Molecular and morphological phylogenetics of chelonine parasitoid wasps (Hymenoptera: Braconidae), with a critical assessment of divergence time estimations

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Abstract

Parasitoid wasps of the subfamily Cheloninae are both species rich and poorly known. Although the taxonomy of Cheloninae appears to be relatively stable, there is no clear understanding of relationships among higher-level taxa. We here applied molecular phylogenetic analyses using three markers (*COI*, *EF1 α* , *28S*) and 37 morphological characters to elucidate the evolution and systematics of these wasps. Analyses were based on 83 specimens representing 13 genera. All genera except *Ascogaster*, *Phanerotoma*, and *Pseudophanerotoma* formed monophyletic groups; *Furcidentia* (**stat. rev.**) is raised to generic rank. Neither *Chelonus* (*Chelonus*) nor *Chelonus* (*Microchelonus*) were recovered as monophyletic, but together formed a monophyletic lineage. The tribes Chelonini and Odontosphaeropygini formed monophyletic groups, but the Phanerotomini *sensu* Zettel and Pseudophanerotomini were retrieved as either para- or polyphyletic. The genera comprising the former subfamily Adeliinae were confirmed as being nested within the Cheloninae. To estimate the age of the subfamily, we used 16 fossil taxa. Three approaches were compared: fixed-rate dating, node dating, and total-evidence dating, with age estimates differing greatly between the three methods. Shortcomings of each approach in relation to our dataset are discussed, and none of the age estimates is deemed sufficiently reliable. Given that most dating studies use a single method only, in most cases without presenting analyses on the sensitivity to priors, it is likely that numerous age estimates in the literature suffer from a similar lack of robustness. We argue for a more rigorous approach to dating analyses and for a faithful presentation of uncertainties in divergence time estimates.

Given the results of the phylogenetic analysis the following taxonomic changes are proposed: *Furcidentia* Zettel (stat. rev.), previously treated as a subgenus of *Pseudophanerotoma* Zettel is raised to generic rank; *Microchelonus* Szépligeti (syn. nov.), variously treated by previous authors, is proposed as a junior synonym of *Chelonus* Jurine; the following subgenera of *Microchelonus* – *Baculonus* Braet & van Achterberg (syn. nov.), *Carinichelonus* Tobias (syn. nov.) and *Scabrichelonus* He, Chen & van Achterberg (syn. nov.), are proposed as junior synonyms of *Chelonus*; a number of new species names are proposed due to homonyms resulting from the above changes and these are listed in the paper.

Keywords

Phylogeny, total-evidence, molecular clock, node-dating, Cheloninae, fixed clockrate

1. Introduction

Approximately every tenth insect species is a parasitoid which completes its development in or on an arthropod host that is eventually killed in the process (Quicke, 2015). Most parasitoids belong to the order Hymenoptera. The study of parasitoids in general and parasitoid Hymenoptera in particular is critical given that they are a key component of almost all terrestrial ecosystems and comprise one of the most species-rich groups of animals. The Cheloninae are a large subfamily of braconid wasps with approximately 1,400 described species in 17 genera worldwide (Table 1) (Yu et al., 2012; Kittel and Austin, 2014). They are sister to five subfamilies together with which they form the microgastroid complex (Murphy et al., 2008), all of which exploit lepidopteran caterpillars as hosts.

Members of the Cheloninae can be easily distinguished from other Braconidae based on the possession of a complete postpectal carina and a carapace formed by the fusion of the first three metasomal tergites (van Achterberg, 1976; Shaw, 1997). The latter character also occurs convergently in a number of other subfamilies (e.g. Brachistinae, Ichneutinae, Microgastrinae, Sigalphinae), but is generally rare outside of the Cheloninae (Dudarenko, 1974; Quicke, 2015). All chelonines are believed to be solitary koinobiont endoparasitoids especially of larval pyralid and tortricid moths, laying their eggs into the host egg. Development is then delayed until the host larva emerges, and the parasitoid eventually kills it in the last larval stage (Shaw, 1997). The final instar wasp larva emerges from the host

larva and feeds from outside before forming a pupal cocoon (Jackson et al., 1978; Huddleston and Walker, 1994). Although some other braconids (a few members of Alysiinae, Helconinae, Ichneutinae, Microgastrinae) also oviposit into host eggs, this strategy is apparently only universal for the Cheloninae (Clausen, 1956; Ruberson and Whitfield, 1996; Quicke, 2015). Chelonines occur on all continents except Antarctica, but the largest number of species are described from the Palaearctic. However, while most genera found in the Palaearctic have a global distribution, numerous genera are apparently endemic to the Neotropics (Table 1) (Yu et al., 2012), while the faunas of the tropics and southern continents are still generally poorly studied.

Although the taxonomy of Cheloninae at the species level is relatively extensive (at least for the Northern Hemisphere) and the generic classification has not changed much during the last few decades (McComb, 1968; Shenefelt, 1973; Huddleston, 1984; van Achterberg, 1990; Zettel, 1992), there is no robust understanding of the phylogeny of the group, which would provide a stable basis for interpreting biogeographic patterns and host associations. Previous attempts to determine the relationships among genera and subgenera were based solely on morphological characters, had limited taxon sampling, and focused only on the Phanerotomini (Zettel, 1990) or Chelonini (Shaw, 1983). Additionally, the Adeliinae have previously been treated as a separate subfamily (Quicke and van Achterberg, 1990; Wharton et al., 1992; van Achterberg, 1993; Shi et al., 2005), but preliminary molecular analyses place them inside the Cheloninae (Belshaw et al., 2000; Belshaw & Quicke, 2002; Dowton et al., 2002). The current classification is likely further flawed by the weak delimitation of some genera; for example, it remains questionable whether *Microchelonus* forms a valid genus or is nested within *Chelonus* (McComb, 1968; Papp, 1995; Lozan and Tobias, 2006; Zhang et al., 2008). In addition, *Leptodrepana* has been treated as a genus (Shaw, 1983) or, after its synonymy with *Ascogaster* (van Achterberg, 1990) subsequently treated as part of *Ascogaster* (Tang and Marsh, 1994; van Achterberg and Polaszek, 1996; Shaw, 1997; Brajkovic et al., 2010; Yu et al., 2012). To date, no comprehensive phylogenetic analysis incorporating molecular data has been undertaken to resolve key questions concerning relationships within this species-rich and biologically interesting group of braconid parasitoids.

Another open question concerns the age of the subfamily and its constituent groups. Ronquist et al. (2012a) have attempted to date the major groups of Hymenoptera, but they only included very few representatives of the Apocrita and, thus, did not provide age

estimates for the ichneumonoid families. There have been very few studies that infer the age of braconid subfamilies, with the Rogadinae and Doryctinae (Zaldivar-Riverón et al., 2008a; Zaldivar-Riverón et al., 2008b) and the microgastroid complex (Whitfield, 2002; Murphy et al., 2008) being notable exceptions. These studies have only utilised a node-dating approach, in which a phylogenetic tree is calibrated by assigning fossils (and their ages) to specific nodes as hard minima. There are severe limitations with this method, most of all the sensitivity to the calibration priors (Inoue et al., 2010).

The aims of this study were to use an extensive dataset comprising sequence data from multiple genes and morphology to investigate chelonine relationships, to test the monophyly of genera and tribes, including the position of members of the former subfamily Adeliinae, and to clarify the status of problematic taxa such as *Microchelonus* and *Leptodrepana*. Furthermore, we compared three different approaches to infer the age of chelonines: fixed-rate dating, node dating, and total-evidence dating, using all described chelonine fossils. The shortcomings of the three methods are discussed both in regard to our dataset and to their application in poorly known groups in general.

N.B. Taxonomic information including the authors of tribes, genera and subgenera are given in Table 1.

2. Material and Methods

2.1 Taxon sampling

For this study, 83 specimens representing 80 species from all major biogeographic regions, 11 of 17 currently recognised extant genera of Cheloninae, and two of the four recognised genera of the former subfamily Adeliinae were sequenced for three molecular markers (see below; Appendix 1). The alignment included two additional sequences of chelonines obtained from GenBank (*Wushenia* and *Chelonus* (*Carinichelonus*)) (see Appendix 1 for GenBank accession numbers; <https://www.ncbi.nlm.nih.gov/genbank/>). Four representatives of other braconid subfamilies were used as outgroup taxa, two being members of the closely related microgastroid subfamilies Microgastrinae (*Microplitis* sp.) and Miracinae (*Mirax* sp.), one from the Ichneutinae (*Ichneutes* sp.) which is the sister clade to the microgastroid complex, and one from the more distantly related Euphorinae (*Leiophron* sp.).

Genera were identified using the keys from Zettel (1990) and Shaw (1997). For distinguishing between *Chelonus* and *Microchelonus*, McComb's (1968) definition was used as follows: female *Microchelonus* with 16 antennomeres, male with a pit present on the posterior metasoma.

2.2 Morphological characters, species identification, and fossils

Thirty-seven morphological characters were coded for all taxa for which we had sequence data (see Appendices 2 and 3). The characters used were mostly adapted from van Achterberg (1984) and Zettel (1990). Character 3 in Appendix 2 ('occipital carina touching hypostomal carina') was identified during the course of this study and was included in the matrix. The program DELTA editor was used to code specimens (Dallwitz et al., 1999).

Sixteen fossil ingroup species representing five genera were used to calibrate the tree in the node dating and total-evidence dating approaches (see Table 2). Morphological characters for these fossil taxa were either examined directly (in the case of *Eobracon cladurus* Cockerell) or coded based on the original description and images (all others), as the fossil taxa in baltic amber described by Brues (1933) have been lost (Shaw, 1983) or difficult to obtain.

2.3 Molecular techniques

2.3.1 DNA extraction protocols

Genomic DNA was extracted from specimens that had been preserved in ethanol (75-100%) or were dry and pin-mounted. One hind leg was removed from each specimen and kept in the fridge for 10 min to let the ethanol evaporate, or a non-destructive approach was applied using the whole specimen. DNA extractions were performed using the Gentra Systems Puregene® DNA Purification Kit (Qiagen). The leg or whole specimen was heated to 55°C in a 300 µl cell lysis solution with 1.5 µl proteinase K solution. After 12-24 h, excessive proteins were removed by adding 100 µl protein Precipitation solution. The DNA was then washed in 300 µl Isopropanol (plus 0.5 µl glycogen) and afterwards in 300 µl ethanol (70%). DNA was rehydrated using 50 µl DNA hydration solution.

