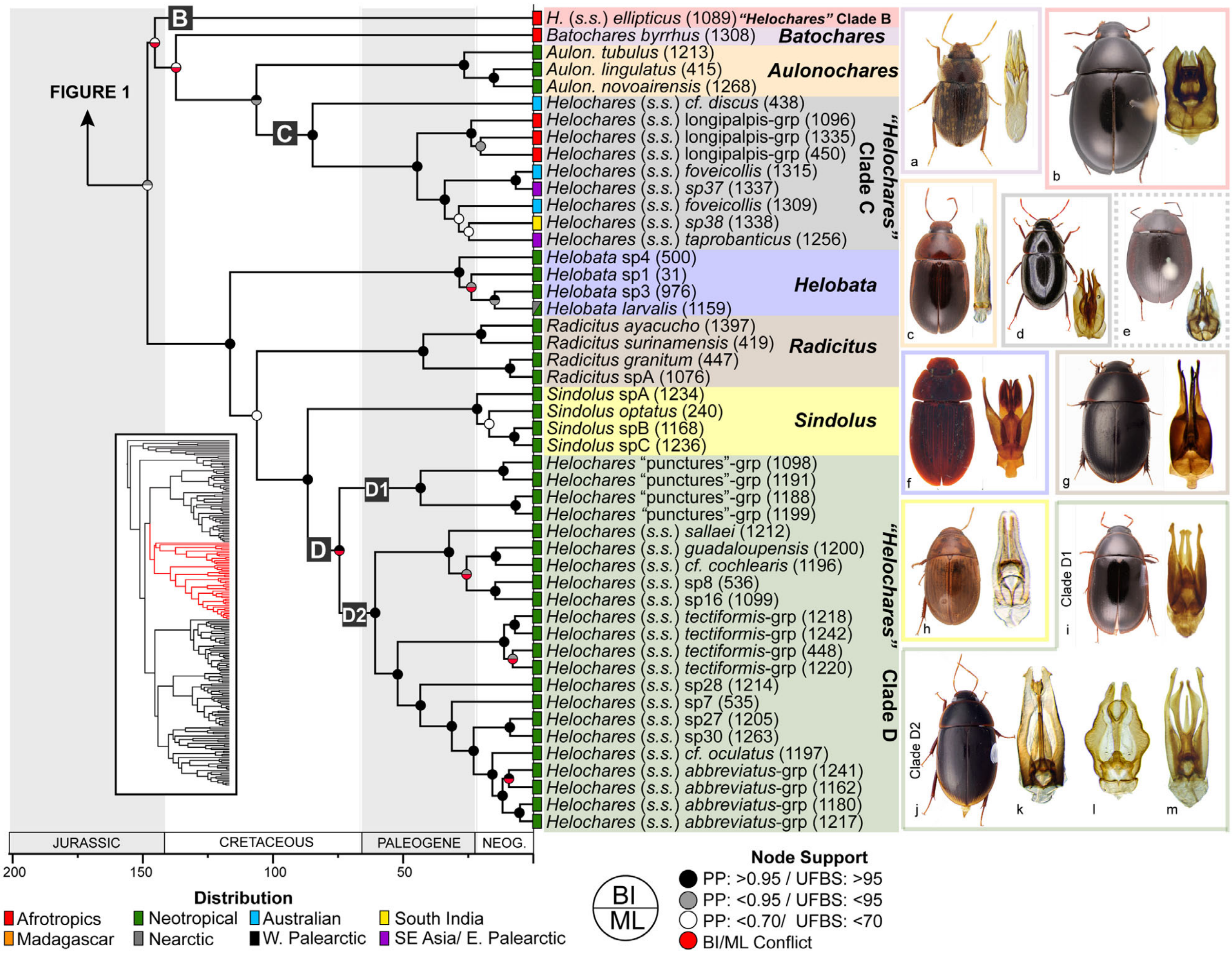

Fig. 2. Phylogeny of the Acidocerinae based on a Bayesian analysis of five genes. Part 2 – *Helochares*-group (in part). Right panel illustrates dorsal views and genitalia of representative species of major clades: (a) *Batochares* sp., (b) *Helochares ellipticus*, (c) *Aulonochares tubulus*, (d) *Helochares longipalpis* (image from Bird *et al.*, 2017), (e) *Peltochares conspicuus*, (f) *Helobata quatipuru* (image from Clarkson & Almeida, 2018), (g) *Radicitus ayacucho*, (h) *Sindolus* sp., (i) *Helochares* n. sp., (j) *Helochares* sp., (k) *Helochares abbreviatus*, (l) *Helochares cochlearis*, (m) *Helochares* n. sp. Bottom panel includes key for distribution and node support. Dashed box around (e) indicate that this genus was not included in the analysis but likely is related to *Helochares* clade C (see discussion). [Colour figure can be viewed at wileyonlinelibrary.com].

future studies. However, while we found strong to modest support for five clades that could potentially serve as tribes, identifying clear morphological synapomorphies for some of them proved difficult. Therefore, we instead propose an informal internal classification that recognizes five genus-groups. The external morphology of many taxa can be deceiving and several characters that have historically been treated as important to the classification of the subfamily are clearly much more homoplasious than previously thought. For example, the presence or absence of elytral serial punctures has long been used as a traditional character for defining genera or subgenera; however, we found repeated instances in which genera (and subgenera) contain species with both character states (e.g. *Helochares* (s.str.), *Hydrobaticus*, *Radicitus*, *Agraphydrus*, *Ephydrolithus*, and others). On the contrary, the form of the male genitalia was rich in characters that appear to more informatively define natural groups. The morphology supporting the proposed genus-groups frequently involves these aedeagal characters which are more consistent and reliable across the subfamily. For example, some lineages maintain presumed plesiomorphic simple tri lobed aedeagal form (e.g., *Agraphydrus*, *Chasmogenus*, most *Tobochares*-group genera; see most aedeagi in Figs 3, 4), whereas more complex and intricate forms have evolved independently and in different ways in other lineages (e.g., *Radicitus*, *Crephelochares*, *Peltochares*, *Helochares* clade A3; see aedeagi in Figs 1g–k, 2d–m). We also emphasize that although our discussions here are necessarily morphologically annotated and informed, a complete genus-level character review is beyond the scope of this paper, however, will be the focus of a separate forthcoming contribution (Girón & Short, in prep).

