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Dennis R. Uit de Weerd [a,b,*], Edmund Gittenberger [b]

[a] School of Science, Open Universiteit, P.O. Box 2960, 6401 DL Heerlen, The Netherlands
[b] Netherlands Centre for Biodiversity Naturalis, P.O. Box 9517, 2300 RA Leiden, The Netherlands

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ABSTRACT

Clausiliidae is one of the most speciose and best-studied families of land snails. The family contributes to land snail diversity on a global scale, with three main centres of diversity: (1) western Eurasia (six subfamilies recognized), (2) East Asia (two subfamilies recognized) and (3) the neotropics (one subfamily recognized i.e. Neniinae). Despite a wealth of shell-morphological and anatomical studies, a well-supported phylogeny is lacking for the family.

To provide a phylogenetic framework and reevaluate morphological and biogeographic observations on the family, we compiled a dataset consisting of partial 28S rRNA, histone H3 and histone H4 nucleotide sequences covering all clausiliid subfamilies, and 23 out of 25 tribes. Our analyses (MrBayes, BEAST, PhyML) divide the family into seven highly supported clades, which were retrieved by at least two of the three markers used, and which are more or less geographically confined. Three of these clades coincide with subfamilies recognized in the current classification (Alopiinae, Garnieriinae, Laminiferinae). The monophyly of four of the remaining six hitherto accepted subfamilies is not supported, with the New World subfamily Neniinae divided across two clades. All shell-morphological characters used in classical clausiliid classification were homoplasious at the subfamily level, with the exception of the type of shell aperture formation. In contrast to previous interpretations, our results suggest that the so-called 'apostrophic' aperture found in the neotropical clausiliids, and in a European (Laminiferinae) and a SE Asian (Garnieriinae) subfamily, is in fact the plesiomorphic condition among extant Clausiliidae. The widespread and fragmented geographic distribution of this type of aperture may therefore be considered relictual. Based on an inferred Late Cretaceous or Early Cenozoic European origin of the clade of extant Clausiliidae, the ancestor(s) of the neotropical Clausiliidae must have colonized the New World after the Atlantic Ocean had opened. A taxonomic revision is proposed.

1. Introduction

Compared to most other families of land snails the family Clausiliidae is both unusually widespread, with a distribution spanning more than half the globe, and locally often extraordinarily speciose. The total number of clausiliid species, most of which live on rocks or on tree bark, is estimated at 1300 (Nordsieck, 2007a). In addition, the family has an extensive fossil record in western Eurasia, particularly in western Europe, consisting of at least 165 species from the Late Cretaceous onwards (see Nordsieck, 2000, 2007a). Nearly all Clausiliidae are easily recognized as such by a complex synapomorphic clausilial apparatus. This device consists of a moveable, door-like plate (clausilium) and associated lamellae, which enable the snail to close off the aperture of its shell.

Clausiliidae have since long attracted the attention of biologists, and several clausiliid genera and species have been the focus of extensive taxonomical, biogeographical, ecological and evolutionary studies (e.g. Giokas et al., 2005; Gittenberger et al., 2006; Uit de Weerd et al., 2006; Douris et al., 2007; Szybiak and Leśniewska, 2008; Maltz and Pokryszko, 2009; Gittenberger et al., 2012). In particular, molecular phylogenetic analyses have provided important new insights into the evolution and palaeobiogeography of certain clausiliid subgroups (Gittenberger et al., 2006; Uit de Weerd et al., 2004; Douris et al., 2007). However, such analyses and knowledge are still lacking for the family as a whole.

Fundamental notions about the interrelationships within the family Clausiliidae are based solely on shell-morphological (conchological) characters, that were later supplemented with genital–anatomical data for selected taxa. Conchological characters are of particular relevance in clausiliid classification because they are in principle available or accessible for all species described and are also applicable to the considerable record of fossilized shells. Nordsieck (2007a) lists seven conchological characters that he considers relevant in Clausiliidae classification. Two of these

* Corresponding author at: School of Science, Open Universiteit, P.O. Box 2960, 6401 DL Heerlen, The Netherlands. Fax: +31 384660248.
E-mail addresses: dennis.uitdeweerd@ou.nl, uitdeweerd@gmail.com (D.R. Uit de Weerd).

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concern the approximate position and length of particular internal lamellae, both gradual characters with ambiguously defined character states. The remaining characters are: (1) the presence or absence of a keel on the last whorl; (2) aperture formation, being either non-apostrophic, semi-apostrophic, or apostrophic (see Fig. 1); (3) the type of clausilial apparatus, with a by-pass canal (N-type) or without (G-type); (4) the clausilium resting on a single ridge (lunella) or on a series of parallel lamellae; (5) separate or connected lamellae on the upper inward (parietal) wall behind the aperture. Based on various combinations of these conchological and genital–anatomical characters (see Table 1), Nordsieck (1978a, 2007a) recognizes nine clausiliid subfamilies: Alopiinae, Baleinae, Clausiliinae, Serrulininae, Laminiferinae, Mentissoideinae, Neniinae, Phaedusinae and Garnieriinae. The subfamily Serrulininae Ehrmann, 1927, was accepted by Nordsieck (1978a) to accommodate taxa that had previously been classified with the Phaedusinae (Nordsieck, 1973). The subfamily Garnieriinae C. Boettger, 1926, was also accepted as such by Zilch (1960) and Nordsieck (1978a, 2005a, 2007a), but was classified with the Neniinae by other authors (Szekeres, 1969, 1998, 1999; Loosjes and Loosjes-van Bemmel, 1973b; Nemeth and Szekeres, 1995).

In terms of the number of subfamilies, Clausiliidae diversity is centered in the area consisting of Europe, the Mediterranean region, and the Anatolian peninsula up to the Caucasus and Northern Iran, together referred to here as western Eurasia. This area comprises the entire ranges of the Clausiliinae, Mentissoideinae, Baleinae, Alopiinae, Serrulininae and Laminiferinae, with the exception of only two taxa: (1) The mentissoideine tribe Boettgeriini consisting of the genus *Boettgeria* from the Madeiran archipelago, the eastern African genus *Macroptychia* and the genus *Sabaeola* from SW Arabia (Neubert, 2002), and (2) the baleine genus *Balea*, of which several lineages colonized Atlantic islands (see Gittenberger et al., 2006). Only three subfamilies have a distribution completely outside western Eurasia, viz. the Phaedusinae and Garnieriinae, which occur in East Asia, and the Neniinae from South America and the Greater Antilles.

Because of the western Eurasian distribution of most extant subfamilies and the fact that the oldest, i.e. Late Cretaceous and Early Cenozoic, fossils assigned to the Clausiliidae (Nordsieck, 2000) are also found in that area, the family is considered to have originated in western Eurasia in the (Late) Cretaceous. Nordsieck (2005a, 2007a) assumes that the ancestors of the East Asian subfamilies Phaedusinae and Garnieriinae independently reached East Asia overland from Europe. This would have happened during the Cretaceous, before the collision of Asia and India, because there are no autochthonous Clausiliidae in peninsular India (Nordsieck, 2005a, 2007a). Likewise, the ancestor of the South-American and Greater Antillean subfamily Neniinae is hypothesized to have colonized the neotropical area via NW Africa in the early Late Cretaceous before the opening of the Atlantic Ocean (Nordsieck, 2007a).

However, the monophyly of the accepted subfamilies is uncertain, as none is supported by any recorded synapomorphies. A phylogenetic interpretation of the morphological characters used in current classifications is hampered by uncertainty about character state polarities, ambiguity of some character states, and lack of congruent patterns of variation among characters (see Table 1). Of all morphological characters used in clausiliid classification, only the type of clausilial apparatus has been examined against an independent, molecular dataset (Uit de Weerd et al., 2004). That study demonstrated extensive homoplasy in this relatively complex character, even among closely related species. The only molecular phylogenetic study to include multiple clausiliid subfamilies with multiple species each, as part of a wider survey, is that by Wade et al. (2006). As that study was not aimed at resolving clausiliid interrelationships, it sampled a total of only nine clausiliid species from the subfamilies Neniinae, Alopiinae, Clausiliinae and Phaedusinae. Of these four subfamilies only the latter three were represented by more than one species. This sampling is inadequate, both in terms of number of subfamilies and number of species per subfamily, to draw any conclusions about the monophyly of the subfamilies recognized by Nordsieck (2007a) or about their interrelationships.

Even if the currently recognized clausiliid subfamilies (Nordsieck, 2007a) are monophyletic entities, it is uncertain when and how they reached their present distribution. First, the ancestors of the Neniinae seem to have left no closely related taxa or fossils in Africa, from where they supposedly (Nordsieck, 1986, 2005a, 2007a) colonized South America during the Cretaceous. All extant African Clausiliidae are classified with otherwise western Eurasian subfamilies. Second, somehow the Clausiliidae failed to colonize North America, although in the Late Cretaceous Europe was located far closer to North America than to South America (see Smith et al., 1994). Third, there is no reason to assume that the collision between the Indian and the Asian plate would by itself have separated the ancestors of the Phaedusinae and the Garnieriinae from the western Eurasian species of Clausiliidae. In fact many species of Phaedusinae are found in the southern Himalayas (see Nordsieck, 1973, 1974), which formed as a result of this collision. Finally, even the opening of the Atlantic Ocean in the Late Cretaceous need not have precluded dispersal of Clausiliidae to South America. Transatlantic dispersal has recently been inferred for the clausiliid genus *Balea*, which apparently reached the remote South Atlantic Tristan da Cunha archipelago from Europe, where it is known from the Pleistocene till Recent (Nordsieck, 2007a, 2007b), or from the Azores (Gittenberger et al., 2006).

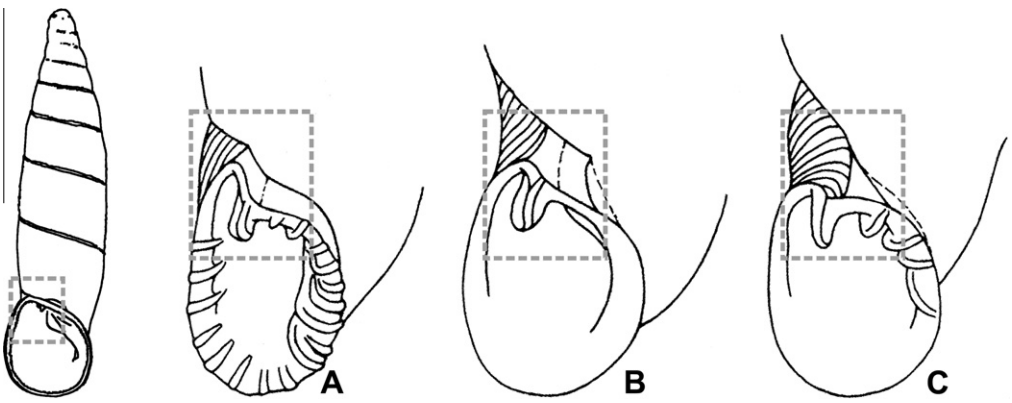

Figure 1. Clausiliid shell with non-apostrophic (A), semi-apostrophic (B) and apostrophic aperture (C), respectively. Modified from Nordsieck (2007a), with permission from publisher and author.