2.3.2 Gene selection and primer design

The selected markers included the mitochondrial gene cytochrome c oxidase subunit 1 (*COI*) and the nuclear genes elongation factor 1 α copy F2 (*EF1 α*) and 28S rRNA D2-D3 region (28S), resulting in a total of 1,454 bp (444 bp *COI*, 378 bp *EF1 α* , and 632 bp 28S) (for primers used, see Table 3). All *EF1 α* sequences were compared with the F1 and F2 copies from the hymenopteran taxa analysed by Klopstein and Ronquist (2013) and were confirmed to belong to the F2 copy.

In order to obtain sequences from older pinned museum specimens, primers were designed for shorter fragments of 28S (Table 3) using the Geneious Pro v. 5.3.6 (Drummond et al., 2011) (by Biomatters, <http://www.geneious.com/>) plugin Primer 3 (Koressaar and Remm, 2007). Consensus sequences were created from these shorter fragments and concatenated with Geneious Pro.

2.3.3 Amplification and sequencing

We used Eppendorf thermal sequencers for PCR amplifications. Each reaction of 25 μ l comprised 2.5 μ l 10 x Taq Gold Buffer, 3 μ l 25 mM MgCl₂, 2 μ l 10 mM dNTPs, 1 μ l of each forward and reverse 5 μ M Primer (see Table 3), 0.1 units (μ l) AmpliTaq Gold® DNA Polymerase (Applied Biosystems Inc.), and 1 μ l DNA (approximately 0.2 μ g DNA). In some reactions, Immolase™ DNA Polymerase (Bioline) was used. PCR settings started with a denaturation step of 9 min at 95°C, followed by 35 cycles of 30 sec 94°C, 30 sec annealing temp (Table 3), and an extension step of 1 min at 72°C. The final extension step was for 6 min at 72°C. PCR products were purified using the Ultraclean™ PCR Clean-up™ Kit (MoBio Biosystems Inc.) and sequenced by the Australian Genome Research Facility Ltd. (AGRF) using capillary separation.

2.3.4 Sequence alignment

The sequences were edited in Geneious Pro and a consensus sequence was created from the forward and reverse sequences. Alignments for each marker were conducted with the Geneious Pro plug-in Clustal W v. 2.1 (Thompson et al., 1994) with the default penalties settings of 15 for gap open costs and 6.66 for gap extend costs. Both *COI* and *EF1 α* had open reading frames, and *COI* had a typical mtDNA AT bias, and therefore was unlikely to belong to a nuclear copy (Chandra et al., 2006). The concatenated alignment of all three genes was

then produced using Geneious Pro (alignment available on TreeBase <http://purl.org/phylo/treebase/phyloids/study/TB2:S17882>).

2.4 Phylogenetic inference

The morphological analysis, separate gene analyses, concatenated genes analysis, and a combined molecular plus morphological (total-evidence) analysis were each conducted using maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) approaches. The best-fitting partitioning scheme and most likely substitution model for each partition were identified using PartitionFinder (Lanfear et al., 2012), applying the Akaike information criterion (Posada and Buckley, 2004) (Table 4).

The MP analyses applied a tree-bisection-reconnection (TBR) heuristic approach as implemented in PAUP* (Swofford, 2003), with 1,000 random stepwise addition replicates each holding 100 trees. The characters were treated as unordered and had equal weight. Bootstrap support was calculated in 1,000 iterations, without replicates for the random stepwise addition step.

The ML analyses were conducted in RAxML v. 7.7.0 (Stamatakis, 2006) with 1,000 random starting trees under the GTRGAMMAI model (GTR with gamma-distributed among-site rate variation and a proportion of invariable sites) (Stamatakis, 2006). Clade support was obtained using 1,000 non-parametric bootstrap iterations, applying fast bootstrapping.

For the BI analyses, MrBayes v. 3.2.2 was used (Ronquist et al., 2012b). Each analysis was run for 20 million generations, with four independent runs of four chains each. Default settings, random starting trees, trees sampled every 1,000 generations, and default heating were used. Topology convergence was verified by a 0.01 threshold of the average standard deviation of split frequencies (Ronquist and Huelsenbeck, 2003), while convergence of the scalar parameters was verified by an effective sample size of more than 200 and the potential scale reduction factor approaching 1. The first 25% of generations were omitted as a burn-in.

The analyses were conducted on tizard, the eResearch South Australia high-performance computing cluster (PAUP* and RAxML), or on the CIPRES Science Gateway V 3.3 (MrBayes) (Miller et al., 2010).

2.5 Dating analyses

We used three different approaches to infer the age of chelonines: fixed-rate dating, node dating, and total-evidence dating, and assessed the reliability of each method. Because the Bayesian framework allows incorporation of complex age priors, we used BI for the dating analyses, all of which were conducted in MrBayes 3.2.3 (Ronquist et al., 2012b). To make sure that we had large enough partitions for the dating analyses, the molecular data were not partitioned as previously, but a simpler partitioning scheme based on molecular evolution was used instead (Simmons et al., 2006) (Table 4), with the first and second codon positions of both *COI* and *EF1 α* forming one partition each, the third codon positions of *COI* and *EF1 α* each forming another, and *28S* represented by a single partition. The fixed-rate analyses were based on the whole gene including the changes at the third codon position of *COI*, and thus the third codon position was included in these analyses. However, in the node-dating and total-evidence dating analyses, the age of the chelonines became much older due to the high saturation in this partition (data not shown), and hence the third codon positions of *COI* were excluded. All analyses were run for 50 million generations, sampling every 1,000 generations, using four independent runs with four chains each, and convergence diagnostics applied as detailed above.

2.5.1 Fixed-rate analyses

Estimates of the number of substitutions per million years can be used to transform substitution branch lengths into time lengths, at least for more recent time scales. We used the general estimate of the rate of evolution for mitochondrial genes in insects as 2.3% per million years (Brower, 1994). As the prior on branch lengths might have a strong impact on the resulting estimates, we took two different approaches; first, we used the default clockrate of 1 and only translated branch lengths into time lengths after running the analysis under the default branch-length prior. Second, we fixed the clockrate to the equivalent of 2.3% for *COI*, calculated for our multi-gene dataset using the rate-multiplier values retrieved from the previous run. These analyses are overly naive when compared to the node-dating and total-evidence dating approaches, where we introduced a time-constraint on the root in addition to the information from the fossils. We also ran the fixed-rate analyses with the tree-age prior set as in the two other approaches (Table 5).

2.5.2 Node dating

For the node-dating analyses, we used the same multi-gene dataset as for the fixed-rate approach, but calibrated specific nodes according to the ages of fossils. We used offset-exponential priors on two calibration points. Due to the topology of the tree and the fact that most fossils belong to stem-groups within their respective genera, only the oldest fossil *Phanerotoma menieri* Belokobylskij et al. could be used to calibrate the Phanerotomini clade, as all the other, younger fossils would only have been used to calibrate the ancestor of all chelonines. The minimum age for this group was thus set to 52 Ma. As a reasonable mean estimate, we used the age of the oldest fossil of Euphorinae (McKellar and Wolfe, 2010), which was also used to provide the minimum age of the root of the tree (including the outgroups). The mean age of the root of the tree was set to 130 Ma following the estimate for the age of the Braconidae calculated by Whitfield (2002). To test the impact of this arguably subjective prior setting, we varied the means of both calibration node priors among analyses (Table 5).

2.5.3 Total-evidence dating

In total-evidence dating, the calibration of the phylogeny is based on fossils included as terminals in the analysis (Ronquist et al., 2012a). To this end, we extended the dataset by including morphological data for the 16 fossil taxa (Table 2), with molecular sequences for fossils coded as missing data. We used a uniform tree prior and the un-correlated gamma-distributed relaxed-clock model (IGR). For an initial analysis, the priors on the clockrate ($\text{lognorm}(-7.08069, 2.458582)$) and on the variance parameter of the IGR model ($\text{exp}(37.12)$) were set as in Ronquist et al. (2012a). After this first step, the IGR variance prior was modified to $\text{exp}(84.65723)$ in an empirical Bayes approach in order to reflect the posterior resulting from this preliminary analysis. The clockrate posterior was very similar to the prior from Ronquist et al. (2012a) and was thus not modified.

The ages of the fossils included as terminals in the dataset were specified according to the ranges of the age estimates of the deposits (Table 2), employing a uniform distribution. Convergence was assessed as above, except the first 50% of generations were omitted as a burn-in, to improve convergence of the clockrate parameter. Random character exclusion was performed to test whether the number of morphological characters included had an impact on the result. *Phanerotoma menieri* was the oldest fossil included in the analysis, but in a non-clock, morphology-only analysis ended up further up the tree than the other fossils (not

shown), and might thus have distorted the analysis. We thus also tested whether the exclusion of *P. menieri* had an impact on the results.

3. Results

3.1 Phylogenetic analyses

The trees from the ML analyses show an overall similar topology to those of the BI analyses, but with lower support values (Fig. 1). However, the tree from the MP analysis was poorly resolved, which is possibly not surprising given the limitations of this method for analysing multi-gene sequence data (Fig. 1). The Cheloninae plus Adeliinae are recovered in all analyses as monophyletic relative to the outgroup subfamilies, with only the single-gene trees for *COI* and *EF1 α* not reaching significant Bayesian posterior probabilities for this clade. The monophyly of the tribes Phanerotomini *sensu* van Achterberg (but including Adeliinae), Chelonini, and Odontosphaeropygini are highly supported in the concatenated analyses, while the single gene analyses and morphology alone show substantial differences. However, the tribes proposed by Zettel (1990), Phanerotomini *sensu* Zettel and Pseudophanerotomini (represented by *Pseudophanerotoma* and *Furcidentia*) are not recovered as monophyletic in all analyses.