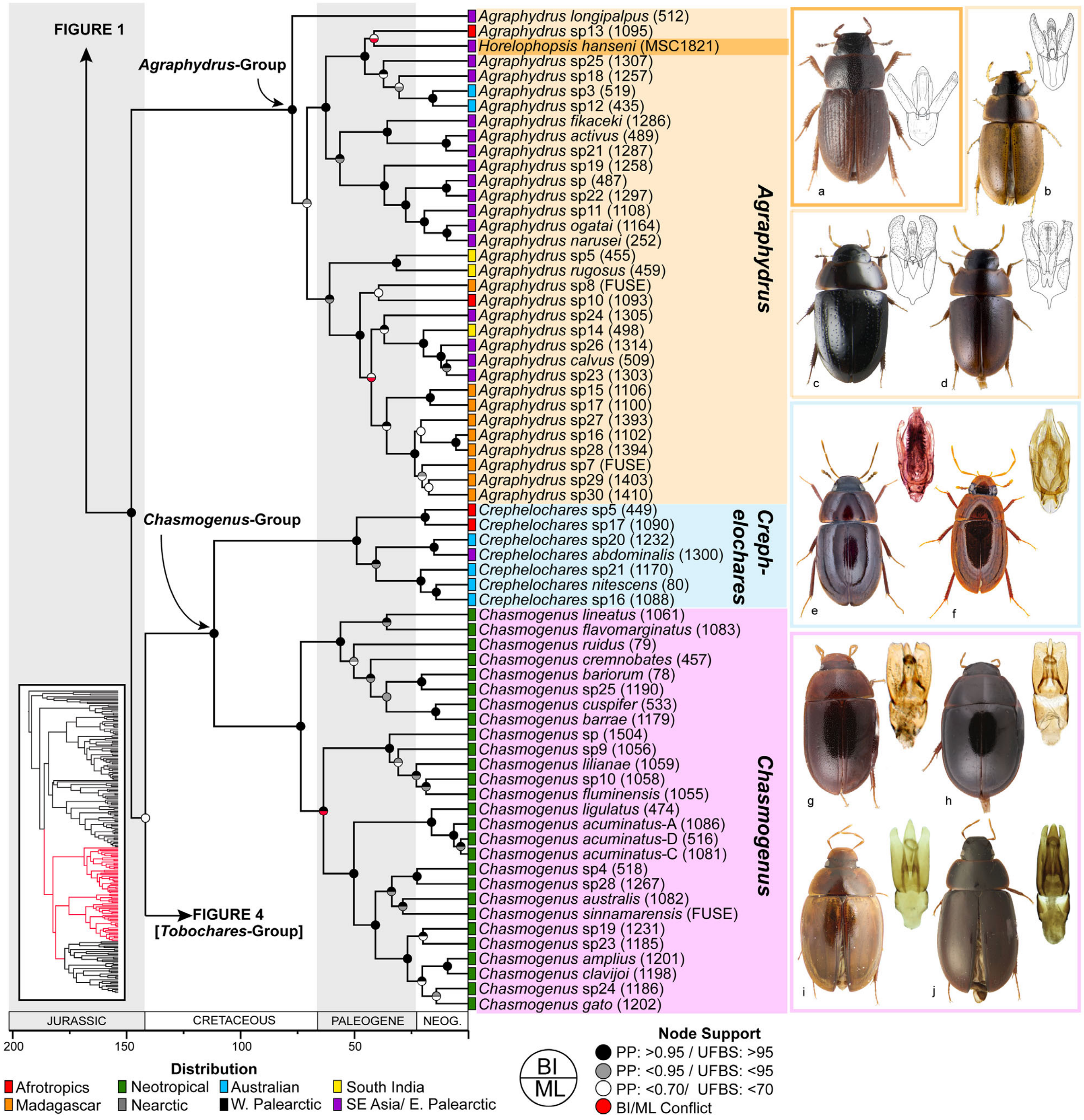

Fig. 3. Phylogeny of the Acidocerinae based on a Bayesian analysis of five genes. Part 3 – *Chasmogenus*-group. Right panel illustrates dorsal views and genitalia of representative species of major clades: (a) *Horelophopsis hanseni*, (b) *Agraphydrus ryukyuensis* (image from Minoshima, 2016), (c) *Agraphydrus insidiator* (image from Minoshima *et al.*, 2015), (d) *Agraphydrus ogatai* (image from Minoshima, 2016), (e) *Crephelochares abdominalis* (image from Jia & Tang, 2018), (f) *Crephelochares cf. patrizii* (image from Bird *et al.*, 2017), (g) *Chasmogenus ruidus*, (h) *Chasmogenus cremnobates*, (i) *Chasmogenus fluminensis* (image from Clarkson & Ferreira-Jr, 2014), (j) *Chasmogenus itatiaia* (image from Clarkson & Ferreira-Jr, 2014). Bottom panel includes key for distribution and node support. [Colour figure can be viewed at wileyonlinelibrary.com].

