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A molecular palaeobiological hypothesis for the origin of aplacophoran molluscs and their derivation from chiton-like ancestors

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Aplacophorans have long been argued to be basal molluscs. We present a molecular phylogeny, including the aplacophorans Neomeniomorpha (Solenogastres) and Chaetodermomorpha (Caudofoveata), which recovered instead the clade Aculifera (Aplacophora + Polyplacophora). Our relaxed Bayesian molecular clock estimates an Early Ordovician appearance of the aculiferan crown group consistent with the presence of chiton-like molluscs with seven or eight dorsal shell plates by the Late Cambrian (approx. 501–490 Ma). Molecular, embryological and palaeontological data indicate that aplacophorans, as well as chitons, evolved from a paraphyletic assemblage of chiton-like ancestors. The recovery of cephalopods as a sister group to aculiferans suggests that the plesiomorphic condition in molluscs might be a morphology similar to that found in monoplacophorans.

Keywords: Polyplacophora; Aculifera; Mollusca; palaeoloricates

1. INTRODUCTION

Molluscs are among the most familiar invertebrates. The well-known shelled taxa, clams, snails and squids, together with some rarer forms, are collectively referred to as conchiferans (Bivalvia, Gastropoda, Cephalopoda, as well as Monoplacophora and Scaphopoda). Our focus here, however, is on a small group of worm-like molluscs known as aplacophorans, which are characterized by a vestigial or completely reduced foot. The mantle (girdle) of aplacophorans is covered by a dense coat of sclerites (often referred to as spicules) [1,2]. There are two distinct groups: the chaetodermomorphs (also called caudofoveates) and neomeniomorphs (solenogastres; figure 1*a*,*b*). The chaetodermomorphs are infaunal selective detrital feeders, whereas most neomeniomorphs feed on cnidarian colonies [1,2]. Aplacophorans share a number of characters such as the vermiform shape, dorsoterminal sense organ, distichous radula, specialized reproductive system and small posterior mantle cavity that indicate their monophyly [4]. Aplacophorans also share many similarities with chitons (Polyplacophora; figure 1*c*,*d*), such as the presence of aragonitic sclerites and papillae in the mantle, and a suprarectal commissure [4].

There are two main hypotheses for the systematic position of aplacophorans. The first, the Testaria hypothesis, posits that aplacophorans are the most proximal outgroup(s) to all other molluscs (Polyplacophora + Conchifera = Testaria) [5–7]. The testarian molluscs share a number of characters absent in aplacophorans, such as a broad ciliary creeping sole, distinctly compartmentalized alimentary tract, radula morphology, tetraneurous nervous system, and aorta. This hypothesis would imply that total-group Aplacophora appeared no later than the Early Cambrian because crown-group Conchifera such as stem bivalves (*Pojetaia runnegari*) [8] and stem gastropods (*Aldanella* and *Pelagiella*) were present by this time [9].

The second, the Aculifera hypothesis, posits that the vermiform morphology of aplacophorans is secondarily modified from a more typical molluscan ancestor, such as a chiton-like form with serial rows of shell plates and a mantle with sclerites [4,10–12]. Embryological evidence for the monophyletic Aculifera hypothesis includes the presence of seven repeated regions devoid of sclerites in a neomeniomorph post-larva [13] (figure 1*f*), and seven dorsal rows of calcium carbonate-secreting cells in a chaetodermomorph larva [3] (figure 1*e*; but see also [14]). These larval forms express a potentially primitive morphology akin to chitons.

This embryological evidence is echoed in fossil forms. The Silurian (Wenlock) aplacophoran-like mollusc *Acaenoplax hayae* [15] (figure 2*c*) from the Herefordshire Lagerstätte possessed seven dorsal shell plates, the last (together with its terminal ventral shell plate) enclosing a possible posterior mantle cavity with gills [15,17] (see alternative interpretation in [18]). Chiton-like fossils with eight plates from the Cambrian and Ordovician, collectively referred to as palaeoloricates [19], typically have extensive tunnels or lacunae inside their shell plates (figure 2*d*–*f*). The presence of these lacunae, which have been homologized to the subapical cavities