Apart from the Bayesian morphology-only analysis, the Adeliinae are recovered within the Cheloninae in all analyses, with *Adelius* and *Paradelius* forming a clade with either *Phanerotomella* or *Dentigaster*, depending on the analysis. Most genera are recovered as monophyletic, with the exception of *Ascogaster*, *Phanerotoma*, and *Pseudophanerotoma*. *Ascogaster* is split in almost all analyses into two clades, a mostly ‘North American’ and a mostly ‘Australian’ clade, thus rendering the genus paraphyletic with respect to *Austroascogaster*, *Leptodrepana*, and *Megascogaster*. The only exception to this finding comes from the morphology-only analysis, where *Ascogaster* is monophyletic. In all analyses, *Phanerotoma* is rendered paraphyletic with respect to either *Huseyinia*, *Phanaustrotoma*, or both. The subgenera of *Pseudophanerotoma* included in the analyses, *P.* (*Pseudophanerotoma*) and *P.* (*Furcidentia*), are retrieved with strong support in all analyses as separate clades, but never together in a single monophyletic clade. The genus *Chelonus s.l.* is recovered as monophyletic with significant support in all analyses. However, neither of the major subgenera, *C.* (*Chelonus*) and *C.* (*Microchelonus*) (CH and MC, respectively, Fig. 1) form a monophyletic group. The only other subgenus, *M.* (*Baculonus*), represented by two species, is monophyletic.

3.2 Placements of fossils

The topology generated in the non-clock analysis that included morphology and the fossils (Fig. 2) is the same, overall, as that obtained for the other analyses (see above), with almost all fossil taxa placed as expected. The fossil *Chelonus* and *Ascogaster* species are recovered with their extant counterparts. *Diodontogaster* is placed among the extant and fossil *Ascogaster* fossils, thus rendering the North American *Ascogaster* paraphyletic. However, *Eobracon* is placed within the Australian clade of *Ascogaster*, together with *Austroascogaster* and *Megascogaster*. The three fossil *Phanerotoma* species did not fall within the extant *Phanerotoma* clade, but rather are either placed basally within the Phanerotomini (*P. extensa* Brues, *P. baltica* Brues) or are recovered as closely related to *Pseudophanerotoma* (*Pseudophanerotoma*) and *Pseudophanerotoma* (*Furcidentia*) (*P. menieri*).

3.3 Comparison of divergence-time estimates

The divergence times estimated under three different approaches using a range of prior settings are summarised in Table 5. In the fixed-rate approach and under the default tree-age prior (gamma (1, 1)), the median age of Cheloninae is estimated as 48 Ma if the evolutionary rate is set to 2.3% for *COI*. Similarly, if the analyses are conducted without the 2.3% changes over time (default clockrate: 1), but translated to the clockrate of 2.3% after the analyses, a median age of 47 Ma is inferred. However, if the tree-age prior is modified, the median age estimates change significantly. For instance, if the mean of the gamma-distributed prior on the tree age is modified from the default of 1 to 49 (gamma (7, 7)), to reflect the posterior from the previous analyses, a median age of 93 Ma is retrieved. The median age estimate for the subfamily becomes even older if the age of the oldest available fossil is used to constrain the root, as in the node-dating and total-evidence dating analyses (i.e., using an offset-exponential prior with minimum = 52 Ma and a mean of 130 Ma): a median age of 139 Ma is then inferred.

In the main node-dating analysis, a median age of 79 Ma is inferred for the chelonines (Table 5). To examine the sensitivity to the node-age priors (Table 5), we also doubled the means of the calibration priors, while leaving their minima, which were based on fossil evidence, unchanged. If the prior for the mean age of either the root, the tribe Phanerotomini,

or both is doubled, then the estimates are much older in all analyses (84 Ma, 100 Ma, or 141 Ma, respectively, Table 5).

The total-evidence dating analyses resulted in the highest estimates of the median age of the chelonines (Table 5). If the same minimum and mean age for the root is applied, as for the previous analyses, a median age of 170 Ma is inferred. If the mean age of the root is doubled, then a median age of 206 Ma is obtained. These results differ only slightly if either 1/3 of the morphological characters are omitted (median age = 193 Ma) or *P. menieri* is excluded (163 Ma).

4. Discussion

4.1 Implications for classification

We here present the first total evidence phylogeny of Cheloninae which, although arguably limited by taxon sampling, nonetheless provides a relatively robust framework for re-evaluating the current classification of the subfamily.

Within Cheloninae, Zettel (1990) proposed four tribes (Table 1), while van Achteberg (1990) argued for just two, Chelonini and Phanerotomini; both authors treating Adeliinae as a separate subfamily. Our results support a classification recognising two tribes *sensu* van Achteberg, a monophyletic Chelonini and a monophyletic Phanerotomini, but the latter also including the adeliine genera without being attributed any higher rank.

Most chelonine genera are well diagnosed by a range of morphological characters (Zettel, 1990), and have also been retrieved as monophyletic in our analyses. However, there are a few noteworthy exceptions. The recovery of *Ascogaster* as paraphyletic in all analyses and particularly the recognition of two *Ascogaster* clades, a mostly ‘North American’ and a mostly ‘Australian’ clade, point to a possible biogeographic signal in the data, but this will require more detailed investigation. The latter is sister to *Leptodrepana*, *Austroascogaster* and *Megascogaster*, while the ‘North American’ clade is sister to all of them. The potentially paraphyletic nature of *Ascogaster* has been discussed previously based on morphological characteristics (Huddleston, 1984; Tang and Marsh, 1994; Shaw, 1997). Given the phylogenetic results here, two scenarios are possible; either the whole clade could be treated as *Ascogaster s.l.* and *Austroascogaster*, *Leptodrepana*, and *Megascogaster* synonymised under it, or the ‘North American’ clade could be treated as *Ascogaster s.str.* (in which the type species of the genus *Ascogaster instabilis* Wesmael belongs) and a new genus (which is

morphologically virtually identical to *Ascogaster*) comprising the ‘Australian’ species be erected. The genera *Leptodrepana*, *Megascogaster*, and *Austroascogaster* would, thus, not be affected in the latter scenario. However, before any nomenclatural changes to *Ascogaster* are considered, a more detailed exploration of the characters defining each group would be required, along with a more comprehensive sampling of taxa representing each group.

The finding that *Phanerotoma* is paraphyletic with respect to *Huseyinia* and *Phanaustrotoma* in all analyses is limited by the fact that both species representing these latter two genera had *EF1 α* sequences missing and incomplete *COI* and 28S sequences. Thus, this relationship needs to be treated with caution until the data set is more complete and additional taxa can be added.

As the subgenera of *Pseudophanerotoma* (*P. Pseudophanerotoma* and *P. Furcidentia*) were distantly related in all analyses, we here propose that the subgenus *Furcidentia* be raised to generic rank (*Furcidentia* stat. rev.) with the following characters to distinguish the genus from *Pseudophanerotoma* as discussed by Zettel (1990): fore wing vein 1-SR+M emanating from the parastigma, clypeus without teeth, and wings infused with white a stripe under the parastigma.

The subgenus *Microchelonus* is not monophyletic, but is nested as multiple clades within *Chelonus* in all analyses, as predicted by van Achterberg and Polaszek (1996). Hence, the subgenus *Microchelonus* (syn. nov.) is synonymised with *Chelonus* as previously suggested by Muesebeck and Walkley (1951), and all species of *Microchelonus* transferred to *Chelonus* (see Appendix 4 for treatment of homonyms). Based on our results, formal synonymy with *Chelonus* is also applied to the included subgenera of *Microchelonus* (*Baculonius* syn. nov., *Carinichelonus* syn. nov.) and *Chelonus* (*Scabrichelonus* syn. nov.). Further, there is no evidence that the other previously recognised subgenera of *Microchelonus* (*Areselonus*, *Megachelonus*, *Parachelonus*, *Rasnichelonus*, and *Stylochelonus*), and of *Chelonus* (*Cubochelonus*) have any taxonomic utility although their synonymy should be confirmed by their inclusion in an expanded phylogenetic analysis.

4.2 Divergence time estimates

4.2.1 Fixed clockrate

For comparison with the other methods, we here included a number of fixed-rate dating analyses, which clearly exposed two shortcomings of this method. First, the divergence time

estimates were highly sensitive to the prior on the tree age, with plausible modifications of the prior resulting in estimates that differed by a factor of two to three. This result is perhaps not too surprising given the large time-scale of at least tens of millions years that we are working with, and the reliance on an evolutionary-rate estimate for mitochondrial DNA. The deeper branches of the phylogeny are especially likely to have experienced a high degree of saturation in the *COI* sequences, and even though the nuclear genes help inform relative branch lengths (because we unlinked the evolutionary rates of the mitochondrial gene in our dataset) the tree-age prior has a major impact on determining the age estimates.

Second, the fixed rate for *COI* as suggested for insect taxa (Brower, 1994) might not be adequate for this group. Several studies have shown that there is a large variation among mitochondrial rates across various insect groups (Crozier et al., 1989; Johnson et al., 2003; Lin and Danforth, 2004; Thomas et al., 2006; Papadopoulou et al., 2010). Furthermore, the time scale employed when estimating evolutionary rates might have an impact on the outcome, with higher rates typically being estimated if more recent divergences are taken into account (Papadopoulou et al., 2010).

Importantly, this study adds to the accumulating evidence that fixed-rate dating analyses should be interpreted with caution. In the context of Bayesian inference, the impact of the prior on the tree age (and/or of the branch-length prior) has to be evaluated carefully, and sensitivity to deviations from the assumed evolutionary rate should be addressed. Over larger time-scales, saturation in mitochondrial genes further diminishes the power of this approach, even when modelled correctly. In the literature, fixed-rate approaches are currently mostly used to date divergences of less than a few million years; our study firmly supports the reservations against its use for estimating older nodes (Zheng et al., 2011).