Primocerus-group. Sister to the remaining Acidocerinae, the *Primocerus*-group contains a single genus, the Neotropical endemic *Primocerus*. The genus was only recently discovered and described (Girón & Short, 2019). All nine described species occur in the Guiana Shield region of South America, where they occur in seepages and less commonly in forested streams. A putative synapomorphy for the *Primocerus*-group is the lack of a sclerotized gonopore, and the presence of a lightly sclerotized projection beyond the apex of the median lobe of the aedeagus. Members of the group are more easily distinguished from most

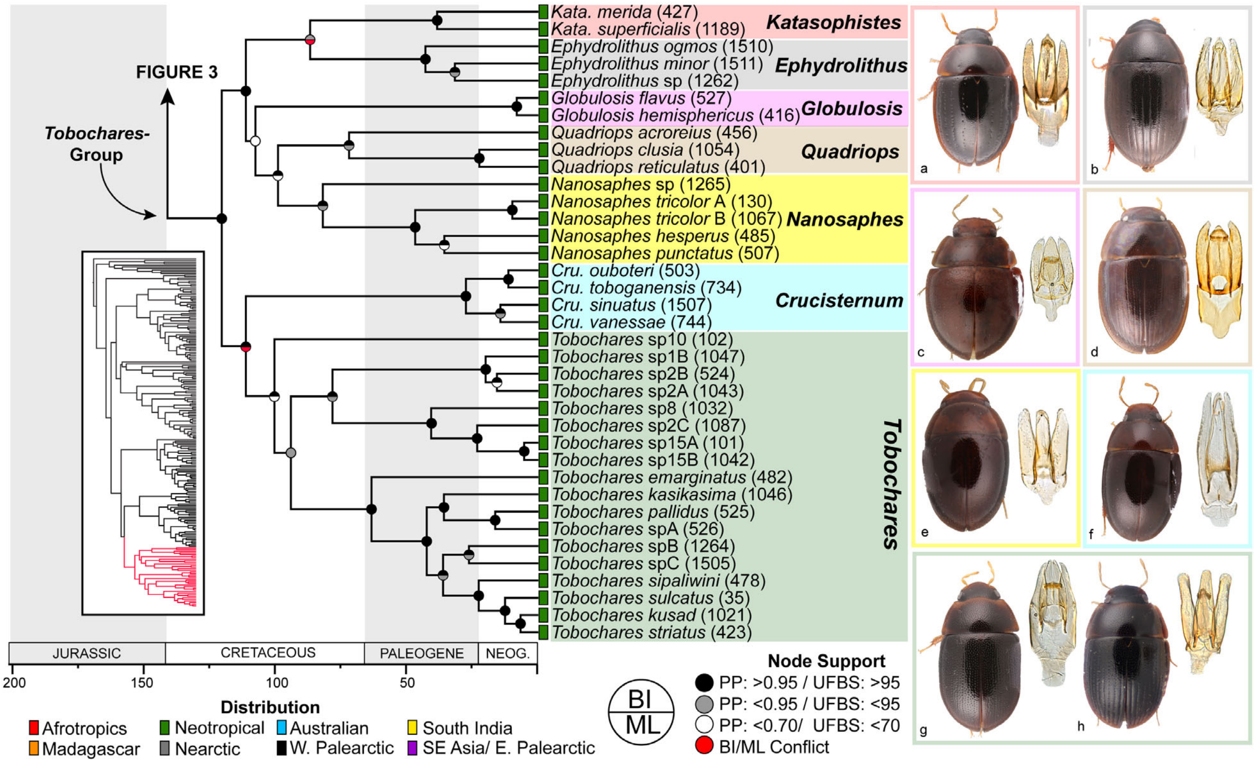

Fig. 4. Phylogeny of the Acidocerinae based on a Bayesian analysis of five genes. Part 4 – *Tobochares*-group. Right panel illustrates dorsal views and genitalia of representative species of major clades: (a) *Katasophistes merida*, (b) *Ephydrolithus ogmos*, (c) *Globulosis hemisphericus*, (d) *Quadriops similaris*, (e) *Nanosaphes punctatus*, (f) *Crucisternum ouboteri*, (g) *Tobochares* sp. 10, (h) *Tobochares sipaliwini*. Bottom panel includes key for distribution and node support. [Colour figure can be viewed at wileyonlinelibrary.com].

Table 1. Results of the BEAST analyses with marginal likelihood estimate comparisons.

Clocks	Tree model	PS MLE	SS MLE	Root median age [95% HPD]	Acido. median age [95% HPD]
1	Birth death	−107 728.030	−107 623.841	204.6932 [192.2314–209.9996]	169.3733 [154.9695–176.9986]
1	Yule	−107 755.670	−107 749.859	204.4752 [192.0975–209.9996]	169.94 [156.3474–176.9947]
2	Birth death	−107 234.883	−107 250.418	191.6841 [171.0495–209.9901]	151.886 [134.6025–169.9236]
2	Yule	−107 233.444	−107 246.004	193.092 [172.6685–209.9543]	151.9293 [135.2724–169.1234]
9	Birth death	−107 079.578	−107 098.325	201.5683 [186.2483–209.9987]	167.1999 [155.0336–176.9987]
9	Yule	−107 082.415	−107 098.830	200.7173 [185.3009–209.9997]	167.4891 [152.2763–176.9988]

In the two-clock analyses, there was one clock each for the mtDNA and nuclear partitions. In the 9 clock analysis, there was one clock for each partition as recovered in PartitionFinder.

PS, path sampling; MLE, marginal likelihood estimate; SS, stepping-stone sampling; 95% HPD, 95% credibility interval.

other acidocerines by the presence of a well-developed sutural stria, shared only with members of the *Chasmogenus*-group.