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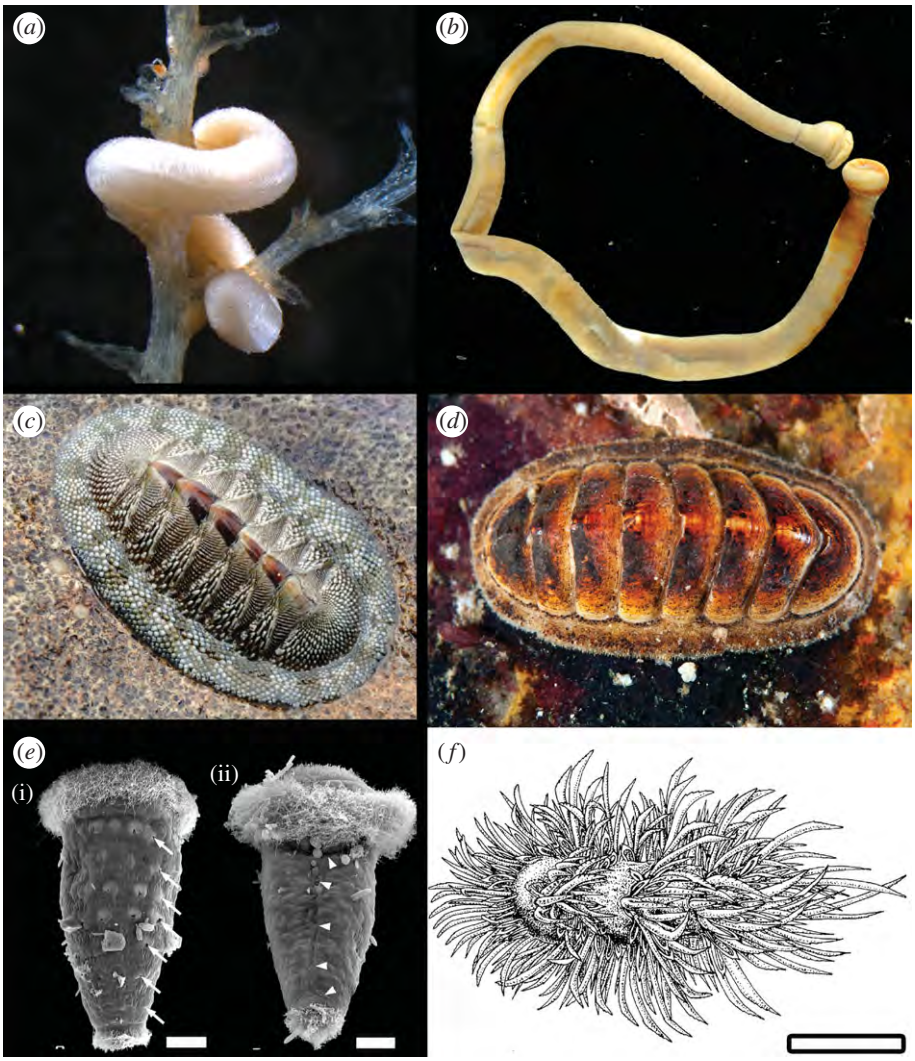

Figure 1. Modern aculiferan diversity. (*a*) An unknown species of aplacophoran neomeniomorph, Antarctica. Yale Peabody Museum of Natural History no. 51789. (*b*) The aplacophoran chaetodermomorph *Chaetoderma intermedium*, Greenland. Zoological Museum of Copenhagen, Denmark, ZMUC-APL-10. (*c*) The chiton *Chiton tuberculatus*, photographed in the intertidal zone, Barbados. (*d*) The chiton *Leptochiton asellus*, underwater photograph, Norway (www.uwphoto.no; copyright © Erling Svensen). (*e*) Late trochophore larva of *Chaetoderma nitidulum* showing seven conspicuous dorsal ridges (i) with calcium carbonate-secreting cells and (ii) the secondarily reduced ventral foot region, from Nielsen *et al.* [3]. Scale bars, 20 μm. (*f*) Neomeniomorph postlarva with seven naked dorsal regions and sclerites arranged in transverse rows and in lateral zones [4]. Scale bar, 0.1 mm.

in the shell plates of forms related to *Acaenoplax* [20,21], is suggestive of a relationship between palaeoloricates and aplacophorans, thus supporting the hypothesis (based on a cladistic analysis [19,22]) that palaeoloricates are a paraphyletic suite of stem-aculiferans as well as stem chitons and aplacophorans [19,22]. According to this interpretation, the possession of a foot and dorsal shell plates, which were subsequently reduced, is primitive for Aplacophora. An Upper Ordovician form (*Helminthochiton thraivensis*) with eight overlapping plates [23] and a mantle that extends ventrally (figure 2*a*), leaving little room for an elaborate foot, pushes the minimum age for an aculiferan divergence back to the Late Ordovician (earlier than 443 Ma) [19]. *Helminthochiton thraivensis* occurs at the same locality as *Septemchiton grayiae* (figure 2*b*), another possible stem-group aplacophoran [19], which has a similar laterally compressed morphology that may indicate a reduced foot. Since the ancestral aculiferan would have been a chiton-like form, a possible age for the origin of the aculiferan crown group, given this scenario, is provided by Late Cambrian (501–490 Ma) forms with serial shell plates, such as *Matthevia* (figure 2*f*) [24,25]. Thus, the fossil record suggests that aplacophorans could have evolved from a chiton-like ancestor. This result, paraphyly of polyplacophorans with respect to aplacophorans, has been obtained in some cladistic analyses [19,22], while others have found all fossil and recent polyplacophorans to be monophyletic [26].

Molecular phylogenetics provides an independent test of morphological and palaeontological hypotheses, but studies to date have not resolved the relationships at the base of the molluscan tree. Most early studies provided support for neither the Testaria nor Aculifera hypothesis, but instead found aplacophorans and chitons to be unrelated and nested within different conchiferan sub-groups [27–29]. Later studies, however, found a relationship between aplacophorans, chitons and cephalopods, but until recently this has been largely unresolved [30–32].