4.2.2. Node dating

The precision of our node-dating approach was rather weak, as evident from the large 95% credibility intervals (Fig. 3). This might be due to the fact that we had to ignore all but one fossil. However, *P. menieri* was somewhat problematic in that its morphology did not conform to the current definition of extant members of the genus (e.g., *P. menieri* has more than 23 antennomeres) and, hence, it could not be assigned to a specific node within the Phanerotomini and had to be placed at the root of the tribe. As *P. menieri* is also the oldest fossil in the dataset, it made all other fossils redundant for our analyses. Unfortunately, such a

situation is rather common when searching the fossil record for specimens that could be used as reliable calibration points, and thus most fossil evidence never finds its way into node-dating analyses (Ronquist et al. 2012a). It has been shown that the results of a node-dating analysis generally improves with the number of fossils or calibration points (Sauquet et al., 2012). Therefore, reconsidering the placement of *P. menieri* and the other two *Phanerotoma* fossils, and assigning them to another more appropriate genus will probably allow for the other chelonine fossils to be used more effectively for this approach.

Our results show median ages between 79 to 140 Ma for the Cheloninae, depending on the prior settings on the calibration ages. The lowest median age of 79 Ma is in concordance with the results of previous studies by Murphy et al. (2008) and Whitfield (2002). Murphy et al. (2008) estimated a mean age of $85 \text{ Ma} \pm 4.31$ for Cheloninae and $103.38 \text{ Ma} \pm 4.41 \text{ Ma}$ for the microgastroid complex as a whole, using a fossil-calibrated penalised-likelihood approach. They included more than 42 fossils, 12 of which belonged to Cheloninae, and 48 sequenced specimens, again with 12 being chelonines. Whitfield (2002) estimated a mean age for the microgastroid complex of $73.7 \text{ Ma} \pm 10 \text{ Ma}$, also calibrated with fossils, albeit far fewer than the Murphy et al. study. However, the similarity of the median ages from our study with the results of these two previous studies does not necessarily mean that the estimates are accurate, but could rather be an artefact of using similar priors and the same node-dating approach.

As in the fixed-rate analyses, we observe a high sensitivity to the choice of prior distributions, in this case on the tree age and the age of the fossil-calibrated node. Once the mean of the calibration priors of the root and the Phanerotomini were modified, the divergence time of the chelonines changed drastically (Table 5). Such a pattern has been observed in previous studies (Inoue et al., 2010; Warnock et al., 2012; dos Reis et al., 2014), and is especially concerning because, although reasonable minima can often be inferred from the fossil record, there is no objective way of setting the mean of calibration distributions (Parham et al., 2012). The results of node-dating approaches thus strongly depend on the priors adopted and are only as good as the *a-priori* assumptions. In our case, we used means that corresponded to age estimates from the literature; even though this approach is used regularly in dating studies and creates the illusion of consistency with previous estimates, it should be rejected as circular. Alternative approaches that use the fossil record in a more informed way to derive age calibrations or estimates are promising in this context (Heath et

al., 2014; Zhang et al., in press), but can only be applied to groups which fossilise comparatively well.

4.2.3 Total-evidence dating

The total-evidence dating approach was the least sensitive to the tree age prior, but estimates still vary from 163 Ma to 206 Ma (Table 5). The 95% credibility interval was narrowest in this approach, but still very large. However, the median ages obtained for the chelonines are much older in the total-evidence approach compared to the other approaches, although the same root constraints were applied. It has been argued that the total-evidence dating approach is preferable from a philosophical point of view (Ronquist et al., 2012a), given that it requires fewer (arbitrary) assumptions regarding the calibration of the tree (i.e., in terms of placement of the fossils or setting of the priors). On the other hand, it makes one rather contentious assumption, which is that the data are at least compatible with a ‘morphological clock’ (Ronquist et al., 2012a; Beck and Lee, 2014). It remains to be shown how clock-like morphological data needs to be for total-evidence dating to work; in any case, our dataset seems to strongly reject this assumption. This is evident from the placement of the oldest fossil, *P. menieri*, which was not recovered as a basal taxon in the phylogeny, but rather is placed on a younger branch (Fig. 2). Furthermore, plotting the morphological branch lengths against molecular branch lengths (Fig. 4) reveals no correlation between the two. This could indicate that our dataset is not suitable for the total-evidence dating approach. Even though the impact of removing one third of the morphological data was rather small, the very low number of morphological characters (37) that we could score is certainly a large impediment. Due to the fact that most fossils have been lost and only the original descriptions of the species are available, we cannot extend the dataset without significantly increasing the amount of missing data.

4.2.3 Estimating divergence time and age of Cheloninae

The tendency for calculating older splits in calibrated trees with more recent analyses is not restricted to the microgastroid complex, but can be found widely among hymenopteran studies. For instance the most recent analyses of the whole order by Ronquist et al. (2012a) estimated an age of 309 Ma for Hymenoptera and of about 260 Ma for Apocrita (including Ichneumonoidea), based on a total-evidence dating approach. On the other hand, considerably

younger ages were inferred with a node-dating approach in a recent study by Misof et al. (2014) who estimated the age of the order at around 240 Ma and Ichneumonoidea about 140 Ma (but using unrealistically narrow node age priors, and none of the more ancient lineages of Hymenoptera). Other studies have estimated a mean age for the Hymenoptera based on node-dating (calibrating nodes of the tree based on fossils) at 220 Ma (Wiegmann et al., 2009), ‘modern Hymenoptera’ at 200 Ma, and Ichneumonoidea to 150 Ma (Grimaldi and Engel, (2005). The oldest described fossil of the order is *Triassoxyela foveolata* Rasnitsyn 1964, with an age range of 235.0 to 221.5 Ma (Rasnitsyn, 1964).

Indeed, there is a general tendency for more recent studies to estimate older ages for wasp lineages than previous attempts and this is likely due to a number of reasons (Thorne and Kishino, 2002; Ronquist et al., 2012a). Positive effects leading to older and possibly more accurate estimates might be attributed to recent studies being more comprehensive (more taxa and a greater number of markers; see Ronquist et al., 2012a), using more sophisticated analytical techniques, and including a greater array of fossils distributed more evenly across the tree (Smith et al., 2010). On the other hand, there are negative effects leading to a younger age, i.e., an incomplete fossil record, biases in the prior settings, model mis-specification, or a combination of these factors (Thorne and Kishino, 2002).

Although our study infers that the subfamily Cheloninae may be considerably older than inferred by the simplistic approaches of a fixed clockrate and node-dating, the total-evidence estimate inferred here is implausible given that it is older than all current estimates for the superfamily Ichneumonoidea. Indeed, several studies came to the conclusion that total-evidence dating can overestimate divergence times (Ericson et al., 2014; Arcila et al., 2015). Only two other braconid subfamilies have been the subject of dating studies, both using a small number of fossils for the calibration and a node-dating penalised likelihood approach. These estimated a mean age of 50 Ma for Rogadinae (36-51 Ma) and 55-59 Ma for Doryctinae (Zaldivar-Riverón et al., 2008a; Zaldivar-Riverón et al., 2008b); both are likely to be significant under-estimates. The total-evidence dating approach is still rather young and more experience with this method is needed to confirm a possible tendency of inferring ages that are too old and potential causes of this, such as a taxonomically biased sampling of morphological characters or an inadequate tree prior (Beck and Lee, 2014; Zhang et al., 2016).

5. Conclusion

This study represents the most comprehensive phylogenetic estimate of relationships for chelonine wasps. The results confirm the presence of two well-supported tribes and the monophyly of most genera. Unfortunately, some rare genera (*Phanerotomoides*, *Sculptomyriola*, *Sinadelius*, *Siniphanerotomella*, and *Wushenia*) could not be included, because specimens were unavailable or the quality or the quantity of their DNA was inadequate. Including more taxa of the rarer genera and a greater geographic spread for the larger genera will improve on our phylogenetic results by providing more robust information on their sister relationships.

Three divergence time estimates were employed, including a fixed clockrate, a node-dating, and total-evidence dating in which molecular and morphological data were combined from extant and fossil taxa. The results show a large range of median ages for chelonine wasps, depending on the analysis and the prior being used. It is likely that none of the three divergence time approaches inferred a realistic age for the chelonines. The fixed clockrate and the node-dating approaches are likely to be flawed and to underestimate ages, while our current dataset for chelonines is not suitable for the total-evidence approach. With a greater array of morphological characters and more fossil taxa, the total-evidence approach has the potential to yield realistic age estimates of the group, but only if the morphological data is sufficiently clock-like. As all three approaches have specific limitations (varying rates for each taxon in fixed clockrate, sensitivity of priors in node-dating, morphological clock requirement for total-evidence), we suggest that the interpretation of all published dating studies be carefully scrutinised. This is crucial because the majority of current studies that estimate divergence time are based on the node-dating approach (Särkinen et al., 2013; Brady et al., 2014). Despite the group being relatively well-represented in the fossil record, our comparison of approaches clearly demonstrates that dating the chelonine tree is currently not possible with reasonable confidence. Divergence dates are repeatedly proposed for groups on an even weaker basis and without careful examination of the sensitivity of the age estimates to the calibration priors.

Acknowledgements

We thank the following people for material and hospitality during visits to various institutions: Yves Braet (Université de Liège) for specimens from French Guiana and the Société Entomologique Antilles Guyane (S.E.A.G.); Mike Sharkey (University of Kentucky)

and his collaborators (Survey in Thailand was supported by National Science Foundation (NSF) grant: DEB-0542864, surveys in Central America were supported by NSF grant DEB-0640015 (Project LLAMA), surveys in Madagascar were supported by NSF grant DEB-0072713 to BL Fisher and CE Griswold and DEB-0344731 to BL Fisher and PS Ward, as well as by the Schlinger Foundation to Mike Irwin and Ev Schlinger, with Harinhala Rinha, who carried out the fieldwork and supervised the processing in Antananarivo; Simon van Noort (Iziko South African Museum); Jim Whitfield (University of Illinois); Julia Stigenberg (Naturhistoriska Riksmuseet, Sweden); Donald Quicke and Gavin Broad (Natural History Museum, UK); Kees van Achterberg (Naturalis Biodiversity Centre, Netherlands); Bob Kula (National Museum of Natural History, USA); Jenő Papp and Gellért Puskás (Magyar Természettudományi Múzeum, Hungary); Gary Taylor (University of Adelaide). We also thank Nick Murphy for the outgroup sequences, Simon Tierney for his computing knowledge, and Steve Cooper and John Jennings for their comments on early drafts of the manuscript. This project was supported financially by the Australian Biology Resources Study (grants ATC212-13 and TTC211-06), Lirabenda Endowment Fund, Sir Mark Mitchell Research Grant, a University of Adelaide PhD scholarship (Adelaide Scholarship International), to RNK, ABRS grant (RF214-16) to ADA, and a Swiss National Science Foundation grant (PA00P3_145377/1) to SK.

