Molecular phylogeny of Megasternini terrestrial water scavenger beetles (Hydrophilidae) reveals repeated continental interchange during Paleocene-Eocene thermal maximum

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Abstract. Megasternini is the largest group of terrestrial water scavenger beetles (Coleoptera: Hydrophilidae) represented by ca. 600 described species distributed worldwide. The highest species diversity is known from tropical areas of all continents. In this study, we used sequences of eight gene regions (five nuclear and three mitochondrial) to investigate the phylogenetic relationships and historical biogeography of this group, implementing maximum likelihood (ML) and Bayesian topology inference, Bayesian divergence dating, ML-based ancestral area estimation and Bayesian diversification analyses. Topology analyses reveal two main lineages of Megasternini characterized by the morphology of male genitalia and surrounding sclerites; these lineages are defined here as subtribes Megasternina Mulsant and Oosternina new subtribe. We identify 12 principal clades of Megasternini, three in Oosternina and nine in Megasternina. These clades group the taxa largely by their geographic distribution rather than morphology, indicating a parallel evolution of morphological characters. Genera Cercyon Leach, Oosternum Sharp, Cetiocyon Hansen, Australocyon Hansen and Pelosoma Mulsant were not recovered as monophyletic. Species of Cercyon, the most diverse genus in the tribe, are found in all principal clades in both subtribes. These results suggest a need for the reorganization of generic concepts in the tribe. The historical biogeography analysis reveals a series of parallel intercontinental dispersal events, including the colonization of South America by Australian members of Oosternina ca. 90 million years ago (mya) and at least five dispersal events between Asia and America ca. 63–55 mya via the Beringia land bridge. The timing of the Asia-America faunal interchange corresponds to the hyperthermal climate of the Late Paleocene and Early Eocene that allowed the expansion of tropical and subtropical biomes towards polar regions. Diversification analyses revealed no effect of intercontinental dispersals on speciation or extinction rates and suggested a possible effect of declining global temperatures in the last 20 million years.

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Introduction

With almost 3000 species distributed worldwide, the family Hydrophilidae (water scavenger beetles) is the largest group in the superfamily Hydrophiloidea (Coleoptera: Polyphaga: Staphylinoformia). As their common name suggests, hydrophilid beetles typically inhabit aquatic habitats like sides of lakes, streams, or waterfalls. The aquatic lifestyle is ancestral for the family and likely also for the whole Hydrophiloidea (Bernhard et al., 2006; Bloom et al., 2014; Song et al., 2014). Nevertheless, around 150 million years ago, the common ancestor of subfamilies Cylinodinae and Sphaeridinae left the water (Bloom et al., 2014; Toussaint & Short, 2018) and gave rise to the most successful lineage of aquatic insects that recolonized terrestrial ecosystems. The members of this lineage have colonized a broad spectrum of habitats, especially in the tropics, where they can be found in a variety of decaying organic substrates as well as in specialized habitats such as ant nests (e.g., Fikáček et al., 2013, Fikáček et al., 2015).

At least half of the terrestrial hydrophilid beetles belong to the tribe Megasternini of the subfamily Sphaeridinae. Despite being, along with its sister Sphaeridiini, the youngest tribe in the family in terms of the number of species and number of habitats colonized. The species diversity of this clade is likely a consequence of an increased diversification rate compared to other hydrophilid lineages (Bloom et al., 2014). Nearly 550 megasternine species are formally described, but the total number of species is likely much higher. It has been estimated to be around 850 species by Bloom et al. (2014). Nevertheless, the proportion of undescribed species found in museum collections (Arriaga-Varela, pers. observ.), as well as recent revisions exemplifying an unexpected species diversity in small tropical areas (e.g., Arriaga-Varela et al., 2017; Szczepański et al., 2018), suggests that the real species richness might be even higher, probably exceeding 1000 species.

Representatives of Megasternini can be found in a wide array of terrestrial habitats like humid leaf litter (Fikáček & Short, 2006), vertebrate dung or carrion (Archangelsky, 1997; Arriaga-Varela et al., 2018a), angiosperm inflorescences (Arriaga-Varela et al., 2018b), debris accumulated by social insects (Spangler, 1962; Fikáček et al., 2013), rotten succulent plants or fruits (Arriaga-Varela et al., 2019b) or beach wrack at the seashore (Suzumura et al., 2019b). Some groups recolonized aquatic habitats, including the phytotelmata in heliconia and ginger plants, or those formed in broken bamboo culms (Archangelsky, 1997; M. Fikáček & D. Kovac, unpubl. data).

Although megasternines are small or rarely medium-sized beetles (1–9 mm long) with compact ovoid bodies, they show a great diversity in morphology. Currently, 51 genera and subgenera are recognized. The genus-level classification is based largely on the shape and proportions of the ventral parts of the thorax (e.g., Hansen, 1991; Fig. 1). The first phylogenetic studies based on DNA sequences (Bernhard et al., 2006; Bernhard et al., 2009; Song et al., 2014) focused on the relationships among hydrophiloid families and only included very few megasternine species. Short & Fikáček (2013) assembled the first taxonomically comprehensive sampling for the family Hydrophilidae that included 12 genera and 16 species of Megasternini. The analysis revealed two main clades within Megasternini: the Cercyon-group and the Oosternum-group of genera. The most species-rich genus in the tribe, Cercyon Leach, was recovered as polyphyletic since the three Cercyon species included in the analysis were placed in different parts of the tree. Already these early results indicated that the current morphology-based classification poorly reflects the phylogeny and is likely obscured by homoplasy and convergence in morphological characters.

Megasternini is distributed worldwide except Antarctica and some distant oceanic islands, with the highest species diversity recorded from humid tropical areas (Hansen, 1999a; Short & Hebauer, 2006; Short & Fikáček, 2011). The global distribution makes it an excellent group for testing hypotheses regarding the processes underlying tropical intercontinental disjunctions. Many genera are of limited distribution, confined to a single continent. In contrast, the genus Cercyon, which comprises almost half of the species diversity in the tribe, is distributed in all biogeographical regions. Other genera like Oosternum Sharp, Cryptopleurum Mulsant, Pelosoma Mulsant and Australocyon Hansen show disjunct continental distributions that make them an interesting target of phylogenetic and biogeographic studies.

The goal of the present contribution is to propose a robust hypothesis on internal phylogenetic relationships within the tribe Megasternini using a multigene dataset obtained from an extensive taxonomic sampling at species and genus levels. We reconstruct and discuss general patterns of species diversity, biogeography and morphological evolution based on the inferred phylogenetic hypothesis and propose a new suprageneric classification of the group. The work is aimed to be a basis for understanding the evolutionary history of the group and the first step towards its natural phylogeny-based classification at the genus level.

Material and methods

Taxon sampling

We assembled a data set of 193 specimens belonging to 189 species. This number represents close to a third of the described species of Megasternini and nearly a fifth of the total number of species estimated by Bloom et al. (2014). The species included in our dataset cover 38 of 51 described genera (75%) (Hansen, 1991, 1999a,b,c; Short & Hebauer, 2006; Short & Fikáček, 2011; Jia et al., 2020). The genera not sampled here are mostly known from a single or a few species and specimens and represent only a minority (ca. 3.5%) of described species in the tribe. Since Megasternini is most diverse in the humid tropical areas, our sampling is mainly focused on species from Neotropical, Oriental, Afrotropical and Australian regions. It includes multiple representatives of the most speciose genera (e.g., Cercyon, Oosternum, Pelosoma and Cryptopleurum) to cover their
morphological diversity and geographic range. We compared our DNA vouchers to specimens from museum collections that hold types for the majority of described species (Orchymont collection in Institute Royal des Sciences Naturelles in Brussels and Sharp and Balfour-Browne collections in the Natural History Museum in London). In many cases, we were not able to identify voucher specimens to species level. Species-level identification, or an approximate one indicated by cf. before species name, was only done for genera and species for which reliable identification tools exist. Our comparison revealed that close to half of the species included in our study is likely undescribed. File S1 lists all taxa used in the analysis. All sequences were generated de novo, except those of *Cetiocyon incantatus* Fikáček & Short (adopted from Short & Fikáček, 2013) and *Cercyon melanocephalus* (Linnaeus) (adopted from Pentinsaari *et al.*, 2014). Eight species representing the remaining tribes of the Sphaeridiinae were sequenced and included as outgroups. The tree was rooted using *Agraphydrus* sp. (Acidocerinae). Voucher specimens are deposited at the National Museum, Prague, Czech Republic.