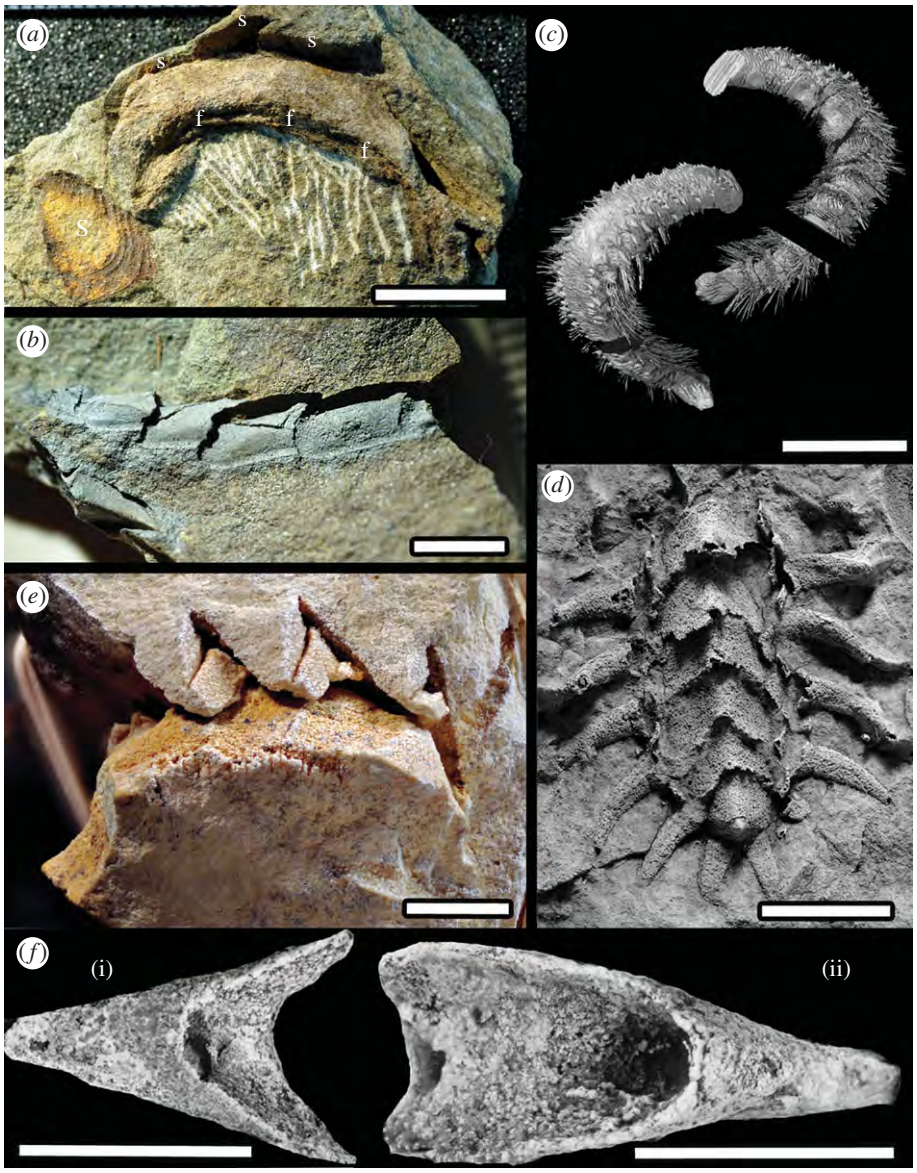

Figure 2. Fossil polyplacophorans and stem group aplacophorans. (*a*) *'Helminthochiton' thraivensis* NHMUK PI G.47253 with a reduced foot region (f) and articulated shell plates (s). (*b*) Anterior region of *Septemchiton*, Natural History Museum, London, UK, NHMUK PI G 47241. (*c*) *Acaenoplax hayae* [15]; reproduced with permission from *Nature* magazine. (*d*) *Echinochiton dufoei* [16], an articulated palaeoloricate, Burpee Museum of Natural History, Rockford, IL, BMNH 1996.045.01. (*e*) Lateral aspect of *E. dufoei*, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA, USNM 517481, showing the extensive lacunae and dorsally projecting conical shell plates characteristic of most paleoloricates, including *Matthevia*. (*f*) Isolated shell plates of *Matthevia*, a Late Cambrian stem-aculiferan, Natural History Museum of Los Angeles County, CA, USA: (i) a presumed intermediate valve, LACMIP 12821, and (ii) a tail valve, LACMIP 13038; photos by M. Vendrasco. (*a*) Scale bar, 1 cm; (*b–e*) scale bar, 5 mm; (*f*) scale bars, 10 mm.

Morphological cladistic analyses have obtained results supporting one or the other hypothesis depending on the coding of characters. Molecular analyses so far have not been able to confirm either of the morphological hypotheses. In order to assess the relative merits of the different morphological hypotheses for the position of aplacophorans, we sequenced seven nuclear housekeeping gene mRNAs [33] from all major classes of molluscs (except monoplacophorans). These data were combined with a representative set of lophotrochozoan and ecdysozoan taxa as outgroups and analysed in a Bayesian phylogenetic framework. We performed two different tests (long branch taxon exclusion and phylogenetic signal dissection) for the robustness of the observed results. Finally, we estimated the origin of chitons and aplacophorans with a Bayesian relaxed molecular clock using calibrations on divergences other than the aplacophorans and polyplacophorans. These results are discussed in the light of previously published morphological cladistic analyses of fossil and recent chitons and aplacophorans.