Table 1
Characters and character states used in the latest intrafamilial classification of extant Clausiliidae (see Nordsieck, 2007a). Rare character states are given between parentheses. Supposedly plesiomorphic character states are indicated with asterisks.

Character(states)	Alopiinae	Baleinae	Clausiliinae	Garnieriinae	Laminiferinae	Mentissoideinae	Neniinae	Phaedusinae	Serrulininae
Conchological									
Body whorl (neck)	R[a] (K)	R[a]/K	R[a]/K	R[a]	R[a]	R[a]/K	R[a]	R[a]	R[a]
Rounded or with weak keel[a]									
Prominent keel									
Aperture formation	N[a]	N[a]	N[a]	A (S)	A	N[a]	A	N[a]	N[a] (S)
Non-apostrophic[a]									
Apostrophic									
Semi-apostrophic									
Clausilial apparatus	N[a]/G	N[a]/G	N[a]/G	N[a]	N[a]	N[a]/G	N[a] (G)	N[a]/G	N[a]
N-type[a]									
G-type									
Palatal plicae	L	L	L	L	L	L	L	P[a]/L	P[a]/L
Plicae-type[a]									
Lunella type									
Parieto-columellar lamellae	L	L	L	L	L	L	L	D[a]	D[a]
Deeply ending inwards, subcolumellar with inner part[a]									
Less deeply ending inwards, subcolumellar without inner part									
Superior lamella	S	C[a]/S	C[a] (S[d])	C[a]/S	C[a]	C[a]/S	C[a]/S	C[a]/S	C[a]
Continuous with spiral lamella[a]									
Separated from spiral lamella									
Subcolumellar lamella	R[a]	R[a]	R[a]	R[a]	R[a]	R[a]	R[a]	S	R[a] (S)
Remaining inwards on the columella[a]									
Shifting inwards to the parietal wall									
Genital–anatomical									
Terminal part of bursa copulatrix	N[b]	N[b]	N[b]	N[b]/B	B/N[b,1]	N[b]	N[b] (B)	B (N[b])	B
Not bent off spermoviduct[b]									
Bent off spermoviduct									
Diverticulum of bursa copulatrix:	L[c]	T	T	L[c] (T)	T	T	L[c]/T	L[c]	L[c]
Like other parts[c]									
Transformed into a glandular tube									
Loop of male copulatory organs	W[c]	T	T	W[c]	W[c]	W[c]	W[c]	W[c]	W[c]
Well-developed, penis of normal length[c]									
Transformed as in Clausiliinae or Baleinae									
Penial retractor	D[c]/N	D[c]/N	D[c]/N	N	D[c]/N	D[c]/N	N	N	N (D[c])
Divided[c]									
Not divided									

[a] Character state in the extinct family Triptychiidae (but see Szekeres, 1998).
[b] Based on ontogenetic origin.
[c] Character state in the majority of extant Stylommatophora.
[d] Only in extinct species.
[1] Based on Gittenberger, 2007.

The uncertainty about the phylogenetic relationships and divergence dates within the Clausiliidae complicates the selection of appropriate ingroup and outgroup species for studies on subgroups (e.g. Schilthuizen et al., 1995; Douris et al., 1998; van Moorsel et al., 2000; Uit de Weerd and Gittenberger, 2004), and hampers the understanding of morphological evolution, relevant to establish the phylogenetic position of fossils (cf. Szekeres, 1998, 1999 and Nordsieck, 1999). It also obstructs the formation of a fossil-calibrated timeframe for the evolution and phylogeography of the clausiliid subgroups (e.g. Uit de Weerd et al., 2004; Douris et al., 2007), and tenable reconstructions of the dispersal of Clausiliidae into their present vast range.

Using a molecular phylogenetic approach, this paper addresses the following four main questions:

(1) Is there molecular support for the clausiliid subfamilies recognized by Nordsieck (2007a)?
(2) What are the estimated ages of the Clausiliidae as a whole and of the clausiliid subfamilies or other main clades?
(3) What is the plesiomorphic condition for the shell characters used in clausiliid classification?
(4) Can all extant Clausiliidae be traced back to a common western Eurasian ancestor?

2. Materials and methods

2.1. Samples used

All clausiliid subfamilies are represented in this study by at least two taxa. Of the 25 tribes distinguished by Nordsieck

(2007a), 23 were included (only Euxinellini and Synprosphymini are missing). In total 67 species, representing 66 clausiliid (sub)genera were studied (see Supplementary material).

Outgroup species were selected using both morphological and molecular criteria. Based on the morphological cladistic analysis by Tillier (1989), the genera *Cerion*, *Autocoptis*, and *Bulimulus* were included as outgroups. In addition, a Discontiguous MegaBLAST search was performed in GenBank, using the clausiliid ITS2 sequences obtained by Wade et al. (2001, 2006). We selected the ITS2 sequences for this purpose rather than the 28S rRNA sequences by Wade et al. (2001, 2006), because we planned to use 28S as a marker in subsequent phylogenetic analyses. Based on the BLAST scores, three additional outgroups were selected: *Arion* (Arionidae), *Mastus* (Enidae) and *Chondrina* (Chondrinidae). By selecting two orthurethran outgroups (*Mastus* and *Chondrina*), we also accommodate for possible affinities between the Clausiliidae and the Orthurethra (Wagner, 1921; Thiele, 1921; Steenberg, 1925; Nordsieck, 1986).

Apart from 12 samples for which DNA had been extracted in previous studies (Uit de Weerd and Gittenberger, 2004; Gittenberger et al., 2006; Uit de Weerd, 2008), new extractions from ethanol-preserved tissue were made for all ingroup samples, using the Qiagen Quick Extraction Kit. All extractions were performed on 1–10 mm^2 of foot tissue, with the exception of *Caspiophaedusa*, for which only genital tissue was available. With the exception of the *Caspiophaedusa* sample and a juvenile *Macroptychia* sample, shells of all samples were kept as vouchers and deposited in the collection of the Netherlands Centre for Biodiversity Naturalis (NCB Naturalis).

2.2. PCR and sequencing

We selected the 28S rRNA region D1–D6, partial histone H3 and the partial histone H4 genes to use in multi-locus analyses. We avoided the internal transcribed spacer 2 (ITS2) sequences used in combination with the 5′ part of 28S rRNA (827–837 bp) by Wade et al. (2001, 2006), as ITS2 is highly variable in length and difficult to align within Clausiliidae (Uit de Weerd and Gittenberger, 2004).

New 28S rRNA (28S), Histone H3 (H3) and Histone H4 (H4) nucleotide sequences were obtained for all samples as part of this study, with the exception of 28S rRNA sequences of *Nenia tridens* (EU409911), *Autocoptis menkeana* (EU409908), *Cerion striatellum* (EU409909), and *Bulimulus cf. diaphanus* (EU409910) (by Uit de Weerd, 2008). The latter sequences were supplemented in the current study with H3 and H4 sequences from the same specimens. The partial 28S, H3 and H4 genes were amplified using the primers given in the Supplementary material. The H3PulF and H4F primers were based on the *Cochlodina laminata* partial H4 sequence (AY559149) obtained by Armbruster et al. (2005). H3PulF corresponds to positions 114–192 (reverse complement), and H4F corresponds to positions 585–608 in this sequence. The H4R primer was designed using H4 sequences of *Biomphalaria glabrata* (GenBank Accession number DQ117979; Bouchut et al., 2006) and *Mytilus galloprovincialis* (GenBank Accession number AY267750; Eirín-López et al., 2004). The H3PulR primer was based on H3 sequences of *Arianta* (Groenenberg, 2012) and *Ophicardelus ornatus* (DQ093512; Giribet et al., 2006). To facilitate amplification of H4 across most taxa sampled, two additional H4 primers were designed: H4Fext and H4Rint. These were based on the *Cochlodina laminata* sequences (AY559149, positions 486–509) and on partial H4 sequences obtained with the regular H4 primers, respectively. For the amplification of 28S, we used different combinations of the following primers: 28S-2F, 28S-Alb607R, 28S-1128F, 28S-1145R, and 28S-2119R, following Uit de Weerd (2008). Together these primers span 28S region D1–D6 (cf. Colgan et al., 2007).

PCR reaction mixes for the amplification of H3 and H4 were similar in composition. In both cases the 25 μl reaction mix consisted of 1× PCR buffer, 0.4 μM of each primer, 0.2 mM of each dNTP, 2.5 units of Taq DNA polymerase and approximately 50 ng of template DNA. No $MgCl_2$ was added. The reaction-mix for the amplification of 28S was similar, but in addition consisted of 1×Q solution and 2.2 mM $MgCl_2$. PCR-reactions for H3 and H4 consisted of 40 cycles with the following steps: denaturation at 94 °C (15 s), annealing at 57 °C (30 s), and extension at 72 °C (40 s). This set of 40 cycles was preceded by 3 min denaturation at 94 °C, and followed by an additional extension step at 72 °C for 1 min. The 28S PCR-reactions started with denaturing at 94 °C (4 min), followed by 40 cycles, each with denaturation at 94 °C (60 s), annealing at 60 °C (60 s), and extension at 72 °C (60–75 s depending upon the length of the amplicon). The reaction ended with 5 min extension at 72 °C. PCR products were either purified directly using Qiagen columns and sequenced on an ABI 377 automated sequencer (PE Biosystems), or they were purified and sequenced at Macrogen Korea or Amsterdam, using an ABI3730XL or ABI3700 sequencer.

All alignments were made in the program CLC Sequence Viewer, version 6.5 (CLC bio). The 28S aligment was edited manually. Regions with multiple alternative indels were considered ambiguously aligned and excluded from all analyses up to the nearest constant positions. Alignments were submitted to TreeBASE (Study Accession URL: http://purl.org/phylo/treebase/phylows/study/TB2:S13423), and the concatenated datamatrix is included in the Supplementary material. The 28S, H3 and H4 datasets were checked for base compositional bias in PAUP* 4b10 (Swofford, 2002), and uncorrected pairwise distances (p-distances) were calculated.

The total dataset was subjected to three types of analyses: (1) time-free Bayesian analyses (in MrBayes v3.2.1), (2) Bayesian analyses enforcing a relaxed molecular clock (employing BEAST v1.6.2), and (3) a maximum likelihood analysis (using PhyML 3.0).