**DNA extraction and amplification**

Specimens were collected and kept in 95% ethanol and stored at ~20°C. Total genomic DNA was extracted from entire beetles using QiaGen Blood and Tissue DNA extraction kit following the manufacturer’s instructions. Gene fragments were amplified using polymerase chain reaction (PCR). We included fragments of three nuclear protein-coding genes (histone 3 (H3), wingless (Wg) and topoisomerase 1 (Top1)), two nuclear ribosomal genes (18S and 28S), two mitochondrial protein-coding genes (cytochrome oxidase I (COI), cytochrome oxidase II (COII)) and one mitochondrial ribosomal gene (12S). COI and 18S sequences were amplified as two or three fragments, respectively. Table 1 lists the primers used and the PCR protocols followed. New primers were designed for regions of the 28S rDNA and cytochrome oxidase I (COI) genes due to problems with amplification using previously published primers. Amplification with standard primers for 28S resulted in unspecific products, including contamination from nematodes, probably present in the substrate from which the beetles were collected. Thus, we designed a forward primer to target a region of the 5’ end of the gene with no variation within the family Hydrophilidae, using the sequence data of Toussaint & Short (2018) combined with part of our novel sequences: HydrEAV5F (forward) 5’-GTCMAAGTCCTTGGCCGAGGGGCCRYTAC-3’. HydrEAV5F primer was coupled with the standard reverse primer LS1041R (Maddison, 2008) to amplify a region of ca. 900bp. In the case of COI, amplification success was limited using primers LCO1490 and HCO2198.
Table 1. List of primers used in this study and the PCR annealing temperatures used.

| Gene   | Primer         | Sequence (5′-3′)                                               | Annealing temperature (°C) | Reference               |
|--------|----------------|---------------------------------------------------------------|----------------------------|-------------------------|
| 12s    | 12s ai         | AAACTACGATTAGATACCTATTAT                                      | 49                         | Simon et al. (1994)     |
|        | 12s bi         | AAGAGCGGACGCGGCAAGTTG                                       | 49                         | Simon et al. (1994)     |
| 18s    | 18Ssend_F_18SS51 | GACAACTGTGTTATCCTTCAGT                                        | 50                         | Shull et al. (2001)     |
|        | 18Ssend_R_18Sb605 | TAACGCCAAGCCTTTTAT                                        | 50                         | Shull et al. (2001)     |
|        | 18Ssc_e_F_18Sa1i | CTTGAGAAACGGTGACTTCACAT                                     | 50                         | Shull et al. (2001)     |
|        | 18Ssc_e_R_18Sb0.5 | GTCACCAACTTCGAACCACT                                       | 50                         | Shull et al. (2001)     |
|        | 18S3_F_18Sa1.0 | GTGGAAATTCCTGGACCCTGTC                                      | 50                         | Shull et al. (2001)     |
|        | 18S3_R_18S3′1  | CACCTACGGAAAACTCTTACGAC                                     | 50                         | Shull et al. (2001)     |
| 28s    | 28s_NLFl184-21 | ACCGGCTGAACTTAACATGAC                                        | 53                         | Van der Auwera et al. (1994) |
|        | 28s LS1041R    | TACGGAACRCATCCATACGGGTTCCTTCCCTGACTTC                       | 53                         | Maddison (2008)         |
| COII   | Tp919R         | GTCTCTTTGCGTYTTRTTRADATYTTYTC                                  |                           |                         |
|        | TP932R         | GGWCCDGCATCDATDGCCCA                                         | 50                         | Wild & Maddison (2008)  |
|        | TP931F         | GACAACTGTGTTATCCTTCAGT                                      | 50                         | Wild & Maddison (2008)  |
|        | TP931R         | GACAACTGTGTTATCCTTCAGT                                      | 50                         | Wild & Maddison (2008)  |
|        | HCO2198        | TAAACTTCAGGGTGACCAAAAAATCA                                    | 48                         | Folmer et al. (1994)    |
|        | HCO1490        | GTTCACAAATCATAAGATATTG                                      | 48                         | Folmer et al. (1994)    |
| COI    | H3aR           | ATATCCTTGGGCATGATGAGTGTGAC                                   | 50                         | Colgan et al. (1998)    |
|        | H3F            | ATATCCTTGGGCATGATGAGTGTGAC                                   | 50                         | Colgan et al. (1998)    |
|        | H3A            | ATATCCTTGGGCATGATGAGTGTGAC                                   | 50                         | Colgan et al. (1998)    |
| TP     | TP643F         | GACGATCGATCAACACTANARAGAATG                                   | 56                         | Wild & Maddison (2008)  |
|        | TP932R         | GGWCCDGCATCDATDGCCCA                                         | 56                         | Wild & Maddison (2008)  |
|        | TP675F         | GAGCAACACGGCAGACGACGCG                                       | 56                         | Wild & Maddison (2008)  |
|        | TP919R         | GTCACCATCCTGCTTCTGCTGTC                                      | 56                         | Wild & Maddison (2008)  |
| Wingless | Wg550F        | ATGCGTCCAGGARTGYAARTGYGCGGTYGATGC                            | 56                         | Wild & Maddison (2008)  |
|        | WgABRZ         | CACTTACCTACCTARCGACGCTGA                                     | 56                         | Wild & Maddison (2008)  |
|        | Wg578F         | TGCACNTGGAARACCYTCCTGGATG                                   | 56                         | Wurd & Downie (2005)    |
|        | WgABR          | ACYTCGACGACCATGGAA                                          | 56                         | Abouheif & Wray (2002)  |

(Folmer et al., 1994), hence a new primer pair for the amplification of a fragment of 625 bp in the 5′ end of the COI gene (barcoding region) was designed using an alignment of 102 coleopteran sequences obtained from BOLD database (Ratnasingham & Hebert, 2007), with a few degenerated bases to cope with the variability found in the alignment. The primer sequences are Coleop_DMEAV5F (forward) 5′-HTGAKCGGWATARTWGG-3′ and Coleop_DMEAV5R (reverse) 5′-RTA WACCTTCCWGGRTGDC-3′; the forward primer region lies ∼50 bp downstream from LCO primer, while the reverse overlaps with the 3′ end of HCO primer.

Sequence editing and alignment

Sequences trace files were uploaded into Geneious (Kearse et al., 2012) for inspection, assembly and editing. All newly generated sequences were submitted to GenBank. The vouchers, associated data and GenBank accession numbers of the sequences used in this study are listed in File S1. Sequences were aligned in Geneious using ClustalW algorithm with default settings. The alignment of DNA sequences was trivial for COI, COII, Top1, H3 and Wg. Alignments for rDNA sequences were inspected by eye and corrected manually or by subsequent iterations of realignments of hypervariable zones using ClustalW or MUSCLE algorithms in Geneious. Concatenation of sequences was performed manually in Geneious software.

Treatment of wildcard taxa

Despite our intention to include as many terminal taxa as possible, some of those included in an original dataset were revealed to be unstable in their position in preliminary analyses, resulting in unresolved larger clades. In most cases, these terminals missed a considerable amount of data in the alignment. These taxa were excluded prior to the final set of analyses and are not listed among taxa examined. Of the final 193 taxa included in the analysis, five of them still showed an unstable ‘wild-card’ behaviour when analysed using the Bayesian inference. For this reason, we assembled two datasets: Wildcard dataset, which includes wildcard taxa and the Mega dataset in which wildcard taxa are not included. Species excluded from the Mega dataset are Cercyon melanocephalus, Cercyon littoralis (Gyllenhal), Evanesternum pulsatum (Orchymont), Pyretus sp. and Cercyon sp (MF1275, a species with an unusual concave mesoventral plate from Madagascar). The unstable position of these species may be caused by missing data; this is especially true for Cercyon melanocephalus (Linneaus) which is only represented by one genetic marker and is included because it is the type species of Cercyon.
Phylogenetic analyses

The concatenated alignments were used to infer the phylogenetic relationships using the Bayesian inference (BI) and maximum likelihood (ML). Protein-coding genes were partitioned by codon position; ribosomal genes were analysed unpartitioned. Best fitting models of substitution were selected using PartitionFinder 2 (Lanfear et al., 2016) with all models included and the greedy algorithm used. The corrected Akaike information criterion (AICc) was used to compare the likelihoods of the models. The ML analyses of both datasets (Wildcard and Mega) were carried out in W–IQ–TREE 1.5 (Nguyen et al., 2014; Trifinopoulos et al., 2016). An ultrafast bootstrap (UFB) with 1000 repetitions (Minh et al., 2013) was applied to estimate the support for the hypothesized nodes. The BI analyses were performed using MrBayes 3.2.6 (Ronquist et al., 2012) implemented on the CIPRES Science Gateway 3.3 (Miller et al., 2010). Four simultaneous independent MCMC runs with six chains each and 100 million generations were used for the analysis, with a tree sampled every 12,500 generations to calculate posterior probabilities (PP). The convergence of the runs and their parameters were assessed in Tracer 1.7 (Rambaut et al., 2018). A conservative 50% burn-in fraction was excluded from the data prior to the construction of the final maximum credibility tree.