2. MATERIAL AND METHODS

(a) *Molecular sequences*

We sequenced the seven nuclear housekeeping genes (elongation factor 1α, aldolase, catalase, methionine adenosyltransferase, ATP synthase β chain, triosephosphate isomerase and phosphofructokinase) from a representative set of molluscs following the protocol of Sperling *et al.* [34]. We sequenced the neomeniomorph aplacophoran *Genitoconia rosea* (Norway); the chaetodermomorph *Chaetoderma* cf. *nitidulum* (Kristineberg, Sweden); the polyplacophorans

Leptochiton asellus (Kristineberg, Sweden), *Chaetopleura apiculata* (Gulf Specimen, Panacea, FL, USA), *Boreochiton ruber* (Gulf of Maine Biological Supply, Pembroke, ME, USA) and *Tonicella lineata* (San Juan Island, WA, USA, collected by J.V.); the scaphopod *Antalis entalis* (Skagerak, Sweden); the gastropods *Strombus alatus* (Gulf Specimen) and *Coryphella* sp. (Marine Biological Laboratory, Woods Hole, MA, USA); and the bivalve *Geukensia demissus* (Marine Biological Laboratory). The cephalopods *Nautilus pompilius*, *Euprymna scolopes*, *Enteroctopus dofleini* and *Octopus bimaculoides* have recently been sequenced and published [35]. These were combined with data from previously published taxa for which at least five of the seven genes were available from either genome or expressed sequence tag (EST) projects on public databases. Sequences have been submitted to GenBank with the accession numbers JN671448–JN671518.

(b) *Phylogenetic analysis*

The amino acid sequences for the seven sequenced genes were aligned manually (alignment available at http://purl.org/phylo/treebase/phylows/study/TB2:S11512) and analysed (2026 characters, with 18.7% of the total cells unfilled owing to missing data or indels) using the Bayesian phylogenetic software PHYLOBAYES v. 3.2f [36] and the molecular substitution models $\mathrm{CAT}+\mathrm{GTR}+\Gamma^4$ and $\mathrm{WAG}+\Gamma^4$. Cross-validation analysis shows that $\mathrm{CAT}+\mathrm{GTR}+\Gamma^4$ is the best overall fitting model for the data (electronic supplementary material, table S1), while $\mathrm{WAG}+\Gamma^4$ is an empirical model commonly applied to amino acid datasets. Markov chain Monte Carlo sampling was carried out until convergence, as determined by comparing the posterior distribution of two independent runs with a suitable discard of the initial sample of trees (burn-in typically between 1000 and 3000 trees of each cycle) and subsampling of every 10th tree. The two independent runs were terminated when the maximum difference between the posterior distributions was around or less than 0.1, as advised in the PHYLOBAYES manual, and summarized as a 50 per cent majority rule consensus tree (figure 3). The posterior probability (PP) of each node is shown.

(c) *Sensitivity tests: phylogenetic signal dissection*

In order to detect systematic errors owing to potentially high signal-to-noise ratios, we performed a so-called slow–fast analysis [37,38], following the procedure outlined by Sperling *et al.* [38]. This method ranks each individual site in the alignment based on their relative rate of substitution, and then divides the sites into two datasets based on those rates. The first dataset includes the fastest-evolving quartile and the invariant sites (1283 characters), while the second contains the three slowest-evolving quartiles, but no invariant sites (772 characters). The phylogenetic trees are shown in electronic supplementary material, figures S2 and S3.

(d) *Sensitivity tests: exclusion of long-branched, unstable and compositionally heterogeneous taxa*

High rates of molecular evolution can lead to increased occurrence of convergent genetic identities and cause unrelated fast-evolving organisms to be grouped together erroneously in molecular phylogenetic analyses, a phenomenon known as long-branch attraction (LBA) [39,40]. Some of the long-branched clades most relevant to the relationships considered here are the aplacophorans and cephalopods. Although the scaphopod does not appear long-branched, its position is unstable and has low support, possibly because just a few genes were sampled from a single taxon to represent the whole molluscan class. In order to test the potential systematic bias of LBA [39], the long-branched and unstable clades were excluded to observe the effect on the topology. First, we removed the cephalopods and scaphopod (electronic supplementary material, figure S4*a*). Subsequently, we excluded the aplacophorans from the total dataset to test whether the long-branched cephalopods would still resolve as a sister group to the short-branched polyplacophorans (electronic supplementary material, figure S4*b*).

We also performed a posterior predictive test with the $\mathrm{CAT}+\mathrm{GTR}+\Gamma^4$ model (as directed in the PHYLOBAYES manual [36]) to generate a matrix with taxa excluded that were estimated to have a significantly heterogeneous amino acid composition. This matrix was then analysed as in §2*b*.