Helochares-group and the Helochares problem. The *Helochares*-group contains more than half of the described species of the Acidocerinae and is composed of the large cosmopolitan genus *Helochares*, as well as the smaller Neotropical genera *Aulonochares*, *Helobata*, and *Radicitus*. Broadly distributed, the *Helochares*-group occurs in all biogeographic regions but is largely concentrated in the Neotropics, Africa, and Southeast Asia. Although we identified no unique synapomorphy that unites all taxa in this group, there is one compelling putative behavioural synapomorphy: most (and potentially all) members of the *Helochares*-group are known to have the female affix the egg case below their abdomen and carry it with them. No other taxon in the family is known to exhibit parental care, although it is also found in the hydrophiloid families Epimetopidae (Archangelsky, 1997) and Spercheidae (Fikáček, 2019).

The taxonomy and definition of the genus *Helochares* has proven frustrating to many prior water beetle taxonomists, with changing circumscriptions and a revolving door of subgenera for more than a century. Our phylogeny reveals why: neither *Helochares* nor its primary subgenera (*Hydrobaticus* and

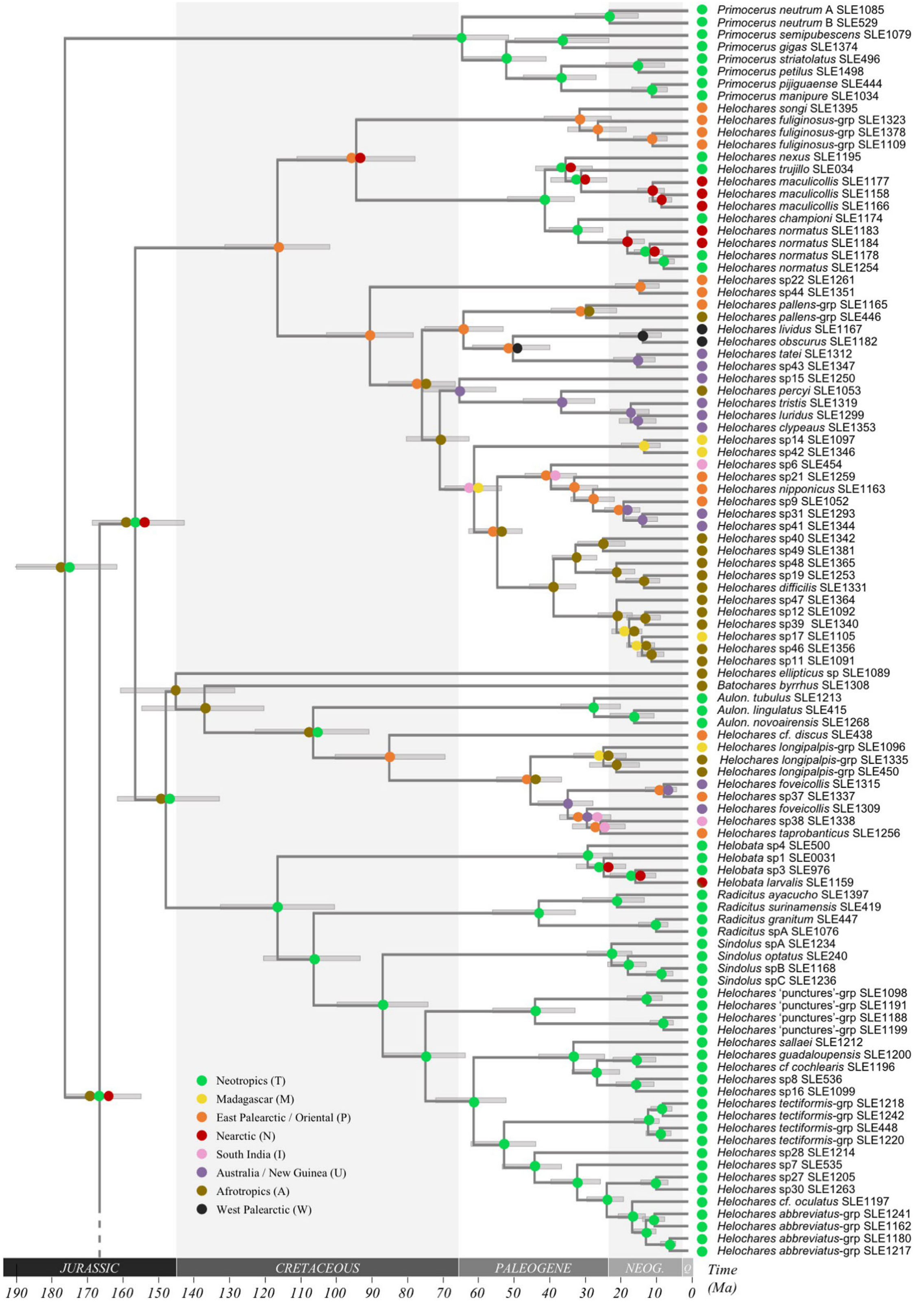

Fig. 5. Historical biogeography of the Acidocerinae. Part 1– Chronogram derived from the BEAST analysis using median age estimates with 95% credibility intervals show as horizontal grey bars. [Colour figure can be viewed at wileyonlinelibrary.com].