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Figures and Table

Table 1. Genera of chelonine wasps and their corresponding tribes according to Zettel (1990) and the former separate subfamily Adeliinae, including the distribution of each genus, number of described species and number of species used in this study. Indented generic names are those variously treated as synonyms or subgenera by various authors.

Tribe	Taxon	Described spp	Taxa incl.	Distribution
Chelonini Förster 1826	<i>Ascogaster</i> Wesmael 1835	154	14	Worldwide
	<i>Leptodrepana</i> Shaw 1983		6	
	<i>Chelonus</i> Jurine 1801	905	10	Worldwide
	<i>Microchelonus</i> Szépligeti 1908		12	
	<i>Areselonus</i> Braet 1999		-	
	<i>Baculonus</i> Braet & van Achterberg 2001		2	
	<i>Carinichelonus</i> Tobias 2000		1	
	<i>Cubochelonus</i> Baker 1926		-	
	<i>Megachelonus</i> Baker 1926		-	
	<i>Parachelonus</i> Tobias 1995		-	
	<i>Scabrichelonus</i> He, Chen & van Achterberg 1997		1	
	<i>Rasnichelonus</i> Tobias 2011		-	
	<i>Diodontogaster</i> Brues 1933	1	-	Fossil
	<i>Eobracon</i> Cockerell 1921	1	-	Fossil
	<i>Megascogaster</i> Baker 1926	1	1	Oriental
	<i>Austroascogaster</i> Kittel & Austin 2014	4	1	Australasian
	<i>Odontosphaeropyx</i> Cameron 1910	6	3	Afrotropical
	<i>Pachychelonus</i> Brues 1924			
Odonto- sphaeropygini Zettel 1990				
Pseudo- phanerotomini Zettel 1990	<i>Pseudophanerotoma</i> Zettel 1990	7	3	Nearctic
	<i>Furcidentia</i> Zettel 1990	3	3	
	<i>Dentigaster</i> Zettel 1990	7	2	Neotropical
	<i>Leptochelonus</i> Zettel 1990	1	0	Neotropical
Phanerotomini Baker 1926	<i>Huseyinia</i> Koçak & Kemal 2008	1	1	Neotropical
	<i>Phanerotoma</i> Wesmael 1838	197	14	Worldwide
	<i>Bracotritoma</i> Csiki 1909			
	<i>Phanerotomella</i> Szépligeti 1900	89	5	Old World
	<i>Phanerotomoides</i> Zettel 1990	1	-	Neotropical
	<i>Siniphanerotomella</i> He, Chen & van Achterberg 1994	2	-	Oriental
	<i>Wushenia</i> Zettel 1990	2	-	Oriental
	<i>Phanaustrotoma</i> Kittel & Austin 2014	2	1	Australasian
	<i>Adelius</i> Haliday 1837	26	1	Worldwide
Adeliini Viereck 1918	<i>Paradelius</i> de Saeger 1942	5	1	Afrotropical, Palaeartic, Nearctic, Oriental
	<i>Sinadelius</i> He & Chen 2000	2	-	Palaeartic, Oriental

<i>Sculptomyriola</i> Belokobylskij 1988	3	-	Palaeartic
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Table 2. Fossils used to calibrate the molecular clock, ordered by age of the deposits.

Taxon	Reference	Deposit	Age range	Age reference
<i>Phanerotoma menieri</i>	Belokobylskij et al., 2010	French amber	52-53 Ma	Nel et al., 1999
<i>Diodontogaster bidentata</i>	Brues, 1933	Baltic amber	40-44 Ma	Ritzkowski, 1997
<i>Ascogaster diletata</i>	Brues, 1933	Baltic amber	40-44 Ma	Ritzkowski, 1997
<i>Ascogaster gracilicornis</i>	Brues, 1933	Baltic amber	40-44 Ma	Ritzkowski, 1997
<i>Ascogaster pinicola</i>	Brues, 1933	Baltic amber	40-44 Ma	Ritzkowski, 1997
<i>Ascogaster praevolans</i>	Brues, 1933	Baltic amber	40-44 Ma	Ritzkowski, 1997
<i>Ascogaster pentagona</i>	Brues, 1933	Baltic amber	40-44 Ma	Ritzkowski, 1997
<i>Ascogaster robusta</i>	Brues, 1933	Baltic amber	40-44 Ma	Ritzkowski, 1997
<i>Ascogaster sylvestris</i>	Brues, 1933	Baltic amber	40-44 Ma	Ritzkowski, 1997
<i>Ascogaster submersa</i>	Brues, 1933	Baltic amber	40-44 Ma	Ritzkowski, 1997
<i>Phanerotoma baltica</i>	Brues, 1933	Baltic amber	40-44 Ma	Ritzkowski, 1997
<i>Phanerotoma extensa</i>	Brues, 1933	Baltic amber	40-44 Ma	Ritzkowski, 1997
<i>Chelonus depressus</i>	Brues, 1910	Florissant shale	33-34 Ma	McIntosh et al., 1992
<i>Chelonus muratus</i>	Brues, 1910	Florissant shale	33-34 Ma	McIntosh et al., 1992
<i>Chelonus solidus</i>	Brues, 1910	Florissant shale	33-34 Ma	McIntosh et al., 1992
<i>Eobracon cladurus</i>	Cockerell, 1920	White River	28-35 Ma	Emry, 1973

Table 3. List of primers used in this study.

Gene	Primer name	Sequence 5' – 3'	Reference and annealing Temp.
COI			47°C
Forward 1	CI-J-1718	GGAGGATTTGGAAATTGATTAGTTCC	(Simon et al., 1994)
Reverse 1	CI-N-2191	CCCGGTAAAATTAATAATAAACTTC	(Simon et al., 1994)
Reverse 2	CI-N-2329	ACTGTAAATATATGATGAGCTCA	(Simon et al., 1994)
Forward 2	M72 (L)	TGATTCTTCGGTCACCCAGAAAGTGTA	(Georges et al., 1998)
Reverse 3	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	(Folmer et al., 1994)
EF1αF2			50°C
Forward	ef1α 1F	AGATGGGYAARGGTCCTTCAA	(Belshaw and Quicke, 1997)
Reverse	ef1α 1R	AACATGTTGTCDCCGTGCCATCC	(Belshaw and Quicke, 1997)
28S			60 °C
Forward	28S D2F	AGAGAGAGTTCAAGAGTACGTG	(Mardulyn and Whitfield, 1999)
Reverse	28S D3R	TAGTTCACCATCTTTCGGGTCCC	(Mardulyn and Whitfield, 1999)
Forward	2379	BGGKCAGCGACGCTACTGCTTT	This study (first third)
Reverse	2380	ATVCCCCRGAATGCGAGAT	This study
Forward	2377	DRAACCCCAAAGATCGAATGAGGAG	This study (second third)
Reverse	2378	AGGRGAAGKGCRCCGCGACA	This study
Forward	2337	GTCCGTGTTTCAAGACGGGTCC	This study (last third)
Reverse	2338	TCGGCGTGCACTTCTCYYYTA	This study

Table 4. Markers, partition schemes, and substitution models according to Partitionfinder.

Marker and partitions	#bp	#var	#par	CG%	Model
COI mtDNA	444	257	224	26.3	
COI_1	148	73	63		TVM+I+ Γ

COI_2	148	46	35		TVM+I+Γ
COI_3	148	138	126		HKY+I+Γ
EF1α F2 nuclear DNA	378	148	108	41.3	
ef1α_1	126	19	8		GTR+I+Γ
ef1α_2	126	15	2		TVM+I+Γ
ef1α_3	126	114	98		TVM+I+Γ
28S nuclear DNA					
28S_123	632	469	357	40.9	GTR+I+Γ
morphology					
n/a	37	32	31	n/a	Mk + Γ
Combined morph and mol	1491				
Morphology, COI_1, COI_2, COI_3, ef1α_1, ef1α_2, ef1α_3, 28S_123					Mk + Γ, GTR+I+Γ
concatenated	1454				
COI_1, COI_2, COI_3, ef1α_1, ef1α_2, ef1α_3, 28S_123					GTR+I+Γ
Total-evidence with fossils	1491				
Morphology, COI_1&2, ef1α_1&2, ef1α_3, 28S_123					Mk + Γ, GTR+I+Γ

Table 5. Divergence-time estimates of three different dating approaches.

Method	Median	lower	upper	priors
Fixed-rate				
	47.39	29.57	73.04	Clockrate: 1 for <i>COI</i> (translating to 2.3% after the analysis)
	48.48	40.24	57.32	Clockrate: 2.3% for <i>COI</i>
	93.42	70.90	120.74	tree-age prior: gamma(1, 1) (mean = 1)
	138.74	92.05	205.51	Clockrate: of 2.3% for <i>COI</i> , tree-age prior: gamma(7, 7) (mean = 49)
				Clockrate of 2.3% for <i>COI</i> , root-age prior: offexp(52, 72)
Node-dating				Calibrations
	78.89	60.06	123.65	Root: offsetexp(72,130), Phanerotomini: offsetexp(52,72)
	100.48	59.58	202.83	Root: offsetexp (72,130), Phanerotomini: offsetexp (52,144)
	84.03	59.27	143.76	Root: offsetexp (72,260), Phanerotomini: offsetexp (52,72)
	140.82	60.16	360.77	Root: offsetexp (72,260), Phanerotomini: offsetexp (52,144)
Total-evidence				
	170.78	105.03	268.47	Root: offsetexp (72,130)
	205.72	122.59	368.06	Root: offsetexp (72,260)
	163.21	95.35	271.93	Root: offsetexp (72,130), without <i>Phanerotoma menieri</i>
	193.41	95.61	291.96	Root: offsetexp (72,130), without 1/3 of the morphological characters

Figure legends.