2.3. MrBayes analyses of the separate and combined datasets

We examined four datasets: 28S, H3, H4, and the combined 28S + H3 + H4 dataset, using MrBayes v3.2.1 (Ronquist and Huelsenbeck, 2003). For each gene or partition (see below), a substitution and rate heterogeneity model was selected using the Akaike information criterion as implemented MrModeltest 2.3 (Nylander, 2004). Apart from this, default MrBayes model and prior settings were used, unless indicated otherwise.

Each MrBayes analysis consisted of two simultaneous runs, each employing four Markov chains and the default heating scheme. Both runs consisted of 10,000,000 generations with a sample frequency of 100. Discarding the first 5,000,000 generations, we thus obtained a total of 2 × 50,000 post-burnin trees. We checked whether the standard deviation of split frequencies between the postburn generations of both runs remained below a threshold of 0.01 (Ronquist et al., 2011). Tracer v1.5 (Rambaut and Drummond, 2009) was used to calculate if the effective sample size for all parameters was higher than 200, as recommended by Drummond et al. (2007). Using AWTY (Wilgenbusch et al., 2004), we checked for between-run convergence in tree topology by plotting the split frequencies of the parallel runs, examining the cumulative split frequencies, and comparing the symmetric tree-difference score within and between runs.

To assess the level of congruence between the MrBayes trees derived from the three independent markers (28S, H3, H4), the three sets of post-burnin trees were analyzed for incompatible bipartitions in the ingroup, employing SplitsTree 4 (Huson, 1998). Taxa missing in one or two sets were removed from all trees. Because of limitations of the program, we sampled 2000 trees out of the total set of 100,000 trees from the Bayesian

analysis of 28S, H3, and H4 each, with fixed intervals of 5000 generations (i.e. 50 trees). Thus the total set of trees for the three markers combined consisted of 6000 trees, with each marker represented by 2000 trees. A network was constructed applying mean edge weights with a threshold of 0.3135, i.e. presence in 31.35% of the combined set of trees. This threshold was chosen so that clades retrieved in the trees from a single marker but nowhere else would still be visible when found in 95% or more of the Bayesian trees based on that particular marker (see also Maureira-Butler et al., 2008). At the same time this approach allows for the adding up and visualization of minority signals from separate markers (see Roberts et al., 2009).

For the combined dataset we compared three partitioning strategies: (1) unpartitioned; (2) two partitions corresponding to rRNA (28S) and protein-coding (H3 and H4) genes; (3) three partitions, with each of the three genes as a separate partition. This was done to avoid under- or overpartitioning. Generally the error induced by underpartitioning is larger than by overpartitioning (Brown and Lemmon, 2007), but the effects of overpartitioning become more severe as the size of the partitions become smaller (Brown and Lemmon, 2007) and more parameters are estimated (Lemmon and Moriarty, 2004). This was a particular concern in this study, given the short length of the H3 and H4 sequences. Furthermore, partitioning by gene can be ineffective when genes have similar overall evolutionary rates (Li et al., 2008).

In the partitioned analyses (28S, H3 + H4; 28S, H3, H4) we unlinked the stationary nucleotide frequencies, the rate matrix parameters, the gamma shape parameter and the proportion of invariable sites across partitions. Rates were allowed to vary between partitions under a flat Dirichlet prior. Initially we assumed a prior exponential distribution of branch lengths with an inverse scale parameter 10 for all analyses. For the partitioned analyses we additionally performed a second analysis employing a shorter branch length prior, assuming a prior exponential distribution of branch lengths with an inverse scale parameter 100 (Marshall, 2010; Ward et al., 2010), corresponding to a mean branch length prior of 0.01 instead of 0.1. This decreases the chance that the runs become trapped in regions of parameter space, which may lead to convergence on erroneously long tree lengths and to unrealistic or strongly contrasting estimates of relative partition rates between runs (Marshall, 2010; Brown et al., 2010).

For the combined dataset the harmonic means of post-burnin tree log likelihood values under the three alternative partitioning schemes were compared using a Bayes factor analysis. Starting from the unpartitioned model, the addition of a partition was accepted only if $2\log_e(B_{10}) > 10$ (see Kass and Raftery, 1995). The 100,000 post-burnin trees under the selected partitioning model were imported in TreeAnnotator v1.6.2 (Drummond and Rambaut, 2011) to summarize Bayesian posterior probabilities (BPPs) on a maximum clade credibility tree. The set of post-burnin trees also served as the input for downstream analyses (shell evolution, biogeographic reconstruction).

2.4. BEAST analyses of the combined dataset

BEAST v1.6.2 (Drummond and Rambaut, 2007) analyses were restricted to the combined dataset. The partitioning schemes, the substitution and rate heterogeneity models per partition, the method of selecting a partitioning scheme, and the checks on effective sample size were as in the MrBayes analysis of the combined dataset. We used a Jeffreys prior for transition-transversion (kappa) and substitution parameters (see Drummond et al., 2002). For the ucld.mean prior we specified a uniform distribution (0,100) with a starting value of 0.0005, and for the ucld.stdev prior a uniform distribution (0,10) with a starting value of 0.05. For other parameters default settings in BEAUti v1.6.2 (part of the BEAST package) were followed. Each main clade previously identified in the MrBayes analyses was defined as a taxon set, without enforcing monophyly. The age of the MRCA of a set of taxa, and the age of the stem leading to that MRCA were both written to the log file. To obtain stem ages we added a tmrca statistic referring to the relevant taxon set with the command line includeStem = "true". For each partitioning scheme four independent BEAST analyses were performed, unlinking the substitution models between partitions (see Supplementary material for the three BEAST files corresponding to the alternative partitioning schemes). Each analysis consisted of a single 12,500,000 generations run, starting with a random tree under a Yule tree prior. Using a burnin of 10,000,000 generations and a sample frequency of 100 generations, this amounted to 100,000 post-burnin trees for the four analyses combined. The number of independent analyses and the burnin period were doubled compared to the MrBayes analyses, because BEAST employs only a single Monte Carlo Markov chain (Crisp and Cook, 2011).

Estimated ages of the clades of extant Clausiliidae were obtained by imposing a relaxed molecular clock model with uncorrelated lognormal rate variation, as recommended by Drummond et al. (2006, 2007). Calibration was based on fossil evidence. Therefore a log-normal prior distribution was assumed for the calibration points (see Ho and Phillips, 2009). The clausiliid subfamilies Alopiinae, Clausiliinae, and Serrulininae were selected for the calibration because of the availability of both old fossils (>15.97 Ma) assigned to the subfamily and more recent fossils of extant subgroups (cf. fig. 1 in Ho and Phillips, 2009). We did not enforce monophyly constraints for any of these three subfamilies. For each we used as a hard minimum bound (off-set), meaning zero probability of younger dates. As a minimum bound (age x) we used the younger boundary of the oldest geological subepoch during which >1 extant subgroups assigned to that subfamily existed according to the fossil record. The mean and log standard deviation were selected based on the resulting prior age distribution, requiring (1) the median value of the prior distribution to coincide with the median age of the geological subepoch with the oldest fossils assigned to the subfamily as a whole (age y); and (2) the 95% upper quantile to be equidistant from the median value as the off-set [=age y + (age y − age x)]. This approach basically extrapolates data on the completeness of the more recent fossil record of the subfamily deeper in time, thus incorporating uncertainty on the maximum ages of the calibration nodes. While such a broad prior distribution may lead to broader 95% highest posterior density (95% HPD) intervals, it should improve the likelihood that the actual clade age is contained within the 95% HPD interval. In order to have a Cretaceous calibration point, we also applied this procedure to the superfamily Urocoptoidea, here represented by *Autocoptis* and *Cerion*. For the numerical settings for the parameters shaping the prior distribution for these node ages, see the Supplementary material and http://purl.org/phylo/treebase/phylows/study/TB2:S13423. We also examined whether the standard deviation of the uncorrelated log-normal relaxed clock (ucld standard deviation) remained within the range 0–1, indicating that the data are reasonably clocklike (Drummond et al., 2007).

Information in the set of post-burnin trees was summarized using Tracer v1.5 and TreeAnnotator v1.6.2. The maximum clade credibility tree and clade BPPs were obtained through TreeAnnotator v1.6.2, as for the MrBayes analysis. Mean values and 95% HPD intervals for the ages of clades and of the stems leading to these clades were calculated using Tracer v1.5.

To examine the effects (see Drummond et al., 2006; see Wertheim et al., 2010) of imposing a relaxed molecular clock model (BEAST analysis) versus using a time-free model (MrBayes analysis) on tree topologies, we compared the BPPs of identical clades for both models. In addition we checked for overlap in the 95%

credible sets of trees of both analyses. A 95% credible set of trees for the post-burnin BEAST trees was obtained using MrBayes v3.2.1.

2.5. Maximum likelihood analyses of the combined dataset

Maximum likelihood (ML) analyses of the combined dataset were performed using the online version PhyML 3.0 (Guindon et al., 2010) on the South of France bioinformatics platform (http://www.atgc-montpellier.fr/phyml/). Starting with 50 random trees, a heuristic search was made using tree bisection and reconnection (TBR) to optimize topology and branch lengths. As this analysis does not allow for data partitions, we implemented the substitution and rate heterogeneity model selected for the combined unpartitioned dataset. All parameter settings within the model were estimated, with the exception of base frequencies, for which empirical equilibrium frequencies were assumed. Bootstrap values were calculated based on 1000 bootstrap replicates. For each replicate the heuristic search was conducted as described above, but starting with a single BIONJ tree (Gascuel, 1997).

2.6. Conchological evolution

Reconstructions of conchological evolution were based on the 100,000 Bayesian trees from both the MrBayes and the BEAST analysis of the combined dataset under the selected partitioning scheme. We examined five conchological characters (see Section 1) used in the classification of fossil and extant species with subfamilies (see Table 1; see Supplementary material). Two characters were excluded because of ambiguously defined character states: (1) the amount of inward extension of the parieto-columellar lamellae, which "in the Phaedusinae and Serrulininae (...) penetrate more or less deeply inwards" (Nordsieck, 2007a: 93), and (2) the position of the subcolumellar lamella, which "in the Phaedusinae (...) shifts inwards to the parietal wall", a shift that "is only recognizable when the subcolumellar ends deeply" (Nordsieck, 2007a: 93). Another concern were two composite characters for which various transitions between the characters states had previously been described: (1) the type of clausilial apparatus (see Nordsieck, 2007a: 91) and (2) the distinction between a single ridge (lunella) on which the clausilium rests, versus parallel lamellae (see Nordsieck, 1982, 2007a: 85). For these the descriptions and assignments of character states by Nordsieck (2007a) were followed as much as possible, using a strict definition of the supposedly apomorphic state. This was done as a conservative approach, making refutation of the supposedly apomorphic state less likely. The states for other characters were scored from the specimen vouchers and/or checked with conspecific shells from the collection of NCB Naturalis.