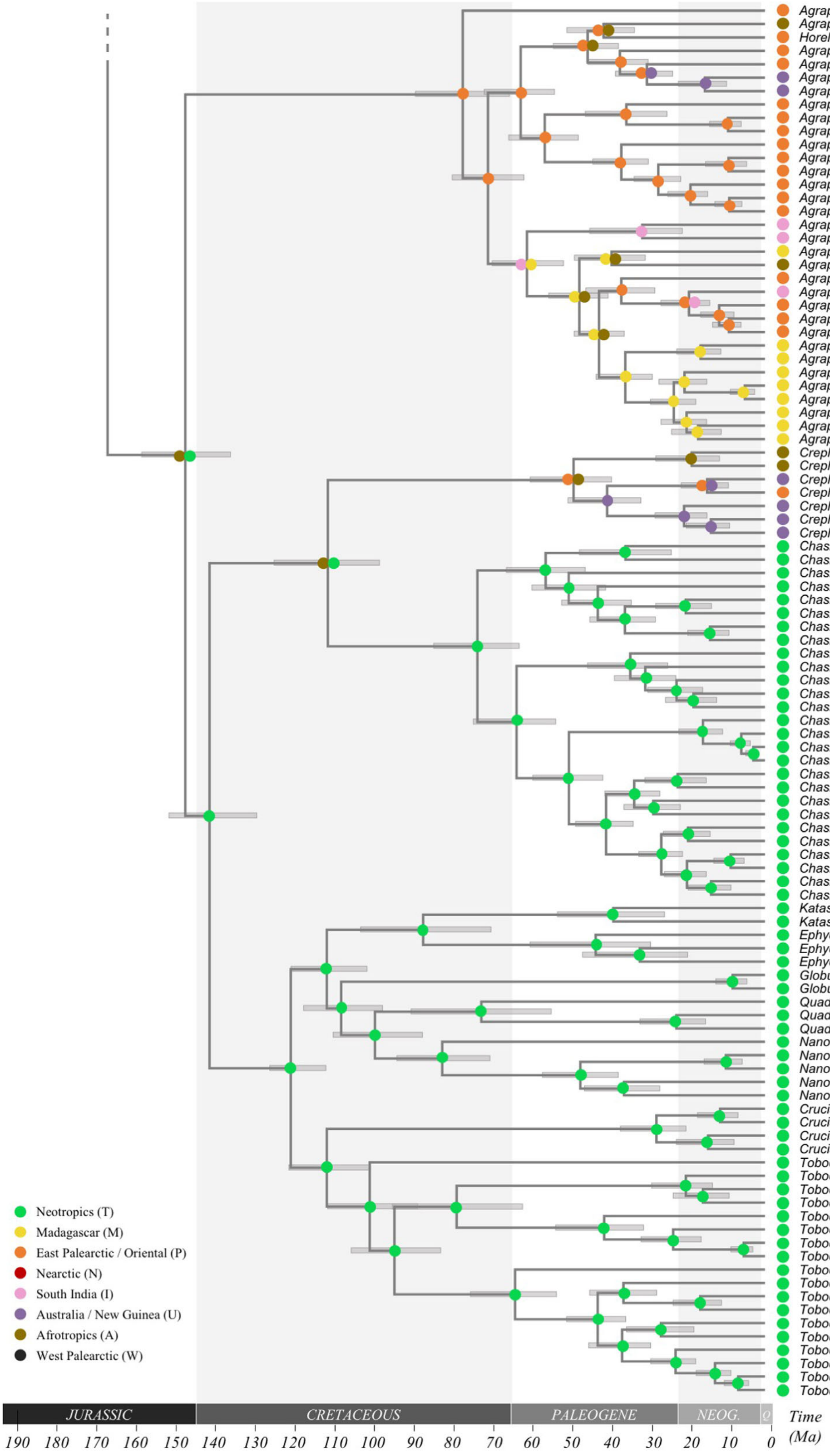

Fig. 6. Historical biogeography of the Acidocerinae. Part 2– Chronogram derived from the BEAST analysis using median age estimates with 95% credibility intervals show as horizontal grey bars. [Colour figure can be viewed at wileyonlinelibrary.com].

Helochares s. str.) are natural groups. Rather, the genus is polyphyletic and composed of at least six distinct clades, each dating to the mid-Cretaceous or earlier. The subgenera *Batochares* and *Sindolus* are both resolved as distinct lineages and we here elevate them to full genera. *Batochares* **stat. n.** contains three species from tropical Africa and is easily diagnosed from all other *Helochares*-group genera by the anteriorly flattened labrum, elytra with irregular pale maculations, and the very long basal piece of the aedeagus. This change creates three new combinations: *Batochares burgeoni* (d'Orchymont) **comb. n.**, *B. byrrhus* (d'Orchymont) **comb. n.**, and *B. corrugatus* (Balfour-Browne) **comb. n.** The genus *Sindolus* **stat. rev.** contains eight Neotropical species and is the only genus in the entire subfamily in which the mesoventrite forms a high, laminar longitudinal keel. This change creates six new combinations: *Sindolus femoratus* (Brullé) **comb. n.**, *S. mesostitialis* (Fernández) **comb. n.**, *S. mini* (Fernández) **comb. n.**, *S. spatulatus* (Fernández) **comb. n.**, *S. talarum* (Fernández) **comb. n.**, and *S. ventricosus* (Bruch) **comb. n.**

The remaining taxa currently assigned to the genus *Helochares* are distributed among four clades, here referred to as *Helochares* clades A through D (Figs 1, 2). *Helochares* clade A (Fig. 1) is by far the largest of these four lineages, and contains the majority of *Helochares* species. This clade is itself composed of three distinct lineages (clades A1–3, Fig. 1), each of which is highly supported (1.0/100). Clade A1 contains a few species from Southeast Asia that are currently placed in *Helochares* (s. str.), whereas clade A2 comprises all species in the subgenus *Hydrobaticus* from the New World (Short & Girón, 2018). Clades A1 and A2 both share a rather distinctive aedeagal form in which the parameres are fused together for most of their length forming a tubular structure (e.g. Fig. 1c, d), otherwise the two clades are rather externally similar. It should be noted that both clades A1 and A2 contain species with and without rows of elytral serial punctures, which had traditionally been used as the primary character to separate members of *Helochares* (s. str.) and *Hydrobaticus*. Clade A3 contains all Old World species placed in the subgenus *Hydrobaticus*, as well as some Old World species currently placed in *Helochares* (s. str.). Species in clade A3 share a distinctive aedeagus in which the apodemes of the median lobe are strongly sclerotized and rod-like, and often extend beyond the margin of the basal piece (e.g. Fig. 1g–k). Clade A3 also includes the type species for both *Helochares* (*H. lividus* (Forster)) and *Hydrobaticus* (*H. tristis* (MacLeay)), which is unfortunately problematic from a nomenclatural viewpoint.