Divergence time estimation

Divergence dating was performed using the Mega dataset in MrBayes 3.2.6. We used a relaxed clock method under the birth-death model. The only fossil megasternine reported by Kubisz (2000) as Cercyon sp. and used as a calibration point by Bloom et al. (2014) and Toussaint & Short (2018) was revealed not to belong to the Hydrophilidae (Arriaga-Varela et al., 2019a). Consequently, no Sphaeriidae fossils is available for calibration, and an external calibration strategy needs to be used. The gene sampling in our Megasternini dataset is not fully compatible with previously published DNA datasets of the Hydrophilidae. Consequently, a preliminary analysis combining Hydrophilidae data published previously with our Megasternini dataset resulted in a largely unresolved backbone topology, probably due to a large amount of missing data. We hence implemented a two-step strategy to date the Megasternini tree. First, we ran a time tree analysis based on a Hydrophilidae dataset (Hydro) containing a comprehensive sampling of the subfamily Sphaeriidae including 66 species of tribe Megasternini. This dataset includes the data for seven genes: 18S, 28S, ArgK, Top1, 16S, COI and COII. The tree was dated using a set of eight fossils, largely following those used by Bloom et al. (2014) and Toussaint & Short (2018) with the following updates and corrections: (i) exclusion of the specimens previously incorrectly identified as Cercyon sp. by Kubisz (2000); (ii) inclusion of Cretocrenis profectaud Arriaga-Varela et al. (44 Ma; Arriaga-Varela et al., 2019a) and Cretocrenis brunanicus Fikáček et al. (99 Ma; Fikáček et al., 2017) to date respective clades of the subfamily Chaetarthriinae. See File S3 for details of this time tree analysis. Results of the Hydrophilidae analysis were subsequently used to constrain the age of basal nodes in the Megasternini-only analysis with all gene fragments included. This second analysis still faced problems in the convergence of the MCMC runs, likely due to a large number of terminal taxa. We hence fixed the topology to that revealed by the ML topology analysis; this topology is fully bifurcated as required for the biogeography and diversification analyses which is why we preferred it to the BI topology. Truncated normal distribution was applied for the constraints as follows: (i) MRCA of Sphaeriidae: lower limit = 140 mya, mean = 158.9 mya, SD = 9.5 mya; (ii) MRCA of Sphaeriidae + Megasternini: lower limit = 117.4 mya, mean = 134 mya, SD = 8.9 mya; and (iii) MRCA of Megasternini: lower limit = 104.5 mya; mean = 120.3 mya, SD = 8.1 mya.

Ancestral range estimation

To estimate the ancestral distribution at the nodes, we carried out the historical biogeography analyses in the R package BioGeoBEARS (Matzke, 2014), using the tree resulting from the Megasternini divergence dating analysis as an input tree; outgroups were excluded. Distribution ranges were coded for main biogeographical realms: O – Oriental; U – Australian; E – Afrotropical; P – Palaearctic; A – Nearctic; and N – Neotropical. We merged Madagascar with Africa and South India with the Oriental region, given the low number (Madagascar) or total absence (South India) of taxa distributed in these areas. BioGeoBEARS implements three models in a maximum likelihood framework: DEC model (Ree & Smith, 2008), DIVALIKE model (a likelihood version of the DIVA model by Ronquist, 1997) and BAYAREALIKE model (a likelihood version of BayArea model by Landis et al., 2013). Founder event speciation can be added to any of the previously mentioned models and left as a free parameter J estimated from the data, adding three extra models. The aim of our analysis was to test whether the historical biogeography of Megasternini is congruent with the history of the continental inter-connections (Lomolino et al., 2010). We hence conducted unconstrained analyses without any geological constraints to estimate the ancestral range on the nodes, implementing all six models and default parameter values. Models were compared using likelihood values and Akaike information criterion. Input files for the biogeography analyses are available in File S5.

Diversification analysis

The analyses above revealed two major clades in the Megasternini, differing in the number of described species (116 species in Oosternina, 426 in Megasternina; Short & Fikáček, 2011) as well as in the expected species diversity. Multiple intercontinental interchanges were moreover revealed in both clades during the Paleocene–Eocene thermal maximum. We hence performed exploratory analyses testing whether the speciation, extinction and diversification rates differ between both major clades and whether they varied through time. First,
we visualized the pattern of accumulation of lineages in the tree by lineages through time (LTT) plots for the whole Megasternini and for each subtribe; the plots were constructed using the ape package in R (Paradis et al., 2004) using the dated tree from which the outgroups were excluded. Diversification rates were estimated and compared between clades and through time using a Bayesian approach under the Episodic Diversification Rate model (Stadler, 2011; Höhna, 2015) as implemented in RevBayes (Höhna et al., 2016). We compared three scenarios differing in the number of time intervals with different rates: (i) a single interval, i.e., no variation of the rates through time; (ii) two intervals modelling different rates before and after the Paleocene-Eocene climatic maximum; and (iii) 10 intervals (for Megasternini) or nine intervals (for both subtribes, taking into account their more recent origin) modelling a more complex variation of rates through the time. All analyses expected the diversified taxon sampling (i.e., a non-random sampling focused on the sampling of major clades and deeper splits; Ronquist et al., 2016). The expected total number of Megasternini was set to the conservative estimate of 1000 species. The taxonomic effort was equal in the tropics of all continents, which are supposed to contribute the most to the diversity of the group, and hence we expect a similar proportion of described versus undescribed species in both subtribes; correspondingly, the total species number was set to 200 species for Oosternina and 800 species for Megasternina. The relative fit of each model to the data was compared by calculating the marginal likelihood of each model and their comparison using Bayes factors. The speciation (b), extinction (d) and net diversification (b-d) rates estimated by the best-performing model were visualized using the RevGadgets package in R.

**Morphology studies**

Voucher specimens and additional specimens of selected species were dissected, with male genitalia (in most specimens) or all body parts (in specimens completely dissected for morphology studies) embedded in a drop of alcohol-soluble Euparal resin. Habitus photographs were taken using a Canon EOS 550D digital camera with an attached Canon MP-E65 mm f/2.8 1–5× macro lens. SEM micrographs of uncoated adults were taken using a Hitachi S-3700N environmental electron microscope at the Department of Paleontology, National Museum (Prague, Czech Republic). Photographs of male genitalia were taken using a Canon D1100 digital camera attached to an Olympus BX41 compound microscope.

**Online data deposition and registration required by International Committee on Zoological Nomenclature (ICZN)**

Final alignments, i.e., the one used for the Mega analysis, the one used for the analysis with the wildcard taxa, and the one used for the Hydrophilidae time tree analysis are available on Zenodo (https://www.zenodo.org) at the following doi:

http://doi.org/10.5281/zenodo.4567675. All newly generated DNA sequences were submitted to GenBank under accession numbers listed in Files S1 and S3. To make the new subtribe described in this study available from the date of the online publication of this paper, the paper was registered in ZooBank under the following ID: http://zoobank.org/urn:lsid:zoobank.org:pub:213029B2-C98C-4ABB-A05B-3CBD8CFC572. The new subtribe was registered under the following ID: http://zoobank.org/urn:lsid:zoobank.org:act:785660AB-4CC8-434E-91F9-4CED58CAC3B1.

**Results**

**Molecular dataset and selection of substitution models**

The final concatenate alignment was 6587 bp long and composed of nine gene fragments as specified in Table 2. PartitionFinder analysis found GTR + I + G to be the best fitting model for all partitions except 28S, the second codon position of H3, and third codon position of Wg, and the first codon position of Top1 for which the SYM + I + G model was found as best fitting model.