Conchological evolution based on the trees from both Bayesian analyses was reconstructed in Mesquite v2.75 (Maddison and Maddison, 2011) and PAUP* 4.0b10, using maximum parsimony (MP). MP was chosen rather than ML or Bayesian character reconstruction, because the implicit assumption of parsimony underlies the use of these characters in classification. For the aperture formation, the non-apostrophic, semi-apostrophic and apostrophic aperture were treated as ordered unpolarized characters. All other characters were two-state. Since all conchological characters examined were specific to the Clausiliidae, the character states in the outgroup taxa were coded as unknown.

First we examined the amount of phylogenetic information present in shell-morphological characters given the Bayesian trees. Characters were plotted on each of the 100,000 trees from the MrBayes and from the BEAST analysis, using the MP criteria in PAUP* 4.0b10. This procedure was repeated on 100,000 trees that were random and equiprobable with respect to ingroup interrelationships. Maximum and minimum tree length for the set of Bayesian trees were subsequently compared to the tree length distribution of random trees.

Second we reconstructed the character states for the most recent common ancestor (MRCA) of the extant Clausiliidae using Mesquite v2.75. This was accomplished by plotting the character states for each character on each of the 100,000 Bayesian trees using the maximum parsimony option in the trace-over-trees module. This approach accommodates for uncertainties both in topology and in character plotting.

2.7. Biogeographic reconstructions

For the biogeographic analysis again the trees from the MrBayes and from the BEAST analysis of the combined dataset were examined. We largely followed the distinction in distribution areas made by Wagner (1920), Loosjes and Loosjes-van Bemmel (1966) and Nordsieck (1978a), except for a subdivision of the Neotropics into the Greater Antilles and the S-American mainland (see Uit de Weerd, 2008), and the addition of sub-Saharan Africa as a separate area. Thus we classified the samples into five categories: (1) western Eurasia including adjacent Africa and Macaronesia, (2) eastern Eurasia, (3) South America, (4) the Greater Antilles and (5) sub-Saharan Africa.

Biogeographic reconstructions were based on Fitch parsimony, as implemented in Mesquite 2.74, and on dispersal–vicariance analysis, as implemented in S-diva 1.9 (Yu et al., 2010). The Fitch parsimony approach (Ronquist, 1994) restricts ancestors to single areas, thus equating subsequent changes in distribution with dispersal. Dispersal–vicariance analysis (Ronquist, 1997) infers ancestral areas based on a vicariance scenario, minimizing the number of dispersal and extinction events. By examining all 100,000 trees from both Bayesian analyses using both reconstruction methods, we accounted for uncertainties both in phylogeny and in biogeographic reconstruction. Outgroups were removed from the trees examined under Fitch parsimony, because the positions of the outgroup taxa relative to the ingroup are uncertain without applying a deeper root. As the S-diva analysis requires an outgroup, we used a single outgroup with an arbitrary unique code. In the S-diva analysis the maximum number of ancestral areas per node was constrained to 2. This was done under the assumptions that (1) ancestral clausiliid species had ranges that were approximately equal in size to that of extant ones, and (2) any ancestral range overlapped with maximally two of the areas considered, given the geographical distance between these areas throughout the Mesozoic and Cenozoic (see Smith et al., 1994). S-diva analyses were performed with the option 'allow reconstruction' in effect, thus calculating the actual frequency of alternative reconstructions per node per tree (see Harris and Xiang, 2009; see Yu et al., 2010). In both the Fitch parsimony and the dispersal–vicariance analysis we calculated the percentage of trees that had a particular area, or combination of areas, as the most parsimonious ancestral distribution for the MRCA of extant Clausiliidae.

3. Results

3.1. Dataset

Partial 28S and H3 nucleotide sequences were obtained for all taxa. Three 28S sequences contained an internal region for which the nucleotide sequence could not be determined. These are the 28S sequence of *Herilla bosniensis rex* (291 bases missing), *Sumelia rolli* (633 bases missing) and *Alinda biplicata biplicata* (22 bases missing). For H4 the combination of the H4F and H4R primers yielded PCR product and sequences for 41 of the samples. Using

other H4 primer combinations (see Supplementary material) we obtained sequences for all but 6 taxa: *Hemiphaedusa (Hemizaptyx) kosakai*, *Incania papillosa papillosa*, *Cyclonenia gibber*, and *Incaglaia adusta tumens* and the outgroup taxa *Mastus cretensis* and *Autocoptis menkeana*.

The 28S sequences ranged in length from 1595 bases in *Alinda biplicata biplicata* to 1637 bases in *Dilataria succineata succineata*. Nearly all of this variation was concentrated in 15 regions, which were excluded from the analyses because of alignment ambiguities. Of the remaining 1522 positions 238 were variable. All H3 sequences were 267 bases long. H4 sequences, when using the standard primer combination H4F-H4R, were 260 bases in length. The primer H4Fext, located in the spacer (see Armbruster et al., 2005) between H3 and H4, amplified an additional 26 bases at the 5′ of H4 plus 54–80 bases of the preceding poorly alignable spacer region for 15 taxa. These positions were excluded from the analyses because they were missing for all other taxa. For 15 taxa the internal H4Rint primer had to be used as a reverse primer, shortening the sequence by 28 bases at the 3′ side. All nucleotide variation in H3 and H4 was synonymous, and most of it resided in third codon positions. Of the 95 variable positions for H3, 86 were third codon positions and 9 were first codon positions. For the 82 variable positions in H4 these numbers were 75 and 7, respectively.

No compositional bias was detected for any of the three genes (Chi-square test, $P = 1.0$). P-distances for the histone H3 and H4 datasets were on average a factor 4.12 and 3.47 (for H4 excluding the 28 3′ positions, see above) higher, respectively, than for 28S. The 28S and H3 + H4 p-distances were positively correlated, with no indication of saturation in one dataset compared to the other, even when considering distances between in- and outgroup species (see Supplementary material).

3.2. Phylogenetic analyses

The substitution models selected by the Akaike information criterion and used in the Bayesian analyses were GTR + I + Γ for 28S and for H3, and HKY + I + Γ for H4. Separate Bayesian analyses for 28S, H3, and H4 produced generally congruent results (see Table 3). The SplitsTree analysis did not reveal any incongruence between datasets with respect to deeper relationships within the family (see Fig. 2).

For the concatenated 28S, H3, and H4 partitions in the three-partition Bayesian analyses we implemented the previously selected models. For the H3 + H4 partition in the two-partition analyses, the Akaike information criterion selected the GTR + I + Γ model, which was used in combination with the GTR + I + Γ model for the 28S partition. The GTR + I + Γ model was also selected for the one-partition analysis.

Employing the default prior inverse scale parameter of 10 for exponential distribution of branch lengths in the two-partition and three-partition analysis in MrBayes, resulted in high tree lengths (95% HPD interval of 4.21–12.45 and 11.03–15.64, respectively) compared to the unpartitioned scheme (95% HPD interval of 1.31–1.66, respectively). The standard deviation of split frequencies never dropped to below 0.02 (two partitions: 0.0206–0.0261; three partitions: 0.0285–0.0317). Furthermore, the posterior effective sample size was inadequate (<200) for estimates of tree length, tree likelihood, alpha, pinvar and the rate multiplier (m). These conditions persisted even when extending both analyses to 20,000,000 generations. Employing an inverse scale parameter of 100 resulted in a prominent decrease in tree length (95% HPD interval for two partitions: 0.96–1.16; 95% HPD interval for three partitions: 0.96–1.17). The standard deviation of split frequencies across post-burnin (5,000,000–10,000,000 generations)

Table 2
Harmonic mean (HM) log likelihood values ($\ln L$) for alternative partitioning schemes and corresponding Bayes factors. Values for the selected partitioning schemes are given in bold.

	One partition (28S, H3, H4) HM $\ln L$	Two partitions (28S) (H3, H4) HM $\ln L$	Three partitions (28S) (H3) (H4) HM $\ln L$	Bayesfactor [$2\log_e(B_{10})$]	Interpretation
MrBayes					
2 versus 1	−10850.93	**−10729.92**[a] (−10938.03)		242.02	Very strong evidence against one partition
3 versus 2		**−10729.92**[a] (−10938.03)	−10736.23[a] (−10909.34)	−12.62	Very strong evidence for two partitions
BEAST					
2 versus 1	−10820.20	**−10779.39**		81.62	Very strong evidence against one partition
3 versus 2		**−10779.39**	−10787.79	−16.80	Very strong evidence for two partitions

[a] Assuming a prior exponential distribution of branch lengths with an inverse scale parameter of 100 rather than 10 (HM $\ln L$ value with inverse scale parameter of 10 is given between parentheses). Bayesfactor [$2\log_e(B_{10})$] applies to the addition of one partition. The interpretation of the Bayesfactors is based on Kass and Raftery (1995).

Table 3
Support for groups based on the individual markers and the combined data set. Significant (BPP > 0.95) support from Bayesian analyses is indicated in bold, both for the individual markers providing such support and for the BPP values from the analysis of the combined dataset. ML bootstrap values >80 are also given in bold, as indicating high support. The seven main clades identified in this study are highlighted in bold. Values from the BEAST analysis are given between parentheses.

Group	>0.50 BPP from MrBayes analysis of	BPP in MrBayes (BEAST) analysis of the combined dataset	ML bootstrap from combined dataset
Alopiinae	**28S, H3**	**1.000 (1.000)**	**98**
Baleinae	None	0.145 (0.320)	<50
Clausiliinae	None	0.759 (0.515)	<50
Garnieriinae	**28S, H4**	**1.000 (1.000)**	**98**
Laminiferinae	**28S, H3, H4**	**1.000 (1.000)**	**100**
Mentissoideinae	None	0.000 (0.000)	<50
Neniinae	None	0.114 (0.020)	<50
Phaedusinae[a]	28S	**0.998 (0.999)**	**87**
Serrulininae	None	0.000 (0.001)	<50
(Phaedusinae + Serrulinae)[a]	**28S, H3, H4**	**1.000 (1.000)**	**100**
(Baleinae + Clausiliinae + Mentissoideinae)	**28S, H3, H4**	**1.000 (1.000)**	**100**
Neniini	**28S,** H4	**1.000 (1.000)**	**94**
Peruiniini[a]	28S, H3, H4	**1.000 (1.000)**	**99**

[a] Taxa missing in this group for histone H4.