(e) *Molecular clock analysis*

Relaxed molecular clock analyses were conducted to estimate the divergence of the Aculifera. The analyses were conducted in PHYLOBAYES v. 3.2f with different sets of parameters as tests of the sensitivity of the divergence estimates (tables S2 and S3 in the electronic supplementary material). These sensitivity tests included (i) estimation of branch lengths using $\mathrm{CAT}+\mathrm{GTR}+\Gamma^4$ and $\mathrm{WAG}+\Gamma^4$ substitution models, (ii) molecular divergence analyses using both the autocorrelated CIR clock relaxation model [41] and the uncorrelated white noise model, (iii) the inclusion versus exclusion of the long-branched cephalopods, and (iv) the effect of divergence estimates when aplacophorans are considered paraphyletic in Aculifera. In all analyses, a birth–death prior on divergence times and a prior age for the root (the divergence between ecdysozoans and lophotrochozoans) were specified to be 600 ± 100 Ma. The chosen root prior is based on the observed presence of both ecdysozoans and lophotrochozoans (protostomes) at the base of the Cambrian indicating an older divergence (earlier than 542 Ma) of this group. Previous molecular clock studies also recovered a divergence of the protostomes at this time [33,42]. The analyses were run initially under the prior in order to confirm that the distribution of ages was sufficiently uninformative for posterior sampling.

The 11 fossil calibrations came from the well-studied fossil records of bivalves, gastropods, brachiopods and arthropods. More controversial fossils discussed in this paper, such as putative early aplacophoran or polyplacophoran representatives, were not used for calibration. The calibration points were adapted from previous molecular clock studies [42], but with some modifications (see electronic supplementary material, table S4 for the full list of calibration points used). Soft bounds were used on all fossil calibrations as permuted by Yang & Rannala [43], with default relaxation (0.05%) as implemented in PHYLOBAYES v. 3.2f. The bivalve–gastropod divergence was assigned a minimum age of 530 Ma based on a time-calibrated correlation [44]. This divergence marks the fossil appearance of the earliest stem-group gastropods, Pelagiellida (i.e. *Pelagiella* and *Aldanella*) [9]. The maximum age of the bivalve–gastropod divergence at 543 Ma was based on a 95% confidence interval of the first appearance of Pelagiellida, estimated using Marshall's [45] method, calculated with the online fossil