**Phylogenetic analyses**

Analyses of the Mega dataset recovered similar topologies both in BI and ML except for few instances of relationships among closely related species (Figs 3A, 4A, 5A) (see File S2 for complete BI and ML trees). Bootstrap support values (UBF) were above 80 for all nodes recovered in the ML analysis and 95–100 in 60% of the nodes. The posterior probability (PP) scores from the Bayesian analysis were comparatively lower, showing approximately 40% of nodes with values below 0.9 and some nodes with critically low support, i.e. below 0.5. The basal-most divergence within the tribe, i.e., the split of the Oosternum and Cercyon group of genera sensu Fikáček (2010), was recovered as strongly supported in ML (UBF = 100) but weakly supported in Bayesian analysis (PP = 0.65). Posterior probability values in the BI analyses were generally above 90% within the first clade, referred hereafter as Oosternina subtribe nov. In the sister clade, referred hereafter as Megasternina Multsant,

| Gene fragment | Length (bp) | Spec. with data | Spec. w/o data | Data coverage (%) |
|---------------|-------------|-----------------|----------------|-------------------|
| 12S rDNA      | 431         | 179             | 23             | 88.6             |
| 18S rDNA      | 1841        | 186             | 16             | 92.1             |
| 28S rDNA      | 1045        | 184             | 18             | 91.1             |
| COI 5′ part   | 625         | 110             | 92             | 54.5             |
| COI 3′ part   | 788         | 104             | 98             | 51.5             |
| COII          | 675         | 104             | 98             | 51.5             |
| Histone 3     | 234         | 147             | 55             | 72.8             |
| Wingless      | 330         | 144             | 58             | 71.3             |
| Topoisomerase 1| 618         | 135             | 67             | 66.8             |
the support was low for some of the deepest nodes at the backbone of the tree, particularly in the clades VII and XII. For the subsequent analyses and discussion of the topology, we decided to follow the systematic hypothesis expressed in the fully bifurcated cladogram resulting from the ML tree reconstruction of the Mega dataset. To better understand the morphology, biology and biogeography of Megasternini, we divided the tribe into 12 clades that were relatively well supported and recognized in all analyses performed. Three of these clades are part of the new subtribe Oosternina (Clades I–III) and nine of Megasternina (Clades IV–XII). Internal relationships among clades within Oosternina were revealed as (I [II [III]]). Internal relationships among clades within Megasternina were as follows: (IV (V (VI (VII (VIII ((IX (X + XI) + (XII)))))),). The relationships among Clades VII, VIII, XII and IX + X + XI remain unclear due to the low PP support at the backbone of the Megasternina in BI analyses.

**Divergence dating**

Time divergence estimates calculated simultaneously with the topology for the Hydrophilidae (Hydro) dataset suggested the stem age of the Megasternini (i.e., its divergence from Sphaeridiini) to be 121.1 (117.4–127.7) mya (mean and 95% credibility intervals) and the crown age for Megasternini to be 111.9 (104.5–118.7) mya. The crown ages of both subtribes were almost identical; 101.7 (92.4–111.5) mya for Megasternina and 95.1 (92.9–112.4) mya for Oosternina.

**Historical biogeography of Megasternini**

Our results of the ancestral range estimation in BioGeoBears showed DIVALIKE+J as the best-fitting model (see File S5 for comparison of all models). This model minimizes the number of dispersal and extinction events (Ronquist, 1997) and allows changes in ancestral ranges via vicariance events. The resulting ancestral range reconstruction is shown in a simplified way in Fig. 7A, and in a complete way with states reconstructed for each ancestor before and after lineage divergence in File S5. Australian-Oriental vicariance was revealed for the root of the Megasternini in the Early Cretaceous (Fig. 7A, node 1). The Oosternina was recovered as ancestrally Australian, with two major vicariant events: the colonization of the Neotropics in the Late Cretaceous (Fig. 7A, node 2) and subsequent dispersal of members of this Neotropical group to the Oriental region during the Paleocene-Eocene (Fig. 7A, node 3). The Megasternina, as well as clades IV, VI and VIII–XI, were recovered as ancestrally Oriental. In contrast, clades V and VII are revealed as ancestrally African and clade XII as ancestrally South American, as a consequence of Early Cretaceous colonization events (Fig. 7A, nodes 4–6). Five colonization events between Asia and America were revealed during the Paleocene-Eocene thermal maximum in clade III (America to Asia, Fig. 7A: node 3), clade VI (Asia to America, Fig. 7A: node 7), clade VIII (Asia to America, Fig. 7A: node 9) and clade XI (Asia to America, Fig. 7A: node 11). The colonization of the Palearctic region from Africa (clade VII, see Fig. 7A: node 8) and the supposed long-distance dispersal from South America to Africa (clade XII, see Fig. 7A: node 12) also happened during this time. Two parallel colonizations of Australia from the Oriental region were revealed in clade VI during the Eocene (Fig. 7A, nodes 13–14), and the colonization of Europe by the North American clade was revealed to happen between Late Eocene and Early Miocene in Clade X (Fig. 7A, node 15).

**Diversification analyses**

The lineage-through-time (LTT) plots without the correction for the unsampled taxa showed a slightly convex shape typical for the incomplete sampling for the whole Megasternini and the Megasternina subclade. The LTT plot of Oosternina indicated a sudden slow-down in diversification around 45 Mya. The model-fitting using Bayesian episodic radiation model corrected for expected total diversity strongly favoured the most complex model with 9 or 10 intervals over the simpler models with two different sets of rates (before and after Eocene climatic optimum) and constant rates through time (Table 3). The 10-interval model revealed rather constant diversification rates through time until the middle Miocene (with a slightly increased diversification rate due to the increased speciation) and a decrease of diversification rate in the last 10 million years. Comparison of Oosternina and Megasternina revealed lower speciation and extinction rates and no evident Miocene increase in the speciation rate in the Oosternina.

| Table 3. Marginal log-likelihoods for the episodic diversification models with different number of time intervals with distinct speciation and extinction rates. |
|-----------------|-----------------|-----------------|
|                 | Megasternini    | Oosternina       | Megasternina    |
| 10 intervals    | −1598.362*      | −379.9424*       | −1111.958*      |
| 9 intervals     | −1743.742       | −405.4038        | −1203.623       |
| 2 intervals     | −1754.833       | −404.6993        | −1221.539       |
| 1 interval      | −1754.833       | −404.6993        | −1221.539       |
| The best-performing models are marked by an asterisk. |

**Systematics**

**Tribe Megasternini Mulsant, 1844**

Tribe Megasternini is a group strongly supported as monophyletic based on molecular as well as morphological characters (Hansen, 1991; Short & Fikáček, 2013) (Fig. 2). Our results corroborate the previously hypothesized subdivision of Megasternini in two diagnosable clades corresponding to the Cercyon and Oosternum groups mentioned by Fikáček (2010), Fikáček & Short (2010), Short & Fikáček (2013) and
Fig. 2. Simplified topology of Megasternini showing representatives and diagnostic characters of the newly proposed subtribes. (A) topology based on a time-calibrated tree obtained under Bayesian Inference with the proposed division of the tribe in two subtribes and 12 clades (taxa currently classified in genera Cercyon, Australocyon, Cetioxyon and Oosternum are colour-coded); (B) Cetioxyon incantatus Fikáček & Short; (C) Oosternum luciae Deler-Hernández et al.; (D) Cetioxyon augai Szczepański et al.; (E) aedeagus of ‘Cercyon’ sp. (Papua New Guinea; MF1651); (F) ninth male sternite of Agna capillata Horn; (G) eighth male sternite of Merosoma sp. (Australia); (H) Cercyon spiniventris Arriaga-Varela et al.; (I) Pachysternum apicatum Motschulsky; (J) Cycreon floricola Arriaga-Varela et al.; (K) aedeagus of Cycreon floricola; (L) ninth male sternite of Cycreon floricola; (M) eighth male sternite of Cercyon haemorrhoidalis (Fabricius). [Colour figure can be viewed at wileyonlinelibrary.com]

Fikáček (2019). Members of both clades differ in the morphology of male terminalia (Fig. 2E–G, K–M). Both clades also show distinct macroevolutionary and biogeographic patterns. In order to formalize these two evolutionary different and morphologically diagnosable lineages, we define them here as new subtribes: Oosternina Arriaga-Varela & Fikáček, new subtribe and Megasternina Mulsant.

Oosternina Arriaga-Varela & Fikáček, new subtribe

Type genus: Oosternum Sharp

Diagnosis

Members of Oosternina can be differentiated from those of Megasternina by the morphology of the male genitalia.
Fig. 3. Phylogenetic relationships and node ages of the subtribe Oosternina (clades I–III). (A) part of the time-calibrated tree obtained under Bayesian inference, node support indicated by colour points (left – posterior probability; right – ML bootstrap). (B–K) morphological structures of selected representatives of this clade: (B) dorsal habitus Cercyon sp. (PNG6175); (C) meso- and metaventrite of Cenebriophilus sp. (MF1615); (D) meso- and metaventrite of Australocyon sp. (MF1616); (E) meso- and metaventrite of Pseudoosternum sp. (MF1619); (F) anterolateral corners of the metaventrite of Platycyon sp. (MF1659); (G) meso- and metaventrite of Kanala punctiventris Fikáček (MF1226); (H) laterodorsal habitus of Oosternum ‘Pemelus’ sp. (MF1661); (I) prosternum of Oosternum ‘grandis group’ sp. (MF1634); (J) prosternum of Sacosternum auribleps Fikáček & Short (Brazil); (K) dorsal habitus of Agna capillata (USA). [Colour figure can be viewed at wileyonlinelibrary.com]

and associated structures: (i) the abdominal sternite 9 is crescent-like, with the median portion not projecting anteriorly (Fig. 2F), (ii) the sternite 8 has a medial projection at the anterior margin (Fig. 2G), and (iii) the median lobe of the aedeagus is firmly joint to the bases of parameters and does not reach deeply into the phallobase (Fig. 2E).