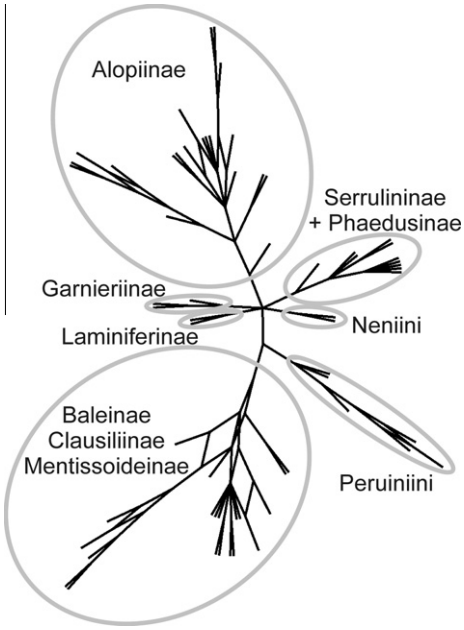

Figure 2. SplitsTree network derived from 2000 trees of the MrBayes analysis of each of the three markers (28S rRNA, histone H3 and histone H4) using mean edge weights with a threshold of 0.3135.

samples remained below 0.01, varying from 0.00427 to 0.00928 for the two-partition and from 0.00357 to 0.00674 for the three-partition analysis. In both cases effective sample sizes well above 200 were reached as well as higher (less negative) log-likelihood values compared to the initial analyses (see Table 2). Therefore, for the two- and three-partition scheme we considered only the estimates from the analyses with an inverse scale parameter of 100.

In both the MrBayes and the BEAST analysis Bayes factors supported the two-partition (28S; H3 + H4) scheme over the two alternative partitioning schemes (see Table 2). Consequently, the set of post-burnin trees obtained in the MrBayes and in the BEAST analysis using two partitions were used in further analyses.

The two-partition MrBayes analysis (harmonic mean log-likelihoods = −10729.92), the two-partition BEAST analysis (harmonic mean log-likelihoods = −10779.39), and the ML analyses (log-likelihood ML tree = −10699.19) of the combined dataset generally recovered identical well-supported clades (see Fig. 3). The mean absolute difference in clade-wise BPPs between the MrBayes and BEAST analysis was 0.0206. The 95% credible sets of trees from the MrBayes analysis (75,073 trees) and the BEAST analysis (68,332 trees) overlapped, sharing 94 identical topologies. The ML tree topology was not contained in the 95% credible sets of trees from either Bayesian analysis.

The MrBayes and BEAST analysis divide the family in seven clades with a BPP of 1.0 (present in all 100,000 trees) in both analyses, and supported by high (94–100%) ML bootstrap values (see Figure 3, see Table 3): (1) Alopiinae, (2) Garnieriinae, (3) Laminiferinae, (4) a clade comprising Phaedusinae plus Serrulininae, (5) a clade consisting of Baleinae, Clausiliinae and Mentissoideinae, (6) a Neniini clade, and (7) a Peruiniini clade. The first five of these are also significantly ($P > 0.95$) supported by at least two datasets in the MrBayes analysis of the individual markers. The analyses of the combined dataset failed to support the monophyly of the subfamilies Neniinae, Serrulininae (paraphyletic with respect to the Phaedusinae), Baleinae and Mentissoideinae, while support for the monophyly of the subfamily Clausiliinae is weak at best (see Table 3). Both Bayesian analyses unite the Baleinae and the Clausiliinae in a significantly (MrBayes BPP = 0.98; BEAST BPP = 1.00) supported subclade, which is also present in the ML tree (<50% ML bootstrap support).

Relationships between the seven clades receive only modest or weak support, with the exception of a clade consisting of the Phaedusinae + Serrulininae, the Garnieriinae, and the Peruiniini combined (BPP = 0.95) that was recovered in the BEAST analysis. That clade receives some support from the MrBayes analysis (BPP = 0.73) and is also part of the ML tree (but ML bootstrap <50%). There is some support for a sister-group relationship between the Alopiinae clade and the Baleinae + Clausiliinae + Mentissoideinae clade from the Bayesian analyses (MrBayes BPP = 0.81; BEAST BPP = 0.77, ML bootstrap support <50%). In the majority of the trees from the Bayesian analyses and in the ML tree the Neniini clade is sister to a clade composed of all remaining Clausiliidae (MrBayes BPP = 0.56; BEAST BPP = 0.67, ML bootstrap <50%).

3.3. Clade ages

The ucld standard deviation in the BEAST analysis with two partitions varied from 0.20 to 0.68 (mean 0.42), indicating that the data are reasonably clocklike (Drummond et al., 2007). Mean ages and age 95% HPD intervals for all clades identified in the BEAST maximum clade credibility tree (Fig. 3) are available from the Supplementary material. The mean estimated age of the MRCA of the extant Clausiliidae is 79.9 Ma (see Table 4) with a 95% HPD interval from 54.9 to 108.6 Ma. The mean estimated ages of the MRCA of each of the seven main clades vary from 21.9 Ma (Garnieriinae) to 47.8 Ma (Baleinae + Clausiliinae + Mentissoideinae) (Table 4), but the mean estimated ages of the stems leading to them are considerably older, between 54.6 Ma (to Garnieriinae and to Phaedusinae + Serrulininae) and 76.3 Ma (to Neniini).

According to the BEAST analysis, the Neniini and the Peruiniini are among the oldest clades, despite their lower taxonomic rank as tribes within the subfamily Neniinae. The Neniini clade has a mean estimated age of 42.1 Ma (95% HPD: 19.4–67.3); the age of the Peruiniini clade is estimated at 40.7 Ma (95% HPD: 25.6–57.4). According to the reconstruction, both tribes shared a last common ancestor at a mean estimated 77.8 Ma (95% HPD: 51.0–108.6). To test the effect of enforcing the combined monophyly of these South American Clausiliidae, we repeated the BEAST analysis of the combined dataset under this constraint. In BEAST such a constrained analysis requires a user-specified starting tree. To this end, for each of the four constrained BEAST runs we selected the first tree from the corresponding unconstrained run that was compatible with the monophyly of the South American Clausiliidae. Using BEAST, we arrived at an estimated age of the enforced Neniinae of 59.5 Ma (95% HPD: 39.6–83.4).

3.4. Conchological evolution

Despite extensive homoplasy, for most of the conchological characters examined the maximum number of transformations required (tree length, TL) across the complete set of Bayesian trees was lower than that on 95% of the random trees ($P < 0.05$) (see Table 5). The only exception is the type of clausilial apparatus ($P_{TL \leqslant 8} = 1.000$). Our analysis retrieves as the optimal states for the MRCA of the extant Clausiliidae: a rounded shell base, an apostrophic rather than a non-apostrophic aperture, a N-type clausilial apparatus, and a lunella rather than plicae (see Table 5). In nearly all (99.9% MrBayes; 99.8% BEAST) of the trees the apostrophic aperture is either the most parsimonious ancestral condition for the extant Clausiliidae, or among the most parsimonious reconstructions. In contrast, only 7.5% of the MrBayes trees and 19.7% of the BEAST trees are consistent with the non-apostrophic aperture being the ancestral character state.

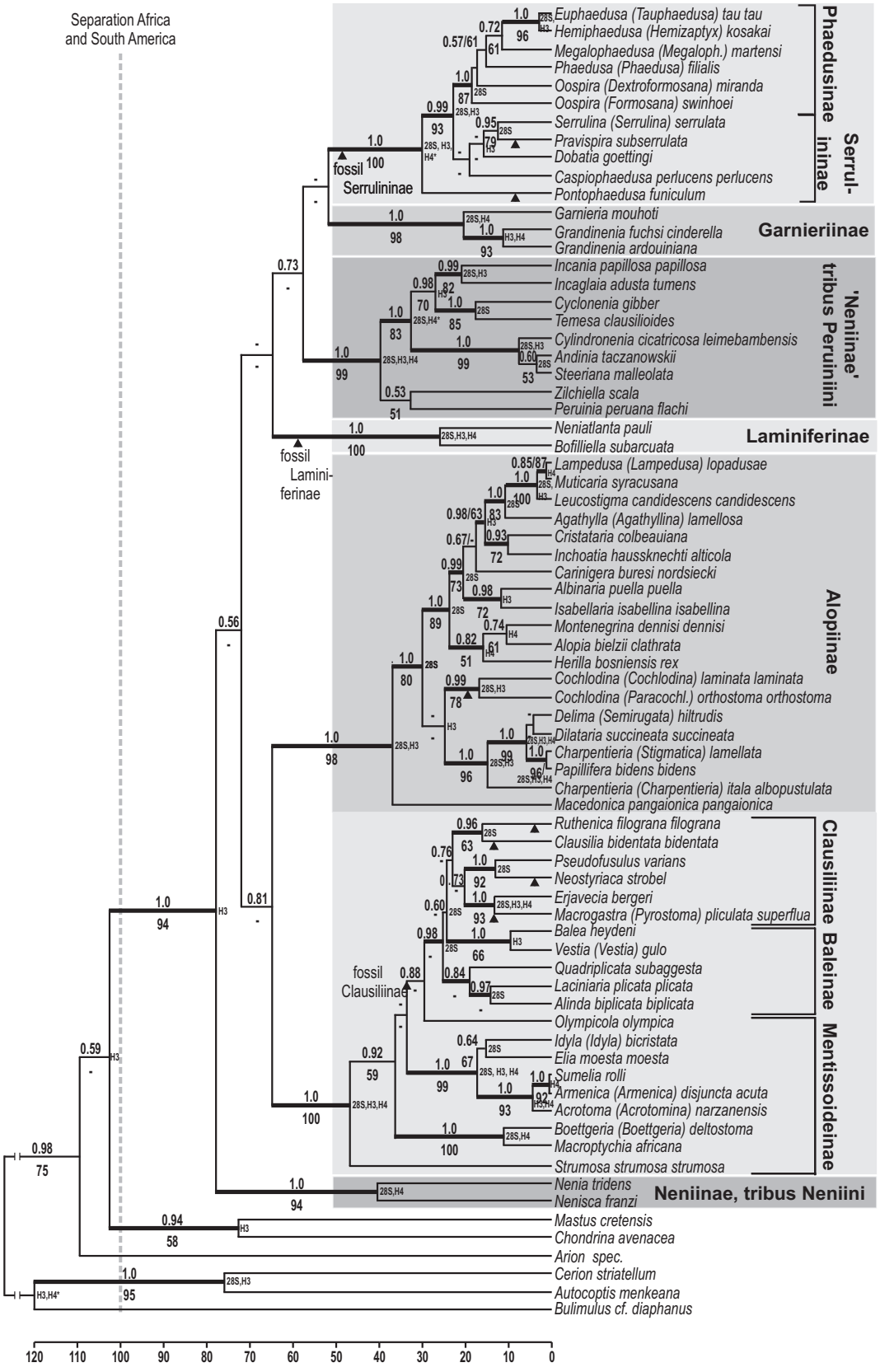

Figure 3. Maximum clade credibility tree from the BEAST analysis. Thick branches indicate clades with Bayesian posterior probabilities (BPP) > 0.95 in the BEAST analysis. MrBayes BPP's > 0.5 are given above branches, ML bootstrap values >50% below branches. Ages in million years (Ma) are given on the bar below. Triangles below branches indicate the age (median age of the geological subepoch) of the oldest fossil evidence assigned to genera sampled or to a particular 'subfamily' (data from Nordsieck, 2007a). The hatched line represents the approximate time of disconnection of Africa and South-America. Individual markers (28S rRNA, histone H3, histone H4) supporting a particular branch by >0.5 BPP when analyzed separately in MrBayes are given on the right side of branches. *One or more of the taxa contained in the clade absent from the histone H4 dataset.