Figure 1. Bayesian consensus tree from the analysis of combined morphological and molecular data. The values under the nodes show the posterior probabilities from Bayesian, bootstrap of maximum likelihood, and bootstrap of parsimony analyses. A dash indicates that this clade was not present in the consensus tree. The squares depict the posterior probabilities of a Bayesian analysis of the individual partitions (morphology, *COI*, *EFLa*, and *28S*). A white square indicates that this branch was not present in the consensus tree. Lines show the position of *Phanaustrotoma* and *Huseyinia* embedded in *Phanerotoma* and *Megascogaster* in *Ascogaster*. Arrows show the placement of *Baculonus* (abbreviated BC), *Carinichelonus* (CA), and *Scabrichelonus* (SC) within *Chelonus*.

Figure 2. Placement of fossils in the Bayesian non-clock analysis of the combined morphological and molecular data. Fossils are marked as such. Numbers next to nodes show posterior probabilities.

Figure 3. Ranges of the 95% credibility intervals in the Bayesian node-dating analysis. Numbers next to nodes show posterior probabilities.

Figure 4. Correlation of morphological and molecular branch lengths. The branch lengths were calculated without the clock-assumption on the maximum *a posteriori* topology inferred in the total-evidence analysis.

Appendices

Appendix 1. Species used in this study, with internal code/voucher number, country of origin and GenBank accession numbers. Annotations: ● single sequence; ♦ (Shi et al., unpublished); ♣ (Murphy et al., 2008); ■ (Kittel and Austin, 2015); * has been included, but is too short for a Genbank accession number.

Taxon (code)	Voucher No.	Country	COI	EF1α	28S
<i>Adelius</i> sp.	RK395	France	KJ472526	-	KJ472639
<i>Ascogaster</i> sp. (AS)	RK91	South Korea	KJ472527	KJ472584	KJ472640
<i>Ascogaster</i> sp. (AS)	RK29	Australia	KJ438543■	-	KJ472641
<i>Ascogaster</i> sp. (AS)	RK36	Australia	-	KJ472585	KJ472642
<i>Ascogaster</i> sp. (AS)	RK28	Australia	KJ438541■	-	KJ472643
<i>Ascogaster</i> sp. (AS)	RK141	Australia	KJ438561■	KJ472586	KJ472644
<i>Ascogaster</i> sp. (AS)	RK167	New Caledonia	KJ472528	-	KJ472645
<i>Ascogaster</i> sp. (AS)	RK85	Australia	KJ438636■	KJ472587	KJ472646
<i>Ascogaster</i> sp. (AS)	RK88	Australia	KJ472529	KJ472588	KJ472647
<i>Ascogaster</i> sp. (AS)	RK301	Australia	KJ438554■	KJ472589	KJ472648
<i>Ascogaster</i> sp. (AS)	RK168	New Caledonia	KJ472530	-	KJ472649
<i>Ascogaster</i> sp. (AS)	RK380	Costa Rica	KJ472531	KJ472590	-
<i>Ascogaster</i> sp. (AS)	RK379	Costa Rica	KJ472532	KJ472591	KJ472650
<i>Ascogaster</i> sp. (AS)	RK385	USA	KJ472533	KJ472592	-
<i>Ascogaster</i> sp. (AS)	RK383	USA	KJ472534	KJ472593	KJ472651
<i>Austroascogaster varia</i> Kittel & Austin (AAG)	RK424	Australia	KJ472535	-	KJ472652
<i>Austroascogaster varia</i> (AAG)●	RK473	Australia	-	-	√ *
<i>Chelonus</i> sp. (CH)	RK100	Guatemala	KJ472536	KJ472594	KJ472653
<i>Chelonus</i> sp. (CH)	RK99	Honduras	KJ472537	KJ472595	KJ472654
<i>Chelonus</i> sp. (MC)	RK217	French Guiana	KJ472538	-	KJ472655
<i>Chelonus</i> sp. (MC)	RK107	Guatemala	-	KJ472596	KJ472656
<i>Chelonus</i> sp. (MC)	RK392	USA	-	KJ472597	KJ472657
<i>Chelonus</i> sp. (CH)	RK384	USA	-	√ *	KJ472658
<i>Chelonus</i> sp. (CH)	RK221	Gabon	KJ472539	-	KJ472659
<i>Chelonus</i> sp. (CH)	RK102	South Korea	KJ472540	-	KJ472660
<i>Chelonus</i> sp. (MC)	RK109	Thailand	KJ472541	-	KJ472661
<i>Chelonus</i> sp. (CH)	RK387	Republic of the Congo	KJ472542	KJ472598	KJ472662
<i>Chelonus</i> sp. (CH)	RK05	Australia	KJ472543	KJ472599	KJ472663

<i>Chelonus</i> sp. (MC)	RK66	Australia	KJ472544	KJ472600	KJ472664
<i>Chelonus</i> sp. (CH)	RK38	Australia	KJ438520■	KJ472601	KJ472665
<i>Chelonus</i> sp. (MC)	RK105	Slovakia	KJ472545	KJ472602	KJ472666
<i>Chelonus</i> sp. (MC)	RK106	Honduras	KJ472546	KJ472603	KJ472667
<i>Chelonus</i> sp. (MC)	RK142	Australia	KJ472547	KJ472604	KJ472668
<i>Chelonus</i> sp. (MC)	RK20	Australia	KJ472548	KJ472605	KJ472669
<i>Chelonus</i> sp. (MC)	RK76	Australia	KJ472549	KJ472606	KJ472670
<i>Chelonus</i> sp. (MC)	RK110	South Korea	KJ472550	-	KJ472671
<i>Chelonus</i> sp. (CH)	RK389	Costa Rica	KJ472551	KJ472607	KJ472672
<i>Chelonus</i> sp. (CH)	RK381	Costa Rica	KJ472552	-	KJ472673
<i>Chelonus</i> sp. (MC)	RK39	Australia	KJ472553	KJ472608	KJ472674
<i>Baculonus</i> sp. (BC)	RK201	French Guiana	KJ472554	-	KJ472675
<i>Baculonus</i> sp. (BC) ●	RK204	French Guiana	-	-	KJ472676
<i>Scabrichelonus</i> sp. (SC)	RK397	Vietnam	KJ472555	KJ472609	KJ472677
<i>Carinichelonus</i> sp. (CA) ●	n/a	China	-	-	AY973198♦
<i>Dentigaster</i> sp. (DG)	RK203	French Guiana	KJ472556	√ *	KJ472678
<i>Dentigaster</i> sp. (DG)	RK214	French Guiana	KJ472557	KJ472610	KJ472679
<i>Huseyinia</i> sp. (FI)	RK401	French Guiana	KJ472558	-	KJ472680
<i>Leptodrepana</i> sp. (LD)	RK96	Costa Rica	KJ472561	KJ472614	KJ472683
<i>Leptodrepana</i> sp. (LD)	RK138	USA	KJ472562	KJ472615	KJ472684
<i>Leptodrepana</i> sp. (LD) ●	RK165	Costa Rica	-	-	KJ472685
<i>Leptodrepana</i> sp. (LD)	RK391	USA	KJ472563	√ *	KJ472686
<i>Leptodrepana</i> sp. (LD)	RK393	USA	KJ472564	KJ472616	KJ472687
<i>Leptodrepana</i> sp. (LD)	RK378	Sweden	KJ472565	KJ472617	KJ472688
<i>Megascogaster</i> sp. (MA)	RK422	Indonesia	√ *	-	KJ472689
<i>Odontosphaeropyx</i> sp. (PC)	RK399	Tanzania	√ *	-	KJ472690
<i>Odontosphaeropyx leucocoxus</i> Braet (PC) ●	RK420	Madagascar	√ *	-	-
<i>Odontosphaeropyx gracilis</i> Braet (PC)	RK421	Madagascar	KJ472566	-	KJ472691
<i>Paradelius rubra</i> (PD)	RK396	USA	KJ472567	KJ472618	KJ472692
<i>Phanerotomella</i> sp. (PL)	RK131	Madagascar	KJ472568	KJ472619	KJ472693
<i>Phanerotomella</i> sp. (PL)	RK170	Madagascar	-	√ *	KJ472694
<i>Phanerotomella</i> sp. (PL)	RK133	Madagascar	KJ472569	KJ472620	KJ472695
<i>Phanerotomella variata</i> Kittel et al. (PL)	RK302	Australia	KJ472570	KJ472621	KJ472696
<i>Phanerotomella</i> sp. (PL)	RK122	Thailand	KJ472571	KJ472622	KJ472697