Genera included in Oosternina

Clade I (undescribed new genus): Undescribed new genus from Papua New Guinea (Fig. 3B).

Clade II (Australian core): Kanala Balfour-Browne (Fig. 3G), Merosoma Balfour-Browne, Platycyon Hansen (Fig. 3F), Cercoy Leach (part.), Ceticyo Hansen Hansen (part.) (Fig. 2B), Cercoydes Broun, Ceronecyton Hansen, Pseudoosternum Hansen (Fig. 3E), Chledocyon Hansen, Australocyon Hansen (except the A. pilocnemoides species group, see clade IV below) (Fig. 3D), Cenebriophilus Hansen (Fig. 3B).

Clade III (Oosternum group sensu novo): Sacosternum Hansen (Fig. 3J), Oosternum Sharp including Pemelus Horn (Figs 1A, 2C, 3H–I), Ceticyo Hansen (in part) (Fig. 2A), Motonerus Hansen, Agna Smetana (Fig. 3K).

Genera included in Oosternina but not sampled for DNA in this study

Notocericy Blackburn and Ercyodes Hansen were not sampled for DNA, but we include both genera in Oosternina on the basis of the morphology of male genitalia and associated structures. Both genera are likely members of Clade II. The morphology of Ercyodes resembles that of Cercoydes in the nearly absent antennal grooves and on the shape of the meso- and metathoracic structures. The affinities of Notocericy are not clear at the moment.

Megasternina Mulsant, 1844

Diagnosis

Members of Megasternina can be differentiated from those of Oosternina by the morphology of the male genitalia and associated structures: (i) the abdominal sternite 9 has a median portion developed as a tongue-like projection (Fig. 2L), (ii) the sternite 8 lacks the anteromedial projection (Fig. 2M), and (iii) the median lobe of the aedeagus reaches deeply into phallobase
and is not firmly joint to the bases of parameres (and hence freely movable in and out of the phallobase; Fig. 2K).

Genera included in Megasternina

Clade IV (Paroosternum clade): Paroosternum Scott, Delimetrium Hansen (Fig. 4C), Australocyon Hansen (in part, A. pilocnemoides group) (Fig. 4B).

Clade V (main South African clade): Cercyon Leach (part of South African species) (Fig. 4D–E).

Clade VI (Megasternum clade): Megasternum Mulsant (Fig. 4G), Giliissius Orchymont, Peltocercyon Orchymont (Fig. 4F), Chimaerocyon Fikáček et al., Pilocnema Hansen, Cercyon Leach (species from China, SE Asia and New Guinea) (Fig. 4I), Bolbonotum Hansen, Deltostethus Sharp (Fig. 4H).

Clade VII (Cercyon core): Cercyon Leach (most European species incl. C. melanocephalus Linnaeus), the type species of the genus, and some species from Asia and Africa; Fig. 4J), Evanesternum Arriaga-Varela et al., Pyretus Balfour-Browne. The assignment of the latter two genera into the clade is based on the ML analyses of the Wildcard dataset and needs to be confirmed by future analyses (Fig. 6).

Clade VIII (Cryptopleurum clade): Cryptopleurum Mulsant (Fig. 1C), Pachysternum Motschulsky, Crytonius Hansen (Fig. 4L), Cercillum Knisch, Cyrillum Knisch (Fig. 4N), Pacrilum Orchymont (Figs. 4K–M), Tectosternum Balfour-Browne.

Clade IX (Oriental clade): Pseudocercyon Orchymont (Fig. 5B), Cercyon Leach (species from China and SE Asia).

Clade X (Armostus clade): Armostus Sharp (Fig. 5C), Cercyon Leach (subgenus Arcocercyon Hebauer, Prostercyon Smetana and species of Cercyon tristis species group) (Fig. 5D–F).
Clade XI (Cycreon clade): Cycreon Orchymont (5G–I), Cercyon Leach (Central American species).

Clade XII (Pelosoma clade): Pelosoma Mulsant (Fig. 5J), Nitidulodes Sharp (Fig. 5M), Morphilus Orchymont (Fig. 5L), Cercyon Leach (incl. All South American species) (Figs 5K, N).

Genera included in Megasternina but not sampled for DNA in this study

Acaryon Hebauer, Parastromus Balfour-Browne, Morastus Orchymont, Nippocercyon Satô, Colerus Hansen, Oreosternum Jia et al., Psucercyon Orchymont, Pelocyon Balfour-Browne, Kahanga Hansen, Emmidolium Orchymont, Quadristernum Balfour-Browne. These genera are included in this subtribe on the basis of the morphology of male genitalia. Parastromus might be a member of Clade VII with which it shares the broad central raised area of the metaventrite with its posterior margin concurrent with the position of femoral lines and the convex anterior margin of the mentum. The shape of meso- and metaventrite of Pelocyon shows a clear resemblance to Paroosternum and Delimetrium from Clade IV (Fig. 4C). Acaryon is very similar to Cycreon in Clade XI. All these hypotheses need to be corroborated by DNA data, and all genera not sampled remain as Megasternina incertae sedis at the moment.

Internal relationships within Oosternina new subtribe (Clades I – III). Clade I (Undescribed new genus) is a previously unknown lineage currently confined to New Guinea and represented by two undescribed species. This clade is much less diverse than its sister clade formed by Clades II + III. These two species were preliminarily identified by us as Cercyon and Merosoma, following current generic concepts. Despite the differences in dorsal sculpture (Fig. 3B) and in the shape of the mesoventral plate, both species share a very short prosternum in front of the procoxae.
**Clade II (Australian core)** is a morphologically diverse lineage containing all Oosternina genera inhabiting Australia, New Caledonia and New Zealand and most taxa from New Guinea. Its earliest branching lineage, *Cercyodes*, is the only megasternine lineage naturally occurring both in Australia and New Zealand. *Cercyodes* is an atypically flattened genus missing antennal grooves on the prothorax; it inhabits rotten beach wrack. Other members of Clade II are associated with animal excrements, leaf litter, or rotten stems of plants (Arriaga-Varela et al., 2019b).

The subsequently diverging clade consists of Australian genera (Hansen, 1990; Fikáček, 2019) and a group of small and flattened species from New Guinea preliminarily assigned to *Platycyon* under the current generic concept. The close relationship of *Australocyon* and *Cenebriophilus* and their recent split (ca. 12.7 mya) is noteworthy since these genera are morphologically characteristic and easy to diagnose (Fikáček, 2019). Both genera share the deep pits on the anteromedial margin of the mesoventrite (Fig. 3C–D), a unique character within Megasternini, which supports their sister relationship and suggests that *Cenebriophilus* could be a highly modified lineage within *Australocyon* with exaggerated dorsal and ventral sculptures and punctuation. A better taxonomic sampling, particularly the inclusion of *A. variegatus* Hansen (type species of the genus) and of the Neotropical representatives of the genus, is needed to understand the relationship between both genera.

*Kanala* is a genus endemic to New Caledonia, with five described species (Fikáček, 2010) relatively diversified morphologically but sharing the mesoventrite broadly connected to the metaventrite (Fig. 3K). *Cetiocyon* lineage includes a variety of species from New Guinea and northernmost Australia (but not the South American *C. incantatus*, see Clade III). This genus contains the largest representatives of Megasternini (Szczepański et al., 2018). Other members of Clade II are taxa bearing the more or less developed ridge demarcating the anterolateral corners of the metaventrite (Fig. 3F). The taxa with distinct ridge are currently classified in *Platycyon* (Hansen, 1999b; Hebauer, 2000, 2001), whereas those with less developed ridge are until today members of the broadly defined genus *Cercyon* and the recently resurrected *Merosoma* (Balfour-Browne, 1939; Fikáček, 2019). Our results confirm that none of these taxa are actually related to *Cercyon*.

**Clade III (Oosternum clade)** is the main Neotropical lineage of the Oosternina. It split from the Australian Clade II at around 95.1 mya and is formed by two subclades (Fig. 3A). The first one groups genera *Motonerus*, *Sacoosternum* (Fig. 3J), *Pemelus* (Fig. 3H) (a genus that was merged under *Oosternum* by Hansen, 1999c) and a lineage formed by *Cetiocyon incantatus* Fikáček & Short (the only Neotropical species classified as *Cetiocyon*, see Fikáček & Short, 2010) and a species fitting the unpublished concept of ‘*Oosternum grandis*’ group of M. Hansen and F. Hebauer. Our results indicate that both *Pemelus* and the species around *Cetiocyon incantatus* deserve a separate generic status from *Oosternum*. This taxonomic rearrangement will be the focus of future publications. The second subclade is composed of the core *Oosternum* species and *Agnia*. *Agnia* is confined to the arid and semiarid zones in the southwestern USA and northern and central Mexico, exclusively inhabiting rotten cacti (Arriaga-Varela et al., 2019b). It has a reduced dorsal and ventral sculpture compared to *Oosternum*. The presence of *Agnia* renders the core *Oosternum* paraphyletic, with the Oriental lineage of *Oosternum* being sister to *Agnia* + Neotropical *Oosternum*. The dispersal of the most recent common ancestor (MRCA) of the Oriental *Oosternum* from the New World is dated to c. 60 mya and likely happened via the Beringia land bridge (see Biogeography discussion).