Helochares clade B (Fig. 2), represented by a single bizarre species, *Helochares* (s. str.) *ellipticus* from central Africa, is isolated in both phylogenetic position as well as morphology. At 14 mm, it is not only the largest species of Acidocerinae, but also putatively the largest known hydrophilid outside of the subfamily Hydrophilinae. Despite its divergent morphology, there are surprisingly no preexisting generic names available for this taxon. It will be redescribed and placed in its own new genus in a subsequent study (Girón & Short, in prep).

Helochares clade C (Fig. 2) is composed of a group of Old World species that are characterized by their (i) larger size (6–10 mm), (ii) typically entirely shiny dark brown dorsal coloration, and (iii) complex genitalia: median lobe with a strongly sclerotized and apically acute main component, accompanied by additional shorter and slender sclerotizations; apical region of parameres usually partly heavily sclerotized and partly membranous, often bifurcated; basal piece strongly reduced; gonopore usually not clearly visible (e.g. Fig. 2d). Most of these features are also shared with the monotypic Afrotropical genus *Peltochares* (see Fig. 2e), which even though was not included in our analyses, we believe this taxon also would fall in this clade, and therefore provide it with a preexisting generic name.

Helochares clade D (Fig. 2) contains all the Neotropical species of *Helochares* that are currently placed in the subgenus *Helochares* (s. str.). This lineage contains two subclades: clade D2 contains all currently described species of Neotropical *Helochares* (s. str.), while D1 (Fig. 2) is composed entirely of undescribed Neotropical species which are morphologically distinct by possessing distinct elytral serial punctures. The monophyly of *Helochares* clade D was strongly supported in the BI analysis (PP: 0.97) but not in the ML analysis, where clade D1 was instead resolved as sister to *Sindolus* but with very low support (UFBS: 39). Although this is an area in the tree that warrants further study, we have chosen the BI topology for the time being as it is most congruent with morphology. Species in *Helochares* clade D1 possess a flat mesoventrite with only a low transverse ridge as those in taxa in *Helochares* clade D2, whereas lacking the strongly elevated longitudinal carina that is the primary synapomorphy for *Sindolus*. It is notable that the aedeagal form of the species falling within clade D1 (Fig. 2i) contain elements in common with both *Sindolus* and *Helochares* clade D2: for example, they possess a deeply forked median lobe as do most *Sindolus*, but also have distinctly toothed paramere apices, a feature common in *Helochares* clade D2.

Agraphydrus-group. The *Agraphydrus*-group is distributed across the Old World tropics. Presently containing 200 species, the lineage still has many undescribed species and the actual species richness is likely to continue to grow (Komarek, 2019). Our analysis strongly supports the placement of the genus *Horelophopsis* **syn. n.** as a highly derived lineage of the diverse genus *Agraphydrus* (Fig. 3). Therefore, we synonymize the former with the latter, creating two new combinations: *Agraphydrus avita* (Hansen) **comb. n.** and *Agraphydrus hanseni* (Satô & Yoshitomi) **comb. n.** A close relationship between *Horelophopsis* and *Agraphydrus* had been previously suggested by both morphological (Minoshima *et al.*, 2013, based on larvae) and molecular data (Short & Fikáček, 2013). A potential synapomorphy for the *Agraphydrus*-group is the V-shaped male abdominal sternite 9 (Minoshima, 2016), however this character has not yet been rigorously examined in all lineages of the subfamily.