<i>Phanaustrotoma pallida</i> Kittel & Austin (PAT)	RK426	Australia	√ *	-	KJ472698
<i>Phanerotoma</i> sp. (PH)	RK119	Costa Rica	KJ472572	KJ472623	KJ472699
<i>Phanerotoma</i> sp. (PH)	RK124	Republic of the Congo	KJ472573	KJ472624	KJ472700
<i>Phanerotoma</i> sp. (PH)	RK136	USA	KJ472574	KJ472625	KJ472701
<i>Phanerotoma</i> sp. (PH)	RK113	Mexico	KJ472575	KJ472626	KJ472702
<i>Phanerotoma</i> sp. (PH)	RK261	Australia	KJ438631■	KJ472627	KJ472703
<i>Phanerotoma</i> sp. (PH)	RK123	South Korea	KJ472576	KJ472628	KJ472704
<i>Phanerotoma</i> sp. (PH)	RK403	South Korea	KJ472577	KJ472629	KJ472705
<i>Phanerotoma</i> sp. (PH)	RK402	South Korea	KJ472578	KJ472630	KJ472706
<i>Phanerotoma</i> sp. (PH)	RK405	South Korea	KJ472579	KJ472631	KJ472707
<i>Phanerotoma</i> sp. (PH)	RK02	Australia	KJ438586■	KJ472632	KJ472708
<i>Phanerotoma</i> sp. (PH)	RK250	Australia	KJ438601■	KJ472633	KJ472709
<i>Phanerotoma behriae</i> Zettel (PH)	RK01	Australia	KJ438611■	KJ472634	KJ472710
<i>Phanerotoma behriae</i> (PH)	RK171	Australia	KJ438616■	KJ472635	KJ472711
<i>Phanerotoma</i> sp. (PH)	RK135	USA	KJ472580	KJ472636	KJ472712
<i>Pseudophanerotoma</i> sp. (PP)	RK218	French Guiana	KJ472581	-	KJ472713
<i>Pseudophanerotoma</i> sp. (PP)	RK314	French Guiana	KJ472582	-	KJ472714
<i>Pseudophanerotoma</i> sp. (PP)	RK114	Guatemala	KJ472583	KJ472637	KJ472715
<i>Pseudophanerotoma</i> (<i>Furcidentia</i>) sp. (LC) ●	RK115	Guatemala	-	KJ472611	-
<i>Pseudophanerotoma</i> (<i>Furcidentia</i>) sp. (LC)	RK116	Guatemala	KJ472559	KJ472612	KJ472681
<i>Pseudophanerotoma</i> (<i>Furcidentia</i>) sp. (LC)	RK315	French Guiana	KJ472560	KJ472613	KJ472682
Microgastrinae (<i>Microplitis</i> sp.)	JW58	Australia	-	KJ472638	KJ472716
Ichneutinae (<i>Ichneutes</i> sp.)	M124	USA	EU106967♣	EU106996♣	EU106928♣
Euphorinae (<i>Leiophron</i> sp.)	JW56	Australia	EU106964♣	EU106993♣	EU106925♣
Miracinae (<i>Mirax</i> sp.)	JW65	Australia	EU106971♣	EU106999♣	EU106929♣

Appendix 2. List of morphological characters and their character states.

Character	Character state
1. Teeth on clypeus	1. absent, 2. one tooth present, 3. two teeth present, 4. three teeth present
2. Mandible with a transverse carina	1. absent, 2. present
3. Occipital carina touching hypostomal carina	1. absent, 2. present
4. Temple	1. swollen, 2. not swollen
5. Shape of eyes	1. oval, 2. round
6. Hairs on eyes	1. glabrous, 2. densely setose

7. Number of female antennomeres	1. 16, 2. 17-22, 3. 23, 4. 24 or more
8. Arrangement of ocelli	1. equilateral, 2. isosceles 'on line', where an imaginary line between anterior margin of posterior ocelli is sometimes touching anterior ocellus, 3. isosceles 'not on line', where and imaginary line between anterior margin of posterior ocelli not touching anterior ocellus
9. Occipital carina	1. complete, 2. reduced/dorsally absent
10. Notauli	1. absent, 2. present
11. Precoxal sulcus	1. absent, 2. present
12. Blister on mesotibia	1. absent, 2. present
13. Teeth on claws	1. absent, 2. present
14. Prepectal carina	1. absent; 2. completely present, 3. partly present
15. Postpectal carina	1. absent, 2. short ventral part present, 3. complete
16. Vein r-m of fore wing	1. absent, 2. sclerotised, 3. unsclerotised
17. r in fore wing	1. absent, 2. present
18. 1-SR+M	1. absent, 2. present, from parastigma, 3. present, from 1-M
19. 2-SR+M	1. antefurcal, 2. interstitial, 3. postfurcal
20. 1-cu1	1. antefurcal, 2. interstitial, 3. postfurcal
21. CU1b	1. absent (subdiscal cell open), 2. present (subdiscal cell closed)
22. SR-1 (+3-SR)	1. complete (sclerotised), 2. incomplete (not sclerotised)
23. (SR1+3-SR)	1. absent, 2. present as SR1+3-SR (interstitial), 3. two present as SR1 and 3-SR (antefurcal), 4. two present as SR1 and 3-SR (postfurcal)
24. R	1. extending to apex of wing, 2. not extending to apex of wing
25. 2-R1	1. absent, 2. present
26. Second cubital cell	1. at least half the length of first, 2. reduced or much shorter than half of first cell, 3. about half as long as first
27. Ratio of pterostigma to 1-R1:	1. same length, 2. much longer, 3. shorter
28. r in hind wing	1. absent, 2. present
29. Carapace (fused metasomal tergites 1-3)	1. absent, 2. present, 3. partly fused
30. Sutures on metasoma	1. absent, 2. present
31. Posterior pit in males	1. absent, 2. present
32. Tip of carapace	1. round, 2. with one central spike, 3. with lobes, 4. with two or four lateral teeth, 5. membranous
33. Shape of metasoma in lateral view	1. posteriorly bent under, 2. flat, 3. broad
34. Shape of metasoma in dorsal view	1. round, 2. elongate/ parallel-sided, 3. oval, 4. with petiole
35. Pairs of abdominal spiracles	1. 4 pairs, 2. 5 pairs, 3. 6 pairs, 4. 7 or more pairs
36. Metasoma is attached to the Mesosoma by	1. a sclerotised median plate, 2. a membranous part of tergum
37. Spiracles of first metasomal tergite	1. in notum, 2. in epipleuron

Appendix 3. List of coded character states per species used in the morphological and total-evidence analyses, in alphabetical order by genera.

Species name	Morphological codings
	00000 00001 11111 11112 22222 22223 33333 33 12345 67890 12345 67890 12345 67890 12345 67
<i>Adelius</i>	
AD_RK395	11221 2?311 21111 11232 12121 22121 15232 11

<i>Ascogaster</i>	
AS_SK_RK91	21221 1?212 21123 32313 21321 21121 11332 11
AS_AUS_RK29	11221 14312 21123 32223 21321 22121 11332 11
AS_AUS_RK36	11221 12312 21123 32213 21321 2?121 11332 11
AS_AUS_RK28	11221 14311 21123 322?3 21322 21121 11332 11
AS_AUS_RK141	11221 1?312 21123 32213 21321 21121 11332 11
AS_NC_RK167	11221 1?112 21123 32223 21321 22121 11332 11
AS_AUS_RK85	11121 14312 21123 32213 21321 22121 11332 11
AS_sp_RK88	11?21 1?212 ?1123 32313 21322 21121 12132 11
AS_AUS_RK301	31121 14312 11123 32223 21321 22121 11332 11
AS_NC_RK168	11?21 1?312 21123 32213 22321 23121 11332 11
AS_CR_RK380	11221 1?312 21123 32313 21321 22121 11332 11
AS_CR_RK379	11?21 1?212 21123 32333 21321 21121 11332 11
AS_USA_RK385	11221 1?212 11123 32333 21321 22121 11132 11
AS_USA_RK383	11221 14212 11123 32333 21321 21121 11132 11
<i>Ascogaster_diletata_fossil</i>	???21 1421? ?1123 32??3 2132? 2?121 11?32 11
<i>Ascogaster_gracilicornis_fossil</i>	???21 1?212 ?1123 32??3 2132? 2?121 11?32 11
<i>Ascogaster_pinicola_fossil</i>	???21 12212 ?1123 32??3 2132? 2?121 11?32 11
<i>Ascogaster_praevolans_fossil</i>	???21 14212 ?1123 32??3 2132? 2?121 11?32 11
<i>Ascogaster_pentagona_fossil</i>	???21 14212 ?1123 32??3 2132? 2?121 11?32 11
<i>Ascogaster_robusta_fossil</i>	???21 14212 ?1123 32333 2131? 2?121 11?32 11
<i>Ascogaster_sylvetris_fossil</i>	???21 14212 ?1123 32??3 2132? 2?121 11?32 11
<i>Ascogaster_submersa_fossil</i>	???21 14212 ?1123 32??3 2132? 2?121 11?32 11
<i>Austroascogaster</i>	
AAG_AUS_RK424	11221 14321 21123 32233 21321 21121 11332 11
AAG_AUS_RK473	11221 14321 21123 32233 21321 21121 11332 11
<i>Chelonus</i>	
CH_GUA_RK100	11121 2?312 11123 321?3 21322 21121 ?1332 11
CH_HON_RK99	11121 2?212 11123 321?3 21322 21121 ?1132 11
MC_FRG_RK217	11?21 2?312 21123 321?3 21321 22121 21332 11
MC_GUA_RK107	11121 21312 11123 321?3 21322 22121 ?1332 11
MC_USA_RK392	11?21 2?312 21123 321?3 21321 22121 ?1332 11
CH_USA_RK384	11121 2?312 21123 321?3 21321 22121 ?1132 11
CH_GAB_RK221	11121 213?2 11123 321?3 21321 21121 ?1132 11
CH_SK_RK102	11121 2?3?2 21123 321?3 21322 21121 ?1132 11
MC_THA_RK109	11?21 ?1312 11123 321?3 21321 21121 ?4332 11
CH_ROC_RK387	11?21 2?212 11123 321?3 2132? 2?121 ?1332 11
CH_AUS_RK05	11?21 ??312 21123 321?3 21322 22121 11132 11
MC_sp_RK66	11?21 21311 ?1123 321?3 21321 22121 2?332 11
CH_sp_RK38	11?21 2?312 ?1123 321?3 21322 22121 ?1132 11
MC_SLO_RK105	11?21 2?312 21123 321?3 21322 22121 2?132 11
MC_HON_RK106	11?21 21212 21123 321?3 21322 22121 ?1332 11
MC_AUS_RK142	11121 ??312 11123 321?3 21322 22121 21132 11
MC_AUS_RK20	11121 11312 11123 321?3 21322 22121 ?1332 11
CH_AUS_RK76	11121 ?1312 11123 321?3 21322 22121 ????2 11
MC_SK_RK110	11?21 21312 21123 321?3 2?322 22121 ?1332 11
CH_CR_RK389	11?21 2?312 21123 321?3 21321 22121 ?1332 11
MC_CR_RK381	11121 21312 21123 321?3 21321 22121 ?1132 11
CH_AUS_RK39	11?21 14312 11123 321?3 21321 22121 ?1132 11
BC_FRG_RK201	11?21 21312 21123 321?3 21321 22121 ?1332 11
BC_sp_RK204	11121 21311 21123 321?3 21322 21121 ?1332 11
SC_sp_RK397	11?11 2?312 11123 321?3 21322 22121 ?1322 11
<i>Chelonus_depressus_fossil</i>	1???1 ???1? ?1123 321?3 2132? 2?121 ?1?32 11
<i>Chelonus_muratus_fossil</i>	1???1 ???1? ?1123 321?3 2132? 2?121 ?1?32 11
<i>Chelonus_solidus_fossil</i>	1???1 ???1? ?1123 321?3 2132? 2?121 ?1?32 11