**Internal relationships within Megasternina (Clades IV – XII).**

**Clade IV (Paroosternum group)** is a collection of dissimilar and disjunct genera (Fig. 4A): *Delimetrium* Hansen from South Africa (Fig. 4C), *Paroosternum* Scott occurring in the Oriental and Afrotropical region, and the Asian species currently classified in the *Australocyon pilocnemoides* species group (Hansen, 2003). *Delimetrium* is revealed as sister to *Paroosternum*. Both genera share well-defined femoral lines on the metaventrite and the subpentagonal mesoventral plate broadly contacting the metaventrite. Members of the *Australocyon pilocnemoides* group need to be transferred to a new genus. Their separate status from *Australocyon* is supported by characters of male genitalia consistent with the subtribal diagnosis and by the loosely defined central part of the metaventrite (Fig. 4B) (this is pentagonal and clearly defined in typical *Australocyon* species). Members of this clade have been found in leaf litter and dung.

**Clade V (South African clade)** is formed by two lineages of *Cercyon* species: (i) a group of closely similar species possibly corresponding to the subgenus *Clinocercyon* Balfour-Browne found on forest litter in the Cape region of South Africa and (ii) three species inhabiting beach wrack of South Africa (*C. aphodioides* Orchymont, *C. gigas* Orchymont and *C. maritimus* Knisch). Both lineages show significant differences in the shape and proportions of the body. Additionally, the beach wrack species are characterized by comparatively flattened tibiae with thick spines on lateral margins. These morphological traits seem to have evolved independently in other non-related beach wrack species from other clades of Megasternini (for example, *Cercyon*, Clade II). All species in Clade V share the mesoventral plate with posterior apex not overlapping the anterior margin of the mesoventrite (Fig. 4D–E).

**Clade VI (Megasternum clade)** is a morphologically diverse lineage that includes genera occurring mainly in the Oriental region. *Megasternum* (Fig. 4G) is part of the earliest-diverging lineage, along with *Gillisius* and *Cercyon undulipenis* Rynedvich et al. *Megasternum* hence does not form a group with other genera with the wide mesoventral plate (see Clade VII) as it was suggested by Hansen (1991). The morphologically aberrant *Chimaerocyon* Fikáček et al., which species are associated with *Pheidole* Westwood ants (Fikáček et al., 2013), is revealed to be sister to typically looking *Cercyon* species from China (i.e., *Cercyon cf. madidus* Orchymont, specimen MF319). This situation is another example of the high morphological plasticity of Megasternini under environmental selection. Clade VI also includes the Oriental *Peltocercyon* strongly supported as a monophylum (Fig. 4F), *Bolbonotum*, the Oriental/Australian
Fig. 6. Phylogenetic position and morphology of the wildcard taxa (marked in yellow). (A) Part of the phylogenetic tree obtained by maximum likelihood analysis in IQTree; (B) meso- and metaventrite of *Evanesternum pulsatum* (Orchymont); (C) mentum of *Pyretus* sp. (MF1367). [Colour figure can be viewed at wileyonlinelibrary.com]

Pilocnema, two lineages of *Cercyon* species from Oriental and Australian regions (Solomon islands), including one lineage with vaguely defined antennal grooves on the prosternum (Fig. 4I), and the American *Deltosthetus*. *Deltosthetus* is estimated to have diverged from the Oriental ancestor c. 62.8 mya and colonize America via the Beringian land bridge (see below).

**Clade VII** (core *Cercyon*) groups species which are classified exclusively in *Cercyon* in the current concept. The result of the ML analysis of the Wildcard dataset indicates that this clade includes *C. melanocephalus* (Linnaeus), the type of the genus *Cercyon* and its close relative *C. haemorrhoidalis* (Fabricius) (Fig. 6). Most specimens in Clade VII have distinct femoral lines on the metaventrite (Fig. 1B), a character absent from *Cercyon* species in other principal clades. Additionally, there is a tendency for the anterior margin of the mentum to be rounded and not distinctly concave. The basal-most divergence in Clade VII is a split between African and Palearctic + Nearctic + Oriental species, which happened c. 64 mya. A more extensive sampling of these species may help to elucidate the dispersal and diversification patterns in the northern hemisphere after the Eocene. The ML analysis of the Wildcard dataset shows *Pyretus* as a sister clade of the whole Clade VII (Fig. 6). However, some details of *Pyretus* morphology (e.g., the shape of the mentum; Fig. 6C) point to its possible relationship to Clade VIII (*Cryptopleurum* clade), and further studies are hence necessary.

**Clade VIII** (*Cryptopleurum clade*) is formed by most genera with a compact convex body, strongly deflexed epipleura, very narrow metastipisterna, elevated and demarcated central part of the prosternum and a broad mesoscutal plate. It largely corresponds to the narrow concept of Megasternini of Horn (1890) except for *Megasternum*, which is placed in Clade VI. A potential synapomorphy for Clade VIII is the subangular lateral edge of the pronotum with a slight indentation in the anterior half caused by the wide antennal grooves (Figs. 1C, 4L). The mentum is characterized by the small rounded or pointed processes in anterolateral corners (Figs. 1C, 4M), a form also found in *Megasternum*. Despite many shared morphological characters, the nodal support at the suprageneric level is low (Fig. 4A). The earliest-divergent group, *Pacrillum* + *Tectosternum*, shows a narrow mesoscutal plate and pentagonal central area of the mesoscutum (Fig. 4K), suggesting a transitional state between the *Cercyon* morphotype and the compact-body form found in most members of this clade (e.g., *Cryptopleurum*, Fig. 1C, and *Pachysternum*). The ancestor of the Neotropical genus *Cycrillum* split from its sister clade composed of *Pachysternum*, *Cryptopleurum*, *Cercillum* and *Cyrtonion* (i.e., genera occurring in Oriental, Afrotropical, Palearctic and Nearctic regions) ca. 60 mya (Fig. 7A node 9). The topology revealed for *Pacriullum + Tectosternum* and *Cercillum + Cyrtionion* is largely supported by morphology, suggesting the need to merge these genera in the future.

**Clade IX** (*Pseudocercyon clade*) is composed of Oriental species of *Cercyon* with *Pseudocercyon* Orchymont nested inside of them. *Cercyon fimbriatus* Mannerheim, a beach wrack
species occurring on the Pacific coast of North America, is sister to all other members of the clade. The *Cercyon* species in this group lack femoral lines. Additionally, the mentum of most species has a straight or slightly concave anterior margin. *Pseudocercyon* specimens differ from other members of this lineage by the distinctly raised medial part of the prosternum and the mesoventral plate widely connected to the anterior margin of the mesoventrite (Fig. 5B).

**Clade X** (*Armostus group*) is formed by *Armostus* and *Cercyon* species currently assigned to subgenera *Prostercyon* Smetana, *Arcocercyon* Hebauer and the *C. tristis-convexiusculus* group of *Cercyon* s. str. (Fig. 5E). The specimens of this group are characterized by the following features: (i) the mesoventral plate is raised and separated from the central part of the mesoventrite by a gap of variable width (Fig. 5C–D, F), and (ii) the anterolateral corners of the mesoventrite are delimited by a ridge (Fig. 3E), which are reduced to a thickened anterior margin in some species classified as *Cercyon*. Some species show a modified medial portion of the prosternum, with median carina or anterior half strongly raised (Fig. 5E). This feature

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was the reason for Smetana (1978) establishing the subgenus Prostercyon for the Nearctic Cercyon roseni Knisch. Nevertheless, this character is also present in species of the European C. convexiusculus Stephens to which C. roseni is the sister clade. The Oriental species Cercyon lineolatus (Motschulsky) is the earliest diverging lineage in this clade, sister to all remaining species; it has the mesoventrite not separated by a gap from the metaventrite.

Clade XI (Cycron group) is formed by the Oriental Cycron and a lineage of Neotropical species currently assigned to Cercyon and lacking femoral lines. Cycron is an Oriental genus whose specimens are associated with the inflorescences of Aracaeae (Arriaga-Varela et al., 2018b). They are flattened beetles with reduced antennal grooves (Fig. 5H), very narrow mesoventral plate (Fig. 5G), and deeply emarginate mentum (Fig. 5I). The Neotropical lineage is estimated to have diverged from the Oriental Cycron approximately 56.3 mya.