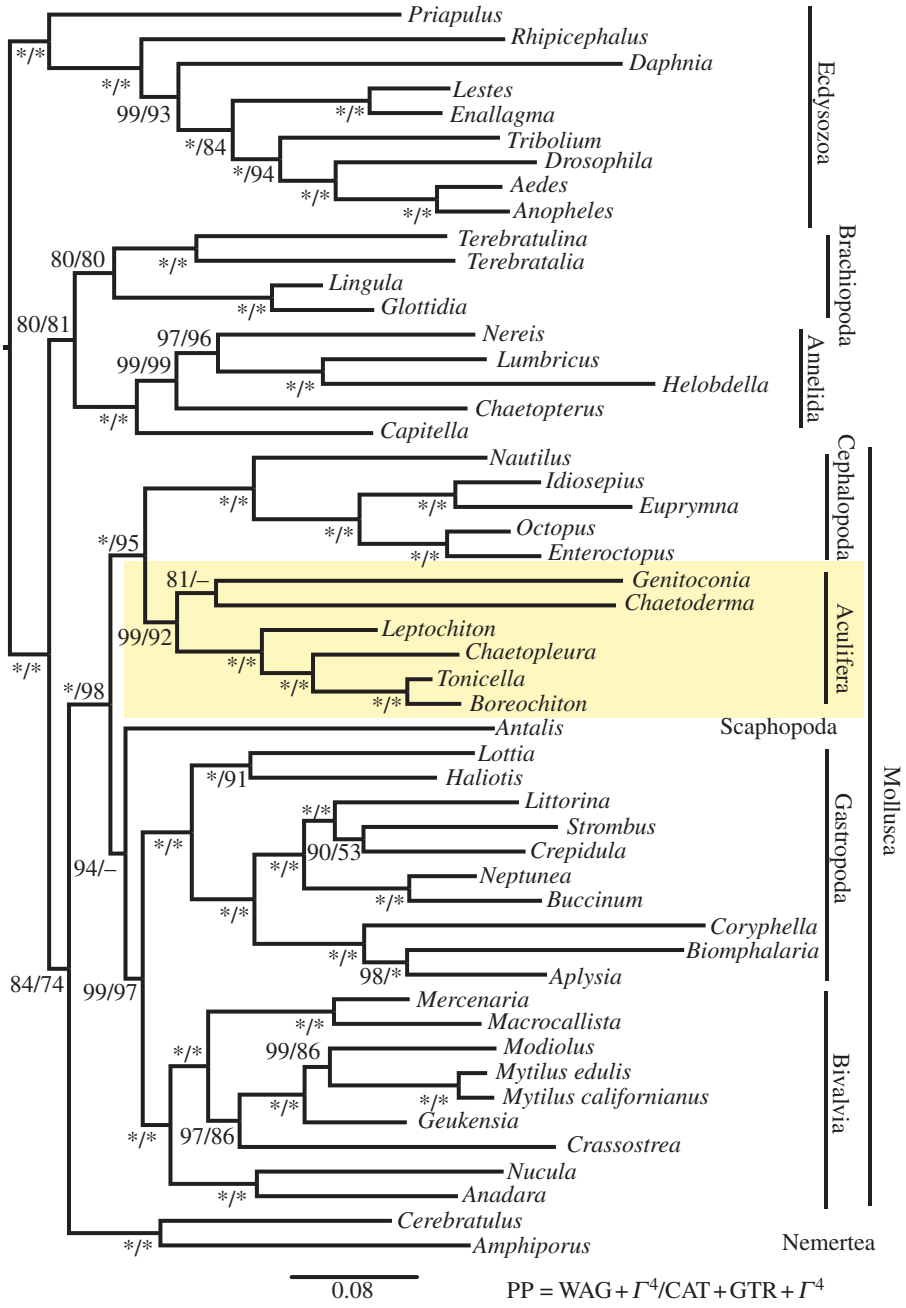

Figure 3. Bayesian phylogenetic analysis of seven nuclear housekeeping genes (2026 amino acids) from 31 molluscs and 20 additional outgroups. Topology shown is the WAG + Γ^4 analysis. Numbers at nodes are the posterior probability under the WAG + Γ^4 model and CAT + GTR + Γ^4 model, respectively. Asterisk denotes PP = 1. Dash denotes that the topology was not recovered under this model.

database www.pbdb.org using the entire permuted record of occurrences of the Early–Mid-Cambrian Pelagiellida. We used a maximum constraint of 549 Myr for crown-group Mollusca, the approximate age of the Nama Group [46,47]. An arguable synapomorphy of molluscs is their mineralized skeleton. The Nama assemblage is an open marine community and yields the earliest biomineralized organisms, such as the widespread *Cloudina* [46,47]. No molluscs, cap-shaped shells with accretionary growth lines, or scale-shaped sclerites are known from the Nama Group, nor from any other *Cloudina*-bearing rock worldwide. This provides a tentative maximum constraint on the appearance of molluscs, given that the preservation of biomineralized fossils in these rocks indicates appropriate conditions to preserve molluscan shells, which are conspicuously absent.

3. RESULTS

(a) *Phylogenetic analysis*

The Bayesian analysis of the concatenated dataset of seven nuclear housekeeping genes recovered a monophyletic Mollusca (PP of 0.98 under the CAT + GTR + Γ^4 model and 1.0 under WAG + Γ^4). In addition, our analyses recovered a monophyletic Aculifera (PP = 0.92 and 0.99, respectively; figure 3 and electronic supplementary material, figure S1). Aplacophorans resolved as a monophyletic clade under the WAG + Γ^4 model (PP = 0.81) and as a paraphyletic clade under the CAT + GTR + Γ^4 model, albeit with very low support for *Chaetoderma* as a sister group to polyplacophorans (PP = 0.63). The cephalopods were recovered as a sister group to aculiferans with high support under both