<i>Dentigaster</i>	
DG_sp_RK203	31?21 1?112 11123 32233 21322 31122 14332 11
DG_FRG_RK214	?1??? ???? ?123 32??3 2?3?? ?122 1???2 11
<i>Diodontogaster</i>	
<i>Diodontogaster_bidentata_fossil</i>	11?21 14??2 ?1123 32333 21311 22121 14332 11
<i>Eobracon</i>	
<i>Eobracon_cladurus_fossil</i>	?1?2? ???? ?1123 32213 21311 23?21 11?32 11
<i>Fischeriella</i>	
FI_sp_RK401	31?22 1?112 12123 32223 21312 23122 15232 11
<i>Furcidentia</i>	
LC_GUA_RK115	11222 1?1?2 22123 32233 21311 23122 13232 11
LC_sp_RK116	11222 1?112 22123 32333 21311 21122 13232 11
LC_FRG_RK315	11222 14112 22123 32232 21311 23122 13232 11
<i>Leptodrepana</i>	
LD_CR_RK96	11221 12112 11123 32313 21321 22121 14332 11
LD_USA_RK138	11?21 121?2 11123 32323 21321 22121 14332 11
LD_CR_RK165	11221 12122 21123 32213 21321 22121 12332 11
LD_USA_RK391	11221 1?122 21123 32323 21321 22121 11332 11
LD_USA_RK393	11?21 1?122 21123 32323 21321 21121 11322 11
LD_SWE_RK378	11221 1?312 21123 323?3 21321 2?121 11322 11
<i>Megascogaster</i>	
MA_IND_RK422	31221 1?322 21123 32213 21322 32121 12322 11
<i>Pachychelonus</i>	
PC_sp_RK399	21221 14112 11223 32313 21321 33222 11132 11
PC_leucocoxus_RK420	21221 14112 21223 32313 21322 31222 11322 11
PC_gracilis_RK421	21?22 14112 21223 32313 21321 31222 11322 11
<i>Paradelius</i>	
PA_RK396	11221 2?311 21111 11332 12121 22122 15232 11
<i>Phanerotomella</i>	
PL_MAD_RK131	11222 14111 11123 32233 11422 23122 13232 11
PL_MAD_RK170	11?22 14111 11123 32233 11422 23122 13232 11
PL_MAD_RK133	11222 14112 11123 32233 11422 22122 1???2 11
PL_variata_sp_nov_RK302	11?22 13111 ?1123 32233 11222 21122 13232 11
PL_THA_RK122	11222 14111 11123 32233 11422 21122 13232 11
<i>Phanaustrotoma</i>	
PAT_AUS_RK426	31222 13112 11123 32233 11311 23122 11222 11
<i>Phanerotoma</i>	
PH_CR_RK119	31222 13112 12123 32223 21311 23122 14232 11
PH_ROC_RK124	41?22 13112 22123 32223 21311 23122 11232 11
PH_sp_RK136	41?22 13121 ?2123 32223 21311 23122 11232 11
PH_MEX_RK113	31?22 13112 22123 32233 21312 21122 13232 11
PH_AUS_RK261	31222 13112 22123 32213 21311 23122 13232 11
PH_SK_RK123	31222 13112 22123 32223 21312 2?122 14232 11
PH_SK_RK403	41222 13112 12123 32223 21311 23122 13232 11
PH_SK_RK402	41?22 13112 22123 32223 21311 21122 11232 11
PH_SK_RK405	31?22 13112 11123 32223 21311 23122 11232 11
PH_AUS_RK02	31?22 13112 22123 32223 21311 23122 14232 11
PH_AUS_RK250	31222 13112 22123 32223 21311 23122 14232 11
PH_sp_RK01	41?22 13111 ?2123 32223 21311 23122 13232 11
PH_AUS_RK171	41222 13111 22123 32223 21311 23122 13232 11
PH_USA_RK135	41222 13112 22123 32223 21311 23122 11232 11
<i>Phanerotoma_baltica_fossil</i>	???21 ?4?12 ?1123 32?33 ?13?? ?122 1??32 11
<i>Phanerotoma_extensa_fossil</i>	???21 14?12 ?1123 32?33 ?13?? ?122 1??32 11
<i>Phanerotoma_menieri_fossil</i>	3?222 14?12 1?123 32231 21311 ?122 1??32 11
<i>Pseudophanerotoma</i>	

PP_FRG_RK218	31122 14112 22123 32333 21311 13122 11232 11
PP_FRG_RK314	31122 1?112 22123 32333 21311 13122 11232 11
PP_sp_RK114	11?22 1?112 ?1123 32333 21311 33122 11232 11
Outgroups	
Euphorinae_JW56 (<i>Leiophron</i> sp.)	1??22 1?112 ?1111 ?232? 11222 21131 11344 2?
Ichneutinae_M124 (<i>Ichneutes</i> sp.)	1??22 1?122 ?1111 ?121? 21322 21111 11234 2?
Miracinae_JW65 (<i>Mirax</i> sp.)	1??22 1?111 ?1111 ?231? 21221 22111 11332 22
Microgastrinae (<i>Microplitis</i> sp.)	???? ???? ???? ???? ???? ???? ???? ??

Appendix 4. Replacement names for homonyms and secondary homonyms.

Senior homonym	Junior homonym	Replacement name for junior homonym
<i>Chelonus</i> (<i>Chelonus</i>) <i>angustiventris</i> Tobias,1974	<i>Chelonus</i> (<i>Microchelonus</i>) <i>angustiventris</i> Tobias,1986	<i>Chelonus fionae</i> nom. nov. Kittel
<i>Chelonus</i> (<i>Microchelonus</i>) <i>apicalis</i> Papp,1971	<i>Chelonus</i> (<i>Chelonus</i>) <i>apicalis</i> Ji & Chen,2003	<i>Chelonus yani</i> nom. nov. Kittel
<i>Chelonus</i> (<i>Chelonus</i>) <i>bicolorus</i> Chen & Ji,2003	<i>Chelonus</i> (<i>Chelonus</i>) <i>bicolorus</i> He,2003	<i>Chelonus billyi</i> nom. nov. Kittel
<i>Chelonus</i> (<i>Microchelonus</i>) <i>brevicornis</i> McComb,1968	<i>Chelonus</i> (<i>Microchelonus</i>) <i>brevicornis</i> Tobias,1989	<i>Chelonus spikei</i> nom. nov. Kittel
<i>Chelonus</i> (<i>Microchelonus</i>) <i>depressus</i> Thomson,1874	<i>Chelonus</i> (<i>Chelonus</i>) <i>depressus</i> Brues,1910	<i>Chelonus williami</i> nom. nov. Kittel
<i>Chelonus</i> (<i>Chelonus</i>) <i>elongatus</i> Szépligeti,1898	<i>Chelonus</i> (<i>Stylochelonus</i>) <i>elongatus</i> Papp,1971	<i>Chelonus ragnhildae</i> nom. nov. Kittel
<i>Chelonus</i> (<i>Chelonus</i>) <i>fischeri</i> Huddleston & Walker,1994 *	<i>Chelonus</i> (<i>Microchelonus</i>) <i>fischeri</i> Tobias,1994 *	<i>Chelonus larsi</i> nom. nov. Kittel
<i>Chelonus</i> (<i>Chelonus</i>) <i>immaculatus</i> Cameron,1905	<i>Chelonus</i> (<i>Chelonus</i>) <i>immaculatus</i> He,1994	<i>Chelonus arthuri</i> nom. nov. Kittel
<i>Chelonus</i> (<i>Chelonus</i>) <i>iranicus</i> Tobias,1972	<i>Chelonus</i> (<i>Microchelonus</i>) <i>iranicus</i> Tobias,2001	<i>Chelonus robi</i> nom. nov. Kittel
<i>Chelonus</i> (<i>Microchelonus</i>) <i>kaszabi</i> Tobias,1989	<i>Chelonus</i> (<i>Chelonus</i>) <i>kaszabi</i> Papp,1992	<i>Chelonus wullii</i> nom. nov. Kittel
<i>Chelonus</i> (<i>Chelonus</i>) <i>mediterraneus</i> Schmiedeknecht & Fahringer,1934	<i>Chelonus</i> (<i>Microchelonus</i>) <i>mediterraneus</i> Tobias,2008	<i>Chelonus hamishi</i> nom. nov. Kittel
<i>Chelonus</i> (<i>Microchelonus</i>) <i>minutissima</i> Tobias,1964	<i>Chelonus</i> (<i>Areselonus</i>) <i>minutissima</i> Braet,1999	<i>Chelonus pauli</i> nom. nov. Kittel
<i>Chelonus</i> (<i>Microchelonus</i>) <i>nigripes</i> Rao & Chalikwar,1971	<i>Chelonus</i> (<i>Microchelonus</i>) <i>nigripes</i> Tobias,1996	<i>Chelonus jeannieae</i> nom. nov. Kittel

<i>Chelonus (Chelonus)</i> <i>scrobiculatus</i> Szépligeti, 1900	<i>Chelonus (Microchelonus)</i> <i>scrobiculatus</i> Tobias, 1986	<i>Chelonus aggieae</i> nom. nov. Kittel
<i>Chelonus (Microchelonus)</i> <i>ubsunuricus</i> Tobias, 1996	<i>Chelonus (Chelonus)</i> <i>ubsunuricus</i> Tobias, 2011	<i>Chelonus jocki</i> nom. nov. Kittel
<i>Chelonus (Megachelonus)</i> <i>uniformis</i> Baker, 1926	<i>Chelonus (Microchelonus)</i> <i>uniformis</i> Tobias, 1994	<i>Chelonus tamkeae</i> nom. nov. Kittel

* Huddleston & Walker (1994) is treated as the senior homonym (see article 24.2 of the ICZN code).

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comparison of molecular versus morphological branch lengths