Chasmogenus-group. The *Chasmogenus*-group occurs in all biogeographic regions except the Nearctic, though the vast majority of species are found in tropical areas. The group is easily distinguished from most other Acidocerinae by the presence of a sharply impressed sutural stria. This feature is otherwise only found in the much rarer genus *Primocerus*, and therefore care should be taken to separate these two genera in the Guiana Shield region of South America where both groups co-occur. Our analysis found *Chasmogenus* as currently defined (*sensu* Hansen, 1991) to be composed of two reciprocally monophyletic clades, which we here elevate to genera in their own right: *Chasmogenus* **sensu n.** and *Crephelochares* **stat. Rest.** *Chasmogenus* as newly circumscribed is restricted to Central and South America, and has a reduction in antennomeres from 9 to 8. *Crephelochares* is broadly distributed throughout much of the Old World tropics and southern Palearctic, and retains the plesiomorphic condition in Hydrophilidae of nine antennomeres. Although differences between complexities in the aedeagus were also thought to separate these two lineages, recent discoveries of highly derived forms in some *Chasmogenus* (s. str.) species has made this character suite less clear as a synapomorphy (Smith & Short, 2020). Although taxonomic stability in both names and concepts is important, and 'subjective' changes to established classification such as this one should be generally avoided, we consider it is appropriate in this circumstance given (i) each lineage is unambiguously diagnosable, (ii) the lineages occur in different, nonoverlapping biogeographic regions, (iii) the old age (>100 Ma) of their divergence, and (iv) that the names of most Old World species of *Chasmogenus* are not widely used in historical literature and therefore there is comparatively minor nomenclatural disruption. The elevation of *Crephelochares* creates 28 new combinations: *Crephelochares abnormalis* (Sharp) **comb. nov.**, *Crephelochares africanus* (d'Orchymont) **comb. nov.**, *Crephelochares balkei* (Short) **comb. nov.**, *Crephelochares cattienus* (Hebauer) **comb. nov.**, *Crephelochares irianus* (Hebauer) **comb. nov.**, *Crephelochares larsi* (Hebauer) **comb. nov.**, *Crephelochares luctuosus* (d'Orchymont) **comb. nov.**, *Crephelochares lycetus* (d'Orchymont) **comb. nov.**, *Crephelochares mauritiensis* (Balfour-Browne) **comb. nov.**, *Crephelochares molinai* (Hebauer) **comb. nov.**, *Crephelochares mollis* (Régimbart) **comb. nov.**, *Crephelochares molluscus* (Hebauer) **comb. nov.**, *Crephelochares nitescens* (Fauvel) **comb. nov.**, *Crephelochares omissus* (Hebauer) **comb. nov.**, *Crephelochares orbus* (Watanabe) **comb. nov.**, *Crephelochares paramollis* (Hebauer) **comb. nov.**, *Crephelochares parorbus* (Jia and Tang) **comb. nov.**, *Crephelochares patrizii* (Balfour-Browne) **comb. nov.**, *Crephelochares punctulatus* (Short) **comb. nov.**, *Crephelochares rhodesiensis* (Hebauer) **comb. nov.**, *Crephelochares ruandanus* (Balfour-Browne) **comb. nov.**, *Crephelochares rubellus* (Hebauer) **comb. nov.**, *Crephelochares rubricollis* (Régimbart) **comb. nov.**, *Crephelochares rudis* (Hebauer) **comb. nov.**, *Crephelochares rusticus* (d'Orchymont) **comb. nov.**, *Crephelochares rutiloides* (d'Orchymont) **comb. nov.**, *Crephelochares rutilus* (d'Orchymont) **comb. nov.**, *Crephelochares szeli* (Hebauer) **comb. nov.**

Tobochares-group. The *Tobochares* group is comprised of seven Neotropical-endemic genera, most of which have only been recently discovered and described. Indeed, the first taxon in the group was described just twenty years ago (*Quadriops*; Hansen, 1999). Most species in the *Tobochares*-group are small to minute, with only a handful of species exceeding 3 mm in length. Some species of *Nanosaphes* are among the smallest of all Hydrophiloidea at just 1.1 mm in total body length (Girón & Short, 2018). Although appearing generally uniform in habitus and superficial appearance, genera in the group vary in the number of antennomeres, the form of the mesoventrite, the relative length of the maxillary palps, the condition of the apical abdominal ventrite, the condition of the eyes, and the presence of serial punctures and elytral striae, among other characters. At this time, no unambiguous morphological synapomorphy is known that would define the group. Dozens of undescribed species still await description in this group (Short & Girón, unpub. data).

Placement and impact of missing genera

Four genera were absent from the analyses, all of which are monotypic: *Acidocerus*, *Helopeltarium*, *Peltochares*, and *Troglochares*. Based on adult morphological similarities, we consider that the first three can be easily accommodated within the *Helochares*-group, whereas the placement of *Troglochares* is less clear.

Acidocerus, the genotype of the subfamily, is known only from a few specimens from the type locality in Mozambique. The type specimens are not in good condition, and the palps are missing (Fig. 1f). However, based on the overall habitus, dorsal sculpturing, size, and distribution we believe it is most likely a member of *Helochares* clade A3 (Fig. 1).

The genus *Helopeltarium* is only known from the type series from Myanmar. We examined two paratype specimens, including a male that we dissected. The form of the genitalia (long, fused, tubular parameres, a simple unmodified median lobe, and relatively short basal piece, Fig. 1e) is nearly an exact match for *Helochares* clade A1 (Fig. 1d). *Helochares* clade A1 also is endemic to Southeast Asia, and we hypothesize that *Helopeltarium* is either a member of, or very closely related to this lineage. Although we are confident in its placement as a member of the *Helochares* group, we refrain from formally synonymizing it with *Helochares* at this time.

Peltochares is a monotypic genus known from tropical Africa distinguished primarily by its very large size and extreme dorsoventrally compressed body form (Fig. 2e). We examined the type series and several additional specimens. The form of the genitalia is quite similar to those in *Helochares* clade C (Fig. 2). Indeed, some species such as *Helochares discus* (which was included in our molecular analysis) are actually closer in body form to the large and explanate *Peltochares* than to other species placed in *Helochares*. We have little doubt that *Peltochares* falls within or adjacent to *Helochares* clade C, which therefore would provide an available genus name for this clade when the classification is formally revised.

Troglochares is known only from the female holotype, which is broken into several pieces. Collected on a wet stalactite from a cave in Ecuador, it has features typical of a troglobiont, namely the loss of eyes and a depigmented cuticle. It lacks a sutural stria, eliminating the *Primocerus* and *Chasmogenus* groups as likely relatives. Given its presence in the Neotropics and its very small size, we hypothesize that a placement within the *Tobochares*-group is most likely; nevertheless, because the *Tobochares*-group has no unambiguous morphological synapomorphy, this placement cannot be presently confirmed. The *Agraphydrus* group, although similar in size and including seepage taxa, is known only from the Old World. The *Helochares* group contains no known species as small as *Troglochares*, especially in the New World, and is very rare in seepage habitats.