Table 4
Estimated mean ages and confidence intervals (in Ma) of the lineage and MRCA of the Clausiliidae, the seven main clades, and other relevant groups. Age of stem refers to the base of the branch leading to a particular clade.

Group	Mean age stem to MRCA (95% HPD interval)	Mean age MRCA in Ma (95% HPD interval)
Clausiliidae	106.3 (70.9–147.9)	79.9 (54.9–108.6)
Main clades		
Alopiinae	66.1 (44.5–90.0)	37.6 (24.5–52.0)
Baleinae + Clausiliinae + Mentissoideinae	67.4 (45.7–91.8)	47.8 (32.7–65.5)
Garnieriinae	54.6 (35.4–76.3)	21.9 (9.0–37.6)
Laminiferinae	68.6 (46.4–94.3)	27.3 (10.9–46.4)
Phaedusinae + Serrulininae	54.6 (36.3–77.2)	30.8 (19.7–43.0)
Neniinae tribus Neniini	76.3 (46.7–108.6)	42.1 (19.4–67.3)
Neniinae tribus Peruiniini	58.7 (39.7–80.2)	40.7 (25.6–57.4)
Other relevant groups		
Phaedusinae	22.7 (13.9–31.8)	18.9 (11.5–27.3)
Neniinae (unconstrained)	98.6 (54.1–143.9)	77.8 (51.0–108.6)
Neniinae (monophyly constraint)	66.1 (44.8–91.6)	59.5 (39.6–83.4)

3.5. Biogeographic reconstructions

Each of the seven main clausiliid clades is more or less geographically confined (see Fig. 4). Two clades span more than one area: (1) the Phaedusinae + Serrulininae clade from eastern and western Eurasia and (2) the Baleinae + Clausiliinae + Mentissoideinae clade from western Eurasia and sub-Saharan Africa. The Fitch parsimony analyses place the ancestor of Phaedusinae + Serrulininae clade in western Eurasia for nearly all Bayesian trees (99.9% MrBayes; 99.8% BEAST), as does the dispersal–vicariance analysis (99.9% MrBayes; 99.8% BEAST). For the Baleinae + Clausiliinae + Mentissoideinae clade, both Fitch parsimony and dispersal–vicariance analysis retrieve a western Eurasian ancestor in all trees (MrBayes and BEAST).

Although the Fitch parsimony and dispersal–vicariance analyses both support an ancestral area for the clade of extant Clausiliidae as a whole that includes western Eurasia, neither can entirely rule out a Greater Antillean origin. In the Fitch parsimony analysis, all Bayesian trees were consistent with a western Eurasian origin of extant Clausiliidae, either as the single most parsimonious reconstruction (43.6% MrBayes; 33.4% BEAST) or as equally parsimonious to a Greater Antillean origin (56.0% MrBayes; 66.5% BEAST) or to other combinations (<1% in either analysis). All trees, from both MrBayes and BEAST, require at least 5 dispersal events between the areas recognized. Only a small proportion (11.6% MrBayes; 2.0% BEAST) of the Bayesian trees are consistent with a single transoceanic or pre-oceanic dispersal event either to or from the New World. All other trees require two dispersal events to explain the New World distribution of both the Neniini and the Peruiniini. According to the dispersal–vicariance analyses the clade of extant Clausiliidae originated either in an area consisting of western Eurasia plus the Greater Antilles (56.1% MrBayes; 66.6% BEAST) or in western Eurasia (43.6% MrBayes; 33.4% BEAST). Other combinations were found in less than 1% of the trees.

4. Discussion

4.1. Congruence of our results with the current classification

This study unambiguously separates all extant Clausiliidae into seven main clades: (1) Alopiinae, (2) Garnieriinae, (3) Laminiferinae, (4) Phaedusinae + Serrulininae (5) Baleinae + Clausiliinae + Mentissoideinae, (6) Neniini, and (7) Peruiniini. Although not all these clades coincide with recognized subfamilies, most are supported by additional data (see below).

The first three clades correspond to subfamilies recognized by Nordsieck (1973, 1978a, 1978b, 2005a, 2007a). The support for the monophyly of these subfamilies is remarkable, given the lack of unique derived characters states shared between all species classified in any of these groups. Apparently, individual conchological or genital–anatomical characters used in classification may exhibit some variation within a clade, but when considered together in a non-cladistic approach may still provide sufficient information to identify these clades nonetheless. As an extreme example, even the clausilial apparatus can be absent in a genus, e.g. *Balea*, yet its placement in the Clausiliidae based on other morphological similarities is confirmed by our analyses.

In addition to these three subfamilies, two of the main clades consist of so-called subfamilies that at some point in the past have been grouped together: (1) Phaedusinae + Serrulininae (Nordsieck, 1973; Nemeth and Szekeres, 1995; but see Nordsieck, 2005a, 2007a), (2) Baleinae + Clausiliinae + Mentissoideinae (Nordsieck, 1978a, 2005a, 2007a).

The Phaedusinae and Serrulininae have been treated as a single subfamily Phaedusinae in the past (Nordsieck, 1973; Nemeth and Szekeres, 1995), and our results now confirm that a separate subfamily Serrulininae, as advocated recently by Nordsieck (2007a) would be a paraphyletic group. According to the description by Nordsieck (2005a, 2007a), both the so-called Phaedusinae and Serrulininae are characterized by the parieto-columellar lamellae, ending deeply inside the last whorl, which could be an autapomorphy for a single clade, although its definition remains somewhat

Table 5
Results of character plots on the 100,000 trees from the MrBayes and the BEAST analysis. Values between parentheses indicate scores for the set of BEAST trees. TL = tree length; HI = homoplasy index. *P*-values are based on the tree length distribution when plotting the character on 100,000 random equiprobable trees. The rightmost column gives the percentage of Bayesian trees with a given character state among the most parsimonious reconstructions for the MRCA of the Clausiliidae. The prevalent character states for the MRCA are given in bold.

	TL	HI	*P* values	Percentage of trees with state present in MRCA	
Body whorl	13–15 (13–14)	0.92–0.93	$P_{TL\leqslant 15} = 0.003$ $P_{TL<13} = 0.000$	**100% (100%)** 0.2% (8.1%)	**Rounded/with weak keel**[a] With prominent keel
Aperture formation	4–8 (4–8)	0.50–0.75	$P_{TL\leqslant 8} = 0.000$ $P_{TL<4} = 0.000$	7.5% (19.7%) 25.6% (30.9%) **99.9% (99.8%)**	Non-apostrophic[a] Semi-apostrophic **Apostrophic**
Clausilial apparatus	7–8 (7–8)	0.86–0.88	$P_{TL\leqslant 8} = 1.000$ $P_{TL<7} = 0.026$	**100% (100%)** 0% (0%)	**N-type**[a] G-type
Palatal plicae	5–7 (6–7)	0.80–0.86	$P_{TL\leqslant 7} = 0.047$ $P_{TL<5} = 0.000$	0% (0%) **100% (100%)**	Plicae-type[a] **Lunella-type**
Superior lamella	8–12 (8–12)	0.88–0.92	$P_{TL\leqslant 12} = 0.003$ $P_{TL<8} = 0.000$	76.2% (46.3%) **97.5% (98.3%)**	Continuous with spiral lamella[a] **Separated from spiral lamella**

[a] Plesiomorphic condition sensu Nordsieck (2007a).

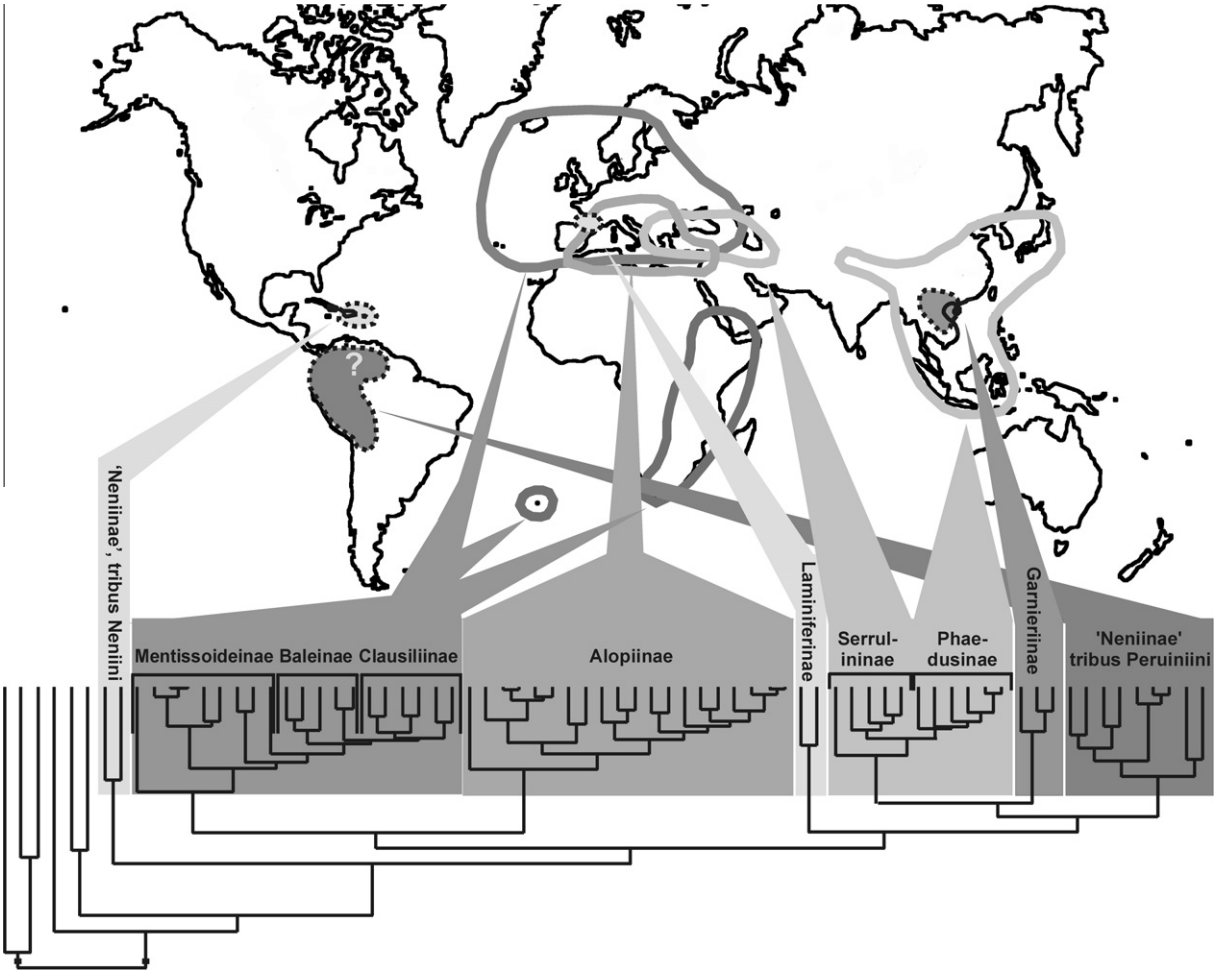

Figure 4. Distribution of the seven main clausiliid clades. Distributional areas of clades characterized by an apostrophic aperture are filled and surrounded by hatched lines. The question mark refers to the South American genus *Neniops*, which was recently (Nordsieck, 2010) transferred from the Peruiniini to the Neniini.

unclear. Moreover, the probably apomorphic plicae-type lunella is only found in this clade, although not in all of its members. The observation that genera classified with Serrulininae occupy the basal branches in the Phaedusinae + Serrulininae clade is consistent with the early emergence of taxa classified as Serrulininae in the fossil record (see Nordsieck, 2000, 2007a) as compared to Phaedusinae. The isolated position of *Pontophaedusa* basal to the other Serrulininae and the Phaedusinae is congruent with the aperture formation. *Pontophaedusa* is unique among the Phaedusinae and Serrulininae in having a semi-apostrophic aperture (Nordsieck, 1978b) rather than an – according to our results – apomorphic non-apostrophic aperture.