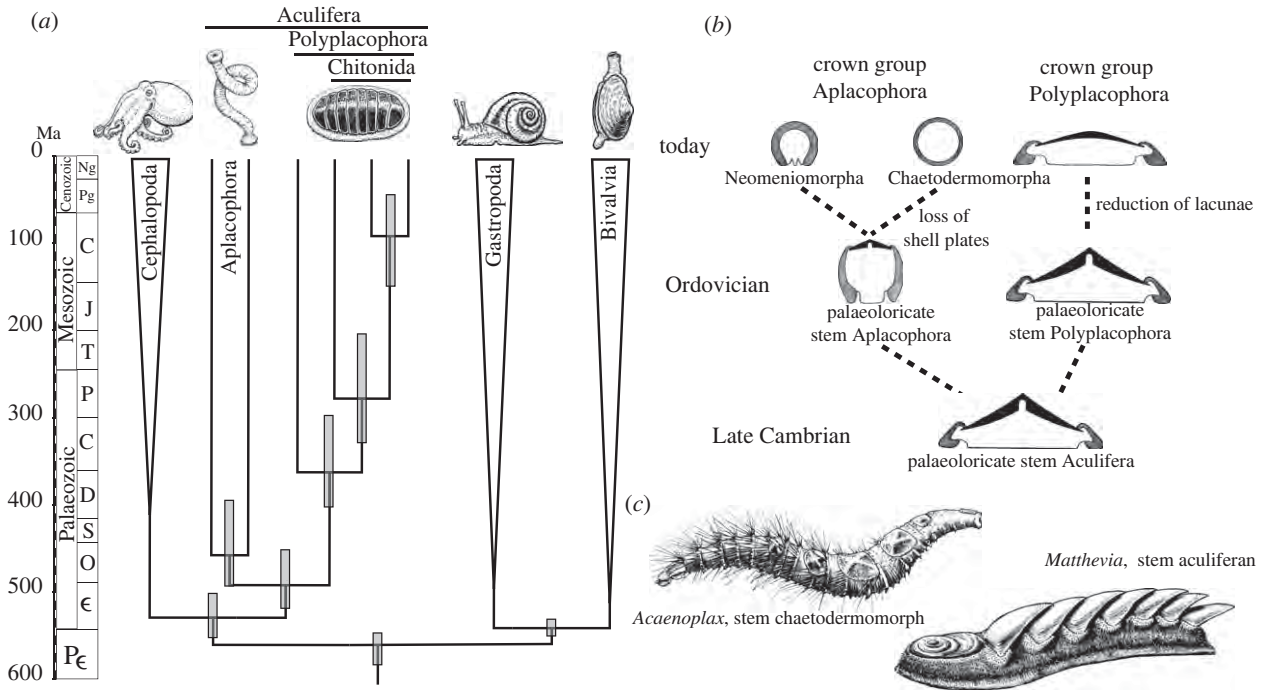

Figure 4. (*a*) Timetree of aculiferans assessed with Bayesian relaxed molecular divergence estimation (see electronic supplementary material, figure S5). Grey bars are 95% credibility intervals. (*b*) Morphological transitions in aculiferan evolution in relation to the foot, shell and mantle, based on molecular evidence presented herein and partly on previous morphological cladistic hypotheses [19,22]. The ancestral aculiferan was a form with a broad ciliary foot and a shell with lacunae (Paleoloricate). The molecular clock analysis and the fossil record indicate that in the Ordovician, the Aculifera had diverged into a stem group aplacophoran with a reduced foot and a laterally compressed body; fossil evidence demonstrates that this form was of palaeoloricate morphology with lacunae in the shell plates. Stem group polyplacophorans also retained the plesiomorphic palaeoloricate condition at this time. The aplacophorans diverged early into the chaetodermomorphs and the neomeniomorphs. Chitons reduced the lacunae in the shells and developed a more dorsoventrally flattened aspect and a smoother dorsal surface. (*c*) Reconstructions of two important fossil forms: *Acaenoplax hayae* (an aplacophoran relative) and *Matthevia* (the earliest known stem group aculiferan with multiple overlapping plates).

models (PP = 0.95 and 1). Scaphopods were recovered as a sister group to gastropods + bivalves in the WAG + Γ^4 analysis (PP = 0.94) and in a basal molluscan polytomy in the CAT + GTR + Γ^4 analysis.

(b) *Sensitivity tests*

It could be posited that the relationship between aculiferans and cephalopods is owing to LBA [32,48]: previous analyses found a sister-group relationship between aplacophorans and cephalopods [29,30,32], which are both long-branched. However, the observed topology was robust to both phylogenetic signal dissection and the sequential exclusion of long-branched taxa, unstable taxa and compositionally heterogeneous taxa.

The dataset with the three slowest-evolving quartiles of amino acid sites yielded essentially the same topology as the total dataset (electronic supplementary material, figures S2*b* and S3*b*) indicating that the fast-evolving amino acid sites—with their potential for adding systematic bias to the analysis—did not compromise the observed topology. Support for Aculifera remained essentially unchanged when compared with the overall topology (electronic supplementary material, figure S2) at 0.98 under WAG + Γ^4, while support for aplacophoran monophyly increased from 0.81 to 0.97. The CAT + GTR + Γ^4 model resolves a monophyletic Aculifera (PP = 0.9) with the two aplacophorans in a polytomy with polyplacophorans (electronic supplementary material, figure S3*b*). The fast-evolving sites (electronic supplementary material, figures S3*a* and S4*a*) did not resolve a monophyletic Aculifera in either of the models used, as the neomeniomorph *Genitoconia* would be located in a basal molluscan polytomy.

Taxon-exclusion analyses indicated that the observed topology (specifically, the monophyly of the aculiferans and the sister-group relationship between the aculiferans and cephalopods) is robust to the exclusion of long-branched and unstable clades. When cephalopods and scaphopods were excluded, the support for aculiferan monophyly increased from PP = 0.99/0.84 to PP = 1/1 in each substitution model (WAG + Γ^4 /CAT + GTR + Γ^4; compare electronic supplementary material, figure S4*a* with figure S1). When the relatively long-branched aplacophorans were excluded, a sister-group relationship of cephalopods and chitons was still recovered with high support under both models PP = 1/0.89 (electronic supplementary material, figure S4*b*).

The posterior predictive test indicated that *Leptochiton*, *Aedes*, *Tribolium*, *Anopheles* and *Drosophila* have significantly heterogeneous amino acid compositions (not shown). The overall topology when analysing a dataset with these taxa excluded did not differ from analysing all available taxa (figure 3).

(c) *Molecular clock analysis*

The results of the relaxed molecular clock analyses are shown in figure 4, and in tables S2 and S3 and figure S5 in the electronic supplementary material. The best-fitting

CIR molecular clock model estimates the age of crown-group Polyplacophora to be 357 Ma (408–298 Ma), and the age of Chitonida was estimated to be 272 Ma (336–201 Ma). These results conform to the fossil record in which the crown group of Polyplacophora has been estimated to have diverged in the Carboniferous [49] and stem members of Chitonida are known from the Early Permian (Leonardian: 270–275 Ma) [50]. The concordance between the geological and genetic records for chitons using external calibration points suggests that our molecular clock can be used to estimate divergences deeper within the Aculifera.

All molecular clock analyses estimated the origin of crown-group Aculifera to be sometime in the Late Cambrian to Early Ordovician. These estimates varied only moderately, regardless of the model used to estimate branch lengths (CAT + GTR + Γ^4 versus WAG + I^4), the relaxed molecular clock model applied (autocorrelated CIR versus uncorrelated white noise) or whether cephalopods were included (tables S2 and S3 in the electronic supplementary material). The most likely age for crown Aculiferans is 488 Ma (credibility interval: 517–453 Ma); this is the estimate derived from the autocorrelated CIR model (figure 4), which has been shown to be the best-fitting model for amino acid datasets [41,51].