Biogeography

Our results support an ancient origin and early diversification of the Acidocerinae c. 175 Ma in West Gondwana (South America + Africa). The diversification events over the next 30 Ma, particularly the divergence of the *Helochares* group, implicate North America but not any components of East Gondwana (Madagascar + India + Australia) as an early area of endemism. This is congruent with the connectivity of these land masses at that time, as West Gondwana and Laurasia (North America) were still connected, but East Gondwana had begun to rift from Africa (though still connected to South America via Antarctica) (Seton *et al.*, 2012). Within the *Helochares*-group, there is an initial split between *Helochares* clade A which originates in Laurasia (Asia) and a clade composed of the remaining lineages which originates in Gondwana (South America + Africa). This split is coincident with the separation of Laurasia and Gondwana, and therefore represents a putative early example of vicariance in the subfamily.

Subsequent to South America's separation from Africa, there remained potential for a 'southern connection' to Australia via Antarctica until as recently as c. 60 Ma (Seton *et al.*, 2012). Evidence for this southern connection is typically shown by either a sister-group relationship between Australian and Neotropical clades or paraphyletic grades that contain taxa from both continents (e.g. Letsch *et al.*, 2020). Signatures for this austral connection are found in other groups of hydrophilids (e.g. Fikáček & Vondráček, 2014). However, no such sister-group relationships between Australia and South America are observed in the Acidocerinae. Indeed, there are no acidocerinae lineages younger than 100 Ma that contain both South American and Australian taxa. Rather, the Australian acidocerine fauna appears relatively young and species poor, and the subfamily is entirely absent from New Zealand. For example, the highly diverse Old World genus *Agraphydrus* is represented in Australia by a single widespread species (*A. coomani* (d'Orchymont)) that also occurs throughout southeast Asia, with just a few additional endemic species present in New Guinea (Komarek, 2019). There is also no evidence for dispersal to or from South America at any point between ca. 100 and 30 Ma, suggesting that the Neotropical acidocerine fauna evolved in isolation for an extended period of time, a scenario perhaps most commonly associated with Mammals (Simpson, 1980).

At first glance, it may not seem as if North America has played much of a role in the diversification of the Acidocerinae. It has substantially lower species diversity than any other biogeographic region or subregion with only four species (including only one endemic), all of which are included in our analysis and we here treat in turn. In the case of *Helobata larvalis* (Horn), it seems clear that the presence of this species in North America is the result of a more recent dispersal event from South America. The Neotropics are resolved as the ancestral area of the genus, and all described species occur there, including the widespread *H. larvalis*. *Helochares sallaei* (Sharp), a primarily Neotropical species known from a handful of specimens from Florida, is thought to be introduced from Central America (Young, 1954). The remaining two North American species are members of *Helochares* clade A2: *Helochares maculicollis* (Mulsant), which is endemic to eastern North America, and *Helochares normatus* (LeConte), which occurs throughout western North America and south to Costa Rica (Short & Girón, 2018). The other members of clade A2 occur in Central America and the Andean region of northern South America. The ancestor of *Helochares* clade A2 is resolved as occurring in the Neotropical region, with two subsequent dispersal events into North America between c. 20–35 Ma. Interestingly, however, the ancestral area of *Helochares* clade A2 + A3 is resolved as North America + Asia/Palearctic. This implies both a dispersal into North America prior to c. 95 Ma, and a later dispersal to South America (coupled with extinction in North America) between 40–95 Ma.

Conclusions

Our study provides the first well-sampled phylogeny of the taxonomically challenging subfamily Acidocerinae. In addition to affirming the monophyly of the subfamily, we found strong support for five primary monophyletic groups of genera. Although we were unable to identify clear morphological synapomorphies for all five lineages, we hold hope that future efforts focused on adult and larval morphology will be successful in this regard and that tribes may be introduced. Although we confirmed most genera are monophyletic as currently circumscribed and made a few relatively simple adjustments to the classification of the subfamily, the situation involving polyphyletic *Helochares* needs more attention to resolve its classification than this study could provide. For the time being, we have identified four morphologically-diagnosable clades within the genus. However, due to the large number of species involved as well as the need to reconcile a number of available genus-group names, we defer further reclassification of *Helochares* to a future study (Girón & Short, in prep).

Our findings suggest that this ancient subfamily likely originated in West Gondwana and that its present-day distribution has signatures consistent with both, the breakup of Gondwana and Laurasia, as well as the fragmentation of West Gondwana itself. Therefore, the Acidocerinae may be added to the list of Mesozoic hydrophilid lineages that bear a distinct imprint of Gondwanan vicariance (Toussaint *et al.*, 2016a; Toussaint & Short, 2018). Our data also support a scenario in which following its separation from Africa, the South American fauna was isolated for ca. 70 million years, and thereafter only experienced more recent interchange with North America.

Now armed with a general phylogenetic blueprint, we hope that this group will be more taxonomically approachable. We further are excited about increased feasibility to study broader patterns of morphological evolution, habitat transitions, and parental care, that make the Acidocerinae such an attractive study system.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1: List of sequenced voucher specimens and GenBank accession numbers

Appendix S2: Nexus file used in the phylogenetic analyses

Appendix S3: BEAST tree file

Appendix S4: IQTree tree file

Appendix S5: BEAST xml file

Appendix S6: Summary of the dispersal rate scaler and adjacency matrices used in BIOGEOBEARS

Appendix S7: Lists of secondary calibrations used in the BEAST analysis and partitions used as identified by PartitionFinder.

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Data availability statement

The data that support the findings of this study are openly available in GenBank at https://www.ncbi.nlm.nih.gov/genbank/ under accessions MW350730 thru MW351619. This article has been registered in the Official Register of Zoological Nomenclature (ZooBank): urn:lsid:zoobank.org:pub:F853ACE7-D597-4F5D-BB07-19FC76A20CA4.

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