The clade consisting of Baleinae, Clausiliinae and Mentissoideinae coincides with the provisional subfamily-group Clausiliinae (Nordsieck, 1978a, 2005a, 2007a). This subfamily-group was defined on the basis of four shared putative morphological apomorphies (Nordsieck, 2005a, 2007a), only one of which is unique to this group (viz. diverticulum of bursa copulatrix transformed into a glandular tube). Additional evidence for a clade consisting of Baleinae, Clausiliinae and Mentissoideinae comes from ITS1&2 rRNA sequences (Uit de Weerd and Gittenberger, 2004), albeit based on only three samples representing each of the subfamilies under the assumption that these are monophyletic. The combined monophyly of the Baleinae and Clausiliinae, as suggested by both Bayesian analyses, is consistent with a transformed loop of the male copulatory organs that is unique to these two subfamilies (Nordsieck, 1978a). Support for a clade of Baleinae, Clausiliinae and Mentissoideinae together with Alopiinae is meager, but intriguingly a keel on the body whorl of fully grown shells is only present in these four subfamilies, though not in all members.

4.2. Neniini and Peruiniini clades

The most surprising finding of this study is the observation that two so-called tribes, i.e. Neniini and Peruiniini, in the subfamily Neniinae represent ancient lineages that that were not retrieved as sister-lineages. They share their MRCA at or near the base of the clausiliid tree. The delineation of the subfamily Neniinae has long been debated. It has been argued that taxa placed in the Garnieriinae by Nordsieck (2005a, 2007a), should be included in the Neniinae because of similarities in radular dentition (Loosjes and Loosjes-van Bemmel, 1973b) and genital anatomy (Szekeres, 1969, 1998). Our analyses suggest that even Nordsieck's narrow definition of the Neniinae is too broad in phylogenetic and cladistic terms. Apart from a lack of any reported unique conchological synapomorphies, the subfamily Neniinae is considered very diverse in genital anatomy (Nordsieck, 2007a). The Peruiniini are distinguished from Neniini by two supposedly apomorphic genital anatomical features: a weaker penis sheath (Thompson, 1998), with the possible exception of *Zilchiella* (Nordsieck, 2005b), and a diverticulum that is transformed into a glandular tube (Nordsieck, 2005a, 2007a). The latter character state is not unique among Clausiliidae, however (see Table 1).

Our study suggests that the clade of the two Antillean genera, *Nenia* and *Nenisca,* represents a relatively old and basal lineage within the Clausiliidae clade. The relatively high estimated

divergence date between these genera is in line with genital–anatomical and conchological observations, which suggest that "*Nenia* and *Nenisca* have had a long and divergent evolutionary history from each other" (Thompson, 1998). The position of the South American genus *Neniops*, which was recently (Nordsieck, 2010) transferred to the tribe Neniini, remains uncertain. That revision was based upon the supposedly symplesiomorphic genital–anatomical character states considered diagnostic for the Neniini (Grego and Szekeres, 2008; Nordsieck, 2010), viz. a strong penial sheath and an untransformed diverticulum. A strong penial sheath is also found in *Zilchiella* (Nordsieck, 2005b; Nordsieck, pers. comm.), a genus that occupies a basal position in the Peruiniini clade. A diverticulum is missing altogether in several genera classified within the Peruiniini (Loosjes and Loosjes-van Bemmel, 1984; Nordsieck, 2005b). Therefore, a position of *Neniops* directly basal to the Peruiniini from the same continent cannot be excluded, and would be more in line with the old phylogenetic split between the Greater Antillean and the South American Clausiliidae as found in this study.

4.3. Age of the Clausiliidae

Our analyses indicate that the MRCA of the extant Clausiliidae dates back to between 54.9 and 108.6 million years ago. This interval is in line with independent fossil finds, not used in the BEAST calibration. The oldest fossils assigned to modern subfamilies, viz. Laminiferinae (see Nordsieck, 2000, 2007a) and, tentatively, Neniinae (Salvador, 2011) are from the Middle to Late Paleocene (55.8–61.2 Ma). The oldest unambiguous Clausiliidae have been described from the Maastrichtian (65.5–70.5 Ma). These fossils were assigned to the species *Proalbinaria undulata*, classified with the tribus Rillyini in the extinct subfamily Eualopiinae (Nordsieck, 2000). Although this age corresponds to our estimate, it is in fact unclear if this lineage branched off before or after the MRCA. The relationships of the Santonian (83.5–85.8 Ma) *Dextrospira minutula* and an unnamed Campanian (70.6–83.5 Ma) fossil to the Clausiliidae clade are even more uncertain, as in both cases the structure and position of the internal lamellae is unknown, and these species do not appear to belong to any better-know clausiliid group (Nordsieck, 2000). Even so, these fossils too are far younger than the 95% HPD upper boundary of 108.6 million years. Within the clausiliid clade, ages of independent fossils not used in the calibration but assigned to genera (Nordsieck, 2007a) sampled in our study, are congruent with the mean age estimates for those genera from the BEAST analysis (see Fig. 3).

The lack of support for any interrelationships between the main clades in the absence of conflicting signal between markers suggests a short period of radiation at the base of the clausiliid tree. According to our results these divergences probably date between 54.6 and 76.3 Ma (range of mean ages of stem lineages, see Table 4), most of them at or shortly after the Cretaceous–Cenozoic boundary. Early Cenozoic radiations have been inferred in other groups of stylommatophoran land snails (Wade et al., 2006; Rowson et al., 2010) and may have been triggered by the extinction of much of the terrestrial biota at the Cretaceous–Cenozoic boundary (Wade et al., 2006; Mordan and Wade, 2008; Rowson et al., 2010).

4.4. Aperture evolution

Of all conchological characters examined, aperture formation (apostrophic, semi-apostrophic or non-apostrophic) is the most informative phylogenetically at the subfamily level (see Table 5). This provides phylogenetic underpinning for the view that "there is no conchological character of higher taxonomical value within the Clausiliidae" [Es gibt kein Schalenmerkmal von höheren taxonomischen Wert bei Clausiliiden] (translated from Ehrmann, 1927). However, our analyses challenge previous assumptions about aperture formation character state polarity, suggesting that the non-apostrophic aperture is the apomorphic rather than plesiomorphic condition.

The notion that the non-apostrophic aperture constitutes the plesiomorphic character state was based on its presence in the extinct family Triptychiidae (=Filholiidae), which is placed together with the Clausiliidae in the superfamily Clausilioidea (Nordsieck, 1976). Two other character states that were considered plesiomorphic based on their occurrence in the Triptychiidae, viz. parallel palatal plicae instead of a lunella and a connected superior and spiral lamella, are probably also apomorphic according to our analyses (Table 5). According to Szekeres (1998) the '*Triptychia* group' is a highly specialized branch of the Clausiliidae, judging from the conchological characters and its relatively young fossil record, which starts in the Middle Eocene. The Triptychiidae/'*Triptychia* group' may therefore be an inappropriate outgroup. In fact, the first occurrence of an aperture that may be apostrophic (Nordsieck, 2000, 2007a) dates back much further to the Campanian (70.6–83.5 Ma) (Nordsieck, 2000, note 4). Ignoring the Santonian (83.5–85.8 Ma) *Dextrospira minutula*, whose classification as a clausiliid species remains problematic (Nordsieck, 2000, 2007a), the oldest non-apostrophic aperture is known from the tribe Rillyini (see Nordsieck, 2000) from the Maastrichtian (65.5–70.6 Ma). It should be noted that it is uncertain whether any of these fossils are part of the Clausiliidae clade, or represent earlier branches. When considering only Clausiliidae that have been assigned to an extant subfamily, the apostrophic aperture is first reported from the Late Paleocene as *Laminifera (Palaeophaedusa) edmondi,* whereas the oldest fossils with non-apostrophic apertures are younger, viz. the Early Oligocene *Canalica manca* (Nordsieck, 2000, 2007a).

Previously, the presence of a supposedly apomorphic apostrophic aperture in Clausiliidae from different continents had to be explained as either synapomorphic (e.g. Nordsieck, 1972; Loosjes and Loosjes-van Bemmel, 1973a, 1973b); or arising from parallel evolution (Nordsieck, 1999, 2005a, 2007a). However, these explanations by themselves fail to account for the complete absence of the supposedly older non-apostrophic Clausiliidae on the Greater Antilles and in South-America (Loosjes and Loosjes-van Bemmel, 1973a). Both explanations implicitly assume that the apostrophic aperture is somehow selectively superior to the supposedly plesiomorphic non-apostrophic aperture, leading either to a greater global distribution of a synapomorphous apostrophic aperture compared to the supposedly older non-apostrophic aperture, or to its repetitive evolution from the non/semi-apostrophic state. It is difficult to reconcile such putative selective advantages of the apostrophic aperture with the observation that in western and eastern Eurasia, where both non-apostrophic and apostrophic Clausiliidae coexist, the non-apostrophic groups currently have larger combined ranges. While the apostrophic subfamily Laminiferinae is now confined to the Pyrenees (see Figure ure4), fossils assigned to it are found in Early Cenozoic sediments throughout western Europe (Ehrmann, 1927; Nordsieck, 2000, 2007a), indicating a major range contraction not observed for the non-apostrophic Clausiliidae from that area. Such contradictions are resolved if the apostrophic aperture is considered the plesiomorphic character state, as is indicated by our results.