Clade XII (Pelosoma group) is composed almost exclusively of Neotropical species classified in Morophilus, Nitidulodes and Pelosoma, and of Neotropical species assigned to Cercyon. The only noteworthy exception is a species from South Africa which lineage diverged from Neotropical ancestors ca. 57.9 mya. Species assigned to Cercyon lack femoral lines. The genus Morophilus shows a general Cercyon morphology with a flattened prosternum without distinct antennal grooves (Fig. 5L) and the subpentagonal mesoventral plate; it is nested among species assigned to Cercyon. Pelosoma species differ from those assigned to Cercyon by an elongate mesoventral plate widely contacting the metaventrite in a straight line (Fig. 5J). Nitidulodes is a large-bodied genus associated with the inflorescences of Aracaeae, so far known in single species from Central America. It is a sister clade to Pelosoma with which it shares the shape of mesoventral plate but differs from it by a very deep emargination of the anterior margin of the mentum (Fig. 5M). Pelosoma species inhabit a wide range of habitats from phytotelmata, leaf litter, inflorescences and rotten fleshy plant stems (Arriaga-Varela et al., 2019b). A group of species assigned to Cercyon is nested in the Pelosoma lineage. These species inhabit dung and carrion and are endemics of the Antilles (Arriaga-Varela et al., 2017) or widespread in South and North America (Cercyon variegatus group).

Discussion

Systematics

We recognized 12 principal clades of Megasternini grouped into two large clades, which are strongly supported as monophyletic by molecular and morphological characters. These two clades are newly defined here as subtribes. In contrast, traditionally used morphological characters correlate rather poorly to the DNA-inferred phylogeny within these subtribes. It seems that the external morphology is remarkably plastic and partly relates to the lifestyle. Formal tests of this assumption need to be performed in the future. The geographic distribution correlates to the phylogeny much better than morphology, as we discuss below. The high amount of convergence naturally complicates the classification, as illustrated by the polyphyly of all large genera (Fig. 2) and will make the morphology-based diagnoses of the newly recognized clades rather challenging.

Future attempts on proposing a natural generic classification of Megasternini require an integrative approach combining molecular data, morphology and distribution. A robust DNA-based phylogenetic hypothesis is needed to re-evaluate the diagnostic relevance of traditionally used morphological characters and to discover new ones better correlated to the phylogeny. Although we did not test the accuracy of particular characters for diagnosing supraspecific taxa, some preliminary conclusions can be formulated. The form of the mesoventral plate (Fig. 1, MP) used traditionally for generic classification (e.g., Hansen, 1991) seems to vary to a large extent even within smaller clades (e.g., within Pelocercyon, Kanala, Deltostethus), and it is hence not advisable to base the generic classification mainly on the shape of this structure. The presence/absence and size of the antennal grooves on the prothorax (Fig. 1, AG) is another example of such a plastic character. There are indications that antennal grooves are correlated with the lifestyle, as they tend to be larger in taxa with the ability to conglobate when disturbed and strongly reduced, e.g., in all beach wrack species. On the other hand, the presence of a ridge dividing the anterolateral corner of the mesoventrite (Fig. 1, ANC) is diagnostic for several clades well-supported by molecular data (e.g. Pelocercyon, Armostus, Arcocercyon or Ceronyton + Cenebiophilus + Australocyon + Pseudosternum clade). The morphology of the sternum and the form of the mentum also seem to be informative and correlate well to the phylogeny.

It is more than evident that the generic boundaries need to be redrawn, especially in the largely polyphyletic genera Cercyon, Oosternum, Cetociyon and Australocyon (Fig. 2). For example, Cercyon species appear in 16 different positions in our phylogenetic tree, indicating the need to split the current genus into multiple monophyletic genera in the future. The rearrangement of Cercyon will be highly challenging because of its rather generalized morphology and a high degree of homoplasy among all unrelated clades currently classified under Cercyon. We suggest not to use the subgenera of Cercyon until such a rearrangement is done, as their final meaning will be significantly different from the current use. The split into multiple genera is also needed for Oosternum, Cetociyon and Australocyon, but the situation is less complex as the morphology is less uniform in these genera. Additional sampling is needed to clarify the generic concepts in New Guinean species of Clade II nowadays classified in Platy- cyon and Merosoma. Our results also indicate that some genera are merely the highly derived internal lineages of another genus (Cenebiophilus in Australocyon, Nitidulodes in Pelosoma, Tectosternum in Pacrillum, etc.). On the other hand, some previously synonymized genera need a resurrection (e.g., Pemelus). Formal taxonomic treatments of these problems will be done in the future after a more detailed analysis of each particular case.

In contrast to the vague definition of many genera, our DNA analyses revealed some traditional genera as strongly supported monophyly. Pelocercyon, Deltostethus and Armostus are examples of such genera defined by only one or two phylogenetically informative diagnostic characters. Surprisingly, some
subgenera and species groups of otherwise largely polyphyletic *Cercyon* were revealed to be well-defined lineages of a rather ancient origin, e.g., the subgenus *Arcocercyon* (the African lineage of Clade X) or the *Cercyon tristis-convexusculus* group (European lineage of Clade X).

**Time tree analyses**

The age inferred for the basal divergence of Oosternina from Megasternina using the Megasternini (*Mega*) dataset was c. 7–13 mya younger than the age recovered in the analysis of the whole Hydrophilidae (*Hydro* dataset) using fossil dating (see File S4). A possible explanation for this difference is the more complete taxon sampling of the Megasternini dataset compared to the Hydrophilidae one since the number of internal nodes impacts the results of dating analyses. Our age estimates for this early split resulting from the analysis of the Megasternini (*Mega*) dataset are closer to those reported by Bloom *et al.* (2014).

**Historical biogeography of Megasternini**

Our analyses demonstrate that geography played an important role in the evolutionary history of the Megasternini. All 12 principal clades recognized are rather restricted in distribution, in extreme cases being endemic for a single continent or region (e.g., clade II for the Australian region, clade V for South Africa, clade IX for the Oriental Region, and clade XII for South America). Geography hence seems to be a better predictor of relatedness than morphology. Yet, our analysis needs to be carefully interpreted as it seems to be biased by our taxon sampling. The bias is most evident at the root and in the backbone of the Megasternini, in which ancestral reconstructions do not correspond to positions of the landmasses in the Cretaceous. We suspect two principal reasons for this bias: (i) the much better sampling of Oriental taxa than those of other continents, and (ii) the limited sampling of African taxa, especially in the Clade IV. African genera *Pelocyon* and *Pseucyon* are likely part of Clade IV based on their morphology closely resembling that of *Parososternum*, the genus itself also occurring in Africa (Hebauer, 2006). Hence, at this moment, we cannot exclude the African origin of Clade IV, which would, together with the South-African clade V, indicate the African origin of the Megasternina. This scenario, including the supposed Gondwanan origin of Megasternini, would correspond to the position of the landmasses in the Cretaceous. It needs to be tested by an expanded taxon sampling in the future.

The Oosternina clade was recovered as ancestrally Australian, with two major vicariant events: the colonization of the Neotropics in the Late Cretaceous (84.9–105.8 mya) (Fig. 7A, node 2) and a subsequent dispersal to the Oriental region during the Paleocene-Eocene (49.1–71.1 mya) (Fig. 7A, node 3). The Australia-South America vicariance can be explained by the dispersal via Antarctica that also separated both landmasses and had tropical to subtropical climate with a maximum summer temperature of nearly 20°C in this period (Pross *et al.*, 2012).

Similar trans-Antarctic dispersals enabled by the warm climate have been reconstructed for plant groups like orchids (Givnish *et al.*, 2016a) and Liliaceae (Givnish *et al.*, 2016b). Continental interchanges between Australia and South America facilitated by the warm climate in Antarctica were possible from the Late Cretaceous to the end of the Eocene, as evidenced by the presence of thermophilous amphibians in Antarctica ca. 40 mya (Mörs *et al.*, 2020). After reaching South America in the Late Cretaceous, the New World members of Oosternina likely dispersed northwards, colonized North America, and reached the Oriental regions via the Beringia land bridge, as discussed below. The history of the dispersal of Oosternina lineages between 95 and 55 mya makes them the first example of an almost complete circumpacific dispersal among beetles, as evidence-based explanations for pan-Pacific distributions are scarce (Kim *et al.*, 2018). Lineages nowadays restricted to New Guinea, including Clade I sister to all remaining Oosternina, diverged during the Late Cretaceous to Eocene, i.e., long before the expected formation of New Guinean landmasses (e.g., Toussaint *et al.*, 2014). All these lineages are hence clearly of Australian origin from where they colonized New Guinea before getting extinct in Australia. This colonization was possible thanks to the intermittent connections of Australia and New Guinea until the Pleistocene (Lamb *et al.*, 2019).