Our study implies that non-apostrophic clausiliids evolved two or three times in western Eurasia, where the non-apostrophic Baleinae + Clausiliinae + Mentissoideinae clade is found, as well as its most probable sister-group the Alopiinae clade, and the basal Serrulininae lineages in the Phaedusinae + Serrulininae clade. These non-apostrophic Clausiliidae must have largely replaced the apostrophic clausiliid subfamilies Laminiferinae and Garnieriinae in western and eastern Eurasia, respectively, but failed to reach South America and the Greater Antilles (see Fig. 4).

4.5. Geographic origin and spread of the Clausiliidae

Our results do not refute the view that the crown clade of modern Clausiliidae originated in western Eurasia and spread from there to other areas. An origin on the Greater Antilles, which was found to be equally parsimonious in 56.0% (MrBayes) to 66.5% (BEAST) of the Bayesian trees is here considered less likely. The clausiliid fossil record in Europe (see Nordsieck, 2000, 2007a) outdates the age of the Greater Antilles. These islands were probably formed in the Eocene, after the proto-Antillean island arc collided with the Bahamas platform (Iturralde-Vinent, 2006; Pindell, 1994). Admittedly, a presence on the proto-Antillean island arc has been hypothesized for two urocoptid lineages of land snails, but as an extension of an originally North American distribution (Uit de Weerd, 2008). A combined origin on the Greater Antilles and western Eurasia of the clade of extant Clausiliidae, as one of the outcomes in our dispersal–vicariance analyses, can be ruled out. Neither the Greater Antillean island arc nor the preceding proto-Antillean island arc were ever connected to western Eurasia (Pindell, 1994; Iturralde-Vinent and MacPhee, 1999). While consistent with a European origin of the Clausiliidae, our results necessitate a revision of the biogeographic scenario proposed by Nordsieck (1986, 2005a, 2007a) with respect to the dispersal to East Asia and to the neotropics.

Our results confirm that two lineages independently reached East Asia from western Eurasia (Nordsieck, 2005a, 2007a): (1) the lineage to the Garnieriinae clade, which diverged around 54.6 Ma (95% HPD: 35.4–76.3) (see Table 4) and (2) the lineage leading to the Phaedusinae clade, which diverged from one of the Serrulininae lineages approximately 22.7 Ma (95% HPD: 13.9–31.8) (see Table 4). Being the elder of the two, the Garnieriinae lineage probably spread to East Asia first, in the Early Cenozoic, even though it currently has the smallest range by far. Pre-Pleistocene eastern Eurasian fossils of Clausiliidae, which could help to fill in the biogeographic history of both lineages in this area, have not been reported to our knowledge. The geographic isolation of the Phaedusinae clade from the Serrulininae, among which it is nested, is probably the result of fragmentation of a once continuous Phaedusinae + Serrulininae range. The Phaedusinae and the Serrulininae are now separated by an arid region (mean annual precipitation <300 mm) extending from central Asia to the Persian Gulf and the Indian peninsula (Miao et al., 2012). The estimated age of the split between the Phaedusinae lineage and its sister-group among the Serrulininae coincides with the aridification of these areas in the Miocene (Guo et al., 2008; Miao et al., 2012).

Dispersal of Clausiliidae to South America by land prior to the opening of the Atlantic Ocean is highly unlikely. The inferred age of 79.9 Ma (95% HPD: 54.9–108.6) of a western Eurasian MRCA of the extant Clausiliidae practically precludes such dispersal by any descendant lineage. The final separation of the South American and African continents is thought to have taken place approximately between 99.6 [Cenomanian] and 112.0 [Late Aptian/Clansayesian] Ma (Pitman III et al., 1993; Smith et al., 1994; Maisey, 2000; Sereno et al., 2004; Gheerbrant and Rage, 2006), and terrestrial dispersal of some vertebrate groups between the continents may have been hampered as early as 120 Ma (Nishihara et al., 2009). We therefore conclude that the ancestors of the New World Clausiliidae most probably colonized the western hemisphere by transatlantic dispersal from Europe or Africa. This conclusion contrasts with molecular phylogenetic studies on other (super)families of land snails, which attributed continental-scale distributional patterns predominantly to the Mesozoic distribution of landmasses (Wade et al., 2006; Herbert and Mitchell, 2008; Uit de Weerd, 2008) with a very limited or no role of transoceanic dispersal.

We find no evidence that the New World Clausiliidae constitute a monophyletic group, although their combined monophyly cannot be entirely ruled out. If the Neniinae clades Neniini and Peruiniini are not sister-groups, this implies two separate transatlantic dispersal events: (1) dispersal of the ancestor of the Neniini to the Greater Antilles, either directly or via South America after a lineage leading to *Neniops* had branched off; and (2) dispersal of the ancestor of the Peruiniini sampled to South America.

According to our analysis, the clade of Greater Antillean Neniini dates back to at least 42.1 Ma (95% HPD: 19.4–67.3). This is consistent with the presumed Eocene age (33.9–55.8 Ma) of the Greater Antilles (Iturralde-Vinent, 2006; Pindell, 1994). The South American Peruiniini must have been in South America at least from 40.7 Ma (95% HPD 25.6–57.4) onwards. This pushes back a transatlantic dispersal of the ancestors or – less likely – ancestor of both clades to the Early Cenozoic, when the distances between Africa and South America were smaller (Smith et al., 1994). The occurrence of a so-called *Temesa magalhaesi* (Trindade, 1953) in Middle to Late Paleocene deposits c. 40 km E of Rio de Janeiro, Brasil (Salvador, 2011), demonstrates that Clausiliidae were present in South America in the Early Cenozoic. Equally, the large geological and geographical gap with the extant South American and Greater Antillean Clausiliidae illustrates the incompleteness of the fossil record and its restricted relevance for the historical biogeography of the Clausiliidae within the continent.

Westward dispersal across the Atlantic Ocean has been inferred for several other groups. At least two vertebrate lineages, the gecko genus *Tarentola* (Carranza et al., 2000) and Amphisbaenians (Vidal et al., 2008), are thought to have reached the Greater Antilles from NW Africa or SW Europe during the Cenozoic. In the same era, many groups reached South America from Africa, (e.g. caviomorph rodents, Poux et al., 2006; Sallam et al., 2009; platyrrhine anthropoids, Poux et al., 2006; seven lineages of gecko's, Gamble et al., 2011; the lizard genus *Mabuya*, Carranza and Arnold, 2003; and opisthocomiform birds, Mayr et al., 2011). Transatlantic dispersal from Africa to South America was invoked as an explanation for the presence of the Paleocene land snail genus *Brasilennea*, with supposedly African affinities, in the South American fossil record (Rowson et al., 2010). However, that genus is now considered part of the New World clade Urocoptoidea, based on conchological similarities (Salvador et al., 2011).

All of the inferred vertebrate dispersals from Africa to South America have been attributed to rafting on floating mats of vegetation. According to Renner (2004) such transport would have been facilitated by surface currents running from central Africa to the eastern tip of South America. The North Atlantic may have provided an alternative dispersal route. A clockwise gyre system was probably already in place there during the Late Cretaceous (see Bush, 1997) and Early Cenozoic (see Barron and Peterson, 1991; see Stille et al., 1996). Such a system would direct rafts from the coastal waters of northwestern Africa or Europe to the Caribbean area and the northern coast of South America.

Dispersal by rafting may account for the insular distribution of some land snails (Dall, 1896), but is generally considered unlikely because of desiccation due to exposure to salt water (e.g. Holland and Hadfield, 2004; Parent and Crespi, 2006; Cowie and Holland, 2006). Even so, some land snails can survive up to 12 h of immersion in salt water (Holland and Cowie, 2007). The extant Greater Antillean Neniini are found mostly on (dead) vegetation or trees (Thompson, 1998; Alvarez and Willig, 1993), and it is therefore likely that their common ancestors also dwelled on floatable plant material. The Peruiniini, in contrast, are generally rock-dwelling (Neubert and Nordsieck, 2005).

Another explanation for trans-oceanic disjunctions in the case of land snails, is aerial dispersal by birds (Rees, 1965; Vagvolgyi, 1975; Parent and Crespi, 2006). Within the Clausiliidae, migrating birds may have acted as vectors in the colonization of the Southern Atlantic Tristan da Cunha archipelago as well as the Northern

Atlantic Azores archipelago by the genus *Balea* (Gittenberger et al., 2006). As in that study, dispersal by birds in this study cannot be easily linked to present-day bird migration patterns. Presently, no bird migration routes span both sides of the tropical or Southern Atlantic Ocean (Renner, 2004). However, such routes may have existed when ancestors or ancestor of the Neniini and the Peruiniini reached the Neotropics, presumably in the Early Cenozoic when migration distances were smaller.

5. Conclusion

5.1. Classification

Based on the seven clades identified in this study, we propose the following revisions with respect to the classification by Nordsieck (2007a). The Serrulininae are merged into an extended Phaedusinae. This restores the Phaedusinae to its original composition before the revision by Nordsieck (1978a). Furthermore, we elevate the so-called Neniinae tribes Neniini and Peruiniini to the status of the separate subfamilies Neniinae and Peruiniinae, respectively. They are treated as subfamilies representing old clades within the Clausiliidae; evidence for a sister-group relationship is lacking. We classify *Neniops* in the Neniinae, following its earlier (Nordsieck, 2010) classification with the Neniini. Finally, we combine Baleinae, Clausiliinae and Mentissoideinae into a single subfamily Clausiliinae. This implies a subdivision of the extant Clausiliidae into seven subfamilies: Phaedusinae, Laminiferinae, Garnieriinae, Neniinae, Peruiniinae, Alopiinae, and Clausiliinae.

5.2. A new scenario for clausiliid evolution and biogeography

Based on our results we propose a scenario for clausiliid evolution and biogeography with two waves of expansion from western Eurasia. The earliest lineages after the MRCA evolved in western Eurasia, and were apostrophic in shell aperture. One lineage spread to Asia, resulting in the Garnieriinae, and one or two to South America to become Neniinae and Peruiniinae. Subsequently, the non-apostrophic aperture evolved twice, or maybe three times, in western Eurasia, in lineages evolving into the Alopiinae, the Clausiliinae (hitherto considered three separate subfamilies, i.e. Baleinae + Clausiliinae + Mentissoideinae), and the Phaedusinae (formerly Phaedusinae + Serrulininae). We suggest that these groups subsequently largely replaced the apostrophic subfamilies in Eurasia, with the Laminiferinae and Garnieriinae surviving in only small ranges (see Fig. 4). The Clausiliinae clade also spread to eastern Africa and to some Atlantic islands. However, the non-apostrophic Clausiliidae apparently failed to reach the neotropics, where particularly the Peruiniinae are still highly diverse. This may be due to widening of the Atlantic Ocean during the Cenozoic. This scenario assumes a selective advantage of a non-apostrophic aperture throughout most of the family's range.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2013.01.011.

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