All the principal clades of Megasternina (IV-XII) diverged from their sister lineages before the K-Pg boundary (65 mya). During the Late Cretaceous (81.3–63.3 mya), Megasternini colonized the New World for the second time by the MRCA of Clade XII. The origin of this colonization remains unclear, considering the probably biased backbone ancestral reconstruction discussed above. During the Cenozoic, South America was isolated from other continents, having only a non-permanent land connection with East Antarctica that lasted until the Eocene (Reguero *et al.*, 2014). The dispersal to South America via Antarctica would imply the historical presence of Megasternina in Australia from where it is currently absent; the complete extinction of this lineage in Australia seems rather unlikely. An alternative scenario would be the colonization of South America from Africa by a trans-Atlantic dispersal, assuming the possible origin of Megasternina in Africa (see above). Another alternative is the colonization of America from Asia via northern hemisphere land bridges during the Late Cretaceous and Paleocene (Brikiatis, 2014). A more comprehensive taxon sampling is necessary to provide evidence for any of these scenarios and to understand the events that shaped the distribution of Clade XII in the Neotropical Region.

We reconstructed seven intercontinental dispersals to happen during the Paleocene and Early Eocene (63–57 mya; Fig. 7A: nodes 3, 7–12). Four of them are dispersals from Asia to America (Fig. 7A: nodes 7, 9–11), one went in the opposite direction (from America to Asia; Fig. 7A: node 3). One event led to the colonization of the Paleartic from Africa (Fig. 7A: node 8), and one to the colonization of Africa from South America (Fig. 7A: node 12). The timing of these events coincides with the increase in global temperature in the late Paleocene and early Eocene (Paleocene-Eocene climatic optimum ca. 65–51 mya; Zachos *et al.*, 2001; Gingerich, 2006; Röhl *et al.*, 2007; Huber
The period was characterized by high mean annual temperatures and mild winters (mesothermal climate) at middle and higher latitudes of the northern hemisphere, allowing the formation of the boreo-tropical zone (Zachos et al., 2001; Cordamine et al., 2012; Brunke et al., 2017; Huang et al., 2019; Meseguer & Cordamine, 2020). This zone was characterized by the presence of frost-intolerant vegetation forming Arctic rainforests that extended up to 76–78°N of palaeolatitude (Basinger et al., 1994; Greenwood et al., 2010). The existence of a high latitude mesothermal biome coincided with periods of sea-level fluctuations that interconnected previously isolated landmasses by land bridges. The four principal land bridges (Beringian, De Geer, Thulean and Turgai) persisted likely until ca. 50 mya (Sammartín et al., 2001; Woodburne, 2010; Condamine et al., 2013a; Brikiatis, 2014; Praz & Packer, 2014). Five of the Paleocene-Eocene dispersal events mentioned above (Fig. 7A, nodes 3, 7, 9–11) most likely went through the Beringian route (Garrouste & Nel, 2019). Only two of these lineages (Cercyon fimbriatus in Clade IX and one species of Deltostethus in Clade VI) survived in North America until today. Representatives of the remaining three lineages went extinct in North America, likely after the tropical forests retreated southwards; they only occur in South America nowadays. A dispersal from/to North America to Asia via Europe (i.e., via DeGeer land bridge and Turgai Strait) cannot be excluded entirely, but it is less likely, considering the absence of any of these clades in Europe today as well as in European fossil record.

The trans-Atlantic dispersal from South America to Africa in Clade XII (Fig. 7A: node 12) might happen through Europe via northern hemisphere land bridges (Sammartin et al., 2001; Smith et al., 2006; Gamble et al., 2011). Crossing the Atlantic Ocean on a raft would be an alternative scenario. However, all known over-water dispersals across the Atlantic went in the opposite direction (Gamble et al., 2011; Mayr et al., 2011; Antoine et al., 2012; Bond et al., 2015). We cannot discard even the possibility of a recent anthropogenic introduction of this unidentified species to Africa.

The only native New Zealand member of Megasternini, Cercydes laevigatus, diverged from its Australian congener ca. 12.8–35.3 mya, implying a long-distance over-water dispersal from Australia to New Zealand. Cercydes is a genus inhabiting rotten beach-wrack (Fikáček, 2019) and adapted to survive in seawater (Leschen & Fikáček, unpubl. data). It can hence use kelp accumulations as rafts transported by extreme weather conditions (Waters et al., 2018).

Additional sampling is needed to reconstruct the history of disjunct distributions of more recent origin. Our current sampling partly covers some of them, e.g., two parallel colonizations of Australia from Asia during the Eocene (Fig. 7A: nodes 13–14) or the Eocene-Oligocene vicariance of North American and European lineages of the Cercyon convexusculus group (Clade X). Additional disjunctions worthy of being studied in the future include Megasternum (Paleartic-Oriental-Nearctic), Tectosternum + Pacirillium (Afrotropical-Oriental-Nearctic), Pelosoma (Afrotropical-Neotropical-Nearctic) and Australocyon (Australian-Neotropical).

**Diversification and biogeography**

The two major clades of Megasternini, defined here as subtribes Oosternina and Megasternina, differ largely in their distribution and most likely also in the number of species. Oosternina is nowadays only distributed in Australia, South America and SE Asia. Today's distribution of Oosternina is a result of the biogeographic history with two major dispersal events only: colonization of America from Australia and colonization of Asia from America. The number of described species of Oosternina is about 120 (Short & Fikáček, 2011). In contrast, members of Megasternina are distributed worldwide, and their evolutionary history was shaped by multiple intercontinental colonizations (our sampling reveals at least 16 dispersal events). Megasternina also has a larger number of described species (ca. 430: Short & Fikáček, 2011). The Paleocene-Eocene climatic optimum was likely the most important period for the colonization of new areas for both clades, as discussed above. It is for this reason that we compared the diversification dynamics of both clades and tested whether the high number of dispersals to new areas in the Paleocene-Eocene resulted in an increased speciation rate.

The lineage-through-time plot (Fig. 8C) indicated a rather constant diversification rate for the whole tribe and Megasternina but suggested a sudden slow-down in diversification for the Oosternina from ca. 45 mya. A possible explanation would be the retreat of tropical areas in Australia and South America following the decrease of global temperatures, which started ca. 50 million years ago (Zachos et al., 2001, Fig. 8B). The Bayesian analysis strongly favoured a more complex diversification model with the rates varying through time rather than differing before and after the Paleocene-Eocene climatic optimum. This result suggests that the high number of Paleocene-Eocene dispersal events was not significantly affecting the diversification regime of the tribe or any of its subtribes. Correspondingly, the plots of the speciation and extinction rates for the multi-epoch model do not show any changes during the Paleocene-Eocene period (Figs. 8D–I). Instead, they suggest rather constant rates through time until the end of the Eocene, followed by the slight increase in diversification rate in the Oligocene and a steep decline in diversification rates in the last 10 million years. A possible interpretation of this result may be the fragmentation of tropical and subtropical forests in the Oligocene due to a further decline of global temperatures, which resulted in a temporarily increased speciation rate. Later, global temperatures continued in decline, leading to a retreat of tropical forests, causing extinctions of Megasternini at higher latitudes. This effect should be larger in Megasternina, which inhabit tropical to temperate regions including those at higher latitudes, and smaller or absent in Oosternina, which is strictly tropical, as also shown by our result (Fig. 8I).

Our analyses are clearly affected by an incomplete taxon sampling (we sampled 189 out of 1000 expected species of Megasternini). Even the correction using the diversified sampling model (Ronquist et al., 2016) cannot remove the effect of incomplete sampling totally, as it requires the information about the total species number of each clade which is unknown.
for Megasternini. We hence consider the analyses performed here as preliminary, allowing us to formulate hypotheses to be tested in the future with a better taxon sampling or more adequate analytical methods. For example, it seems evident that the current diversity and distribution of the Megasternini was affected by the evolution of ecological traits, e.g., the larger or smaller ability to survive in a non-tropical environment. Consequently, the phylogenetic niche conservatism (Wiens & Graham, 2005; Donoghue, 2008; Crisp et al., 2009) needs to be taken into account for future analyses of the evolutionary history of the Megasternini (e.g., see Li et al., 2020; Meseguer & Cordamine, 2020).

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 Megasternini specimens and the GenBank accession numbers of their DNA sequences.

Appendix S2. Results of non-dated analyses of the Megasternini dataset.

Appendix S3. Time tree analysis of the family Hydrophilidae.

Appendix S4. Megasternini time tree.

Appendix S5. Historical biogeography analysis.

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