4. DISCUSSION

(a) *Aplacophorans are derived chiton-like molluscs*

The fossil record of polyplacophorans and aplacophorans suggests that chitons and aplacophorans appeared in the Ordovician and that both evolved from a chiton-like ancestor with seven or eight dorsal plates [19], which first appears in the fossil record in the Late Cambrian [26]. The molecular phylogenetic analyses (figure 3) recovered a monophyletic Aculifera with high support, and our estimated divergence times are concordant with the known fossil record of crown-group Aculifera arising in the Early Ordovician (figure 4*a*). Thus, our analysis rejects the traditional scenario of aplacophorans as a basal grade of molluscs [6,7], as this hypothesis meets neither the predicted topological nor temporal predictions as found here.

The bulk of the divergence estimates of crown-group Aculifera (tables S2 and S3 in the electronic supplementary material) post-dates the first appearance of palaeoloricates such as *Matthevia* and *Chelodes* in the Late Cambrian [25]. This supports the hypothesis that these forms represent stem aculiferans [19], and that aplacophorans lost the shell plates and reduced the ventral foot region secondarily from such an ancestor (figure 4*b*). Thus, both the molecular data (presented herein) and the fossil record [19,22] support the hypothesis of aplacophorans as derived chiton-like molluscs [4]. However, while the empirical WAG + I^4 model supports Aplacophora as a monophyletic group, aplacophorans are recovered as a paraphyletic grade leading to chitons with very low support under the overall best-fitting model (CAT + GTR + I^4), which collapses to a polytomy when only the slowly evolving sites are analysed. Thus, details of the origin and evolution of Aplacophora remain to be assessed. Nonetheless, while we cannot reject that aplacophorans evolved their vermiform and reduced bodyplan twice, both the fossil record and aplacophoran development suggest that the chiton-like morphology is primitive for crown-group Aculifera, consistent with the hypothesis that Aplacophora is indeed a monophyletic group. Recently, Kocot *et al.* [52] recovered a similar topology, finding strong support for both a monophyletic Aculifera and a monophyletic Aplacophora.

(b) *Cambrian aculiferans*

The Aculifera diverged from the conchiferan molluscs in the Early Cambrian, according to our molecular clock analysis, but chiton-like forms are not known in the fossil record before the Upper Cambrian [24,25,53]. Older sclerite-bearing taxa, the sachitids (Early–Mid-Cambrian) [54], have been interpreted as aculiferan relatives [24,55–57], including *Halkieria* [56,58], *Maikhanella* [55], *Orthrozanclus* [59] and *Wiwaxia* [60,61], all taxa known as partial to completely articulated specimens with a variable number of shell plates and sclerites arranged in morphological zones similar to aculiferans [57]. The sclerites are constructed of longitudinal fibres of presumed aragonite, a structure similar to that of chiton sclerites, and are hollow with a branching canal system that has been compared with the aesthete canals in the shell plates of modern chitons and the sclerites in the extinct multiplacophorans [57]. These forms would extend the aculiferan stem lineage into the earliest Cambrian, eliminating a long aculiferan ghost range.

(c) *Ancestral molluscan body plan: a monoplacophoran morphology?*

In most morphological scenarios of molluscan evolution, the aplacophorans and chitons are considered a sister group to all other molluscs (the Conchifera) [6]. However, several independent molecular studies [30–32,62], as well as this one, have recovered the Aculifera and cephalopods as a clade relative to gastropods and bivalves. This implies a paraphyletic Conchifera leading to the Aculifera, and suggests that the conchiferan condition is ancestral to the phylum. Fossil evidence shows that most conchiferan classes were primitively limpet-shaped, with monoplacophoran-like ancestors [63]; interestingly, the few molecular studies on extant monoplacophorans recover them in close affinity with chitons [29,64]. Thus, a clade consisting of monoplacophorans, cephalopods and Aculifera relative to bivalves and gastropods (and perhaps scaphopods) is possible.

An alternative explanation for this pattern is that the root of the molluscs is misplaced—our unrooted molluscan topology is consistent with the monophyly of Conchifera. Such a monophyletic Conchifera would simply require shifting the position of the root by one node, from between Aculifera + Cephalopoda and all the other molluscs to between Aculifera and all the other molluscs. This topology was recently found by Kocot *et al.* [52]. Recent studies investigating rooting issues in other phyla have demonstrated that subtle misrooting can be very difficult to detect, but can be discerned through experiments designed to separate historical signal from systematic error [65,66]. Such experiments on our dataset, such as slow–fast analysis, the exclusion of compositionally heterogeneous taxa and the exclusion of long-branched taxa, did not change our results. Thus, while misrooting remains a concern, there is no evidence at present that the rooting position in

figure 3, which results in a paraphyletic Conchifera, is a result of systematic error. Whether cephalopods are the sister group to the remaining conchiferans, as found by Kocot *et al.* [52], or to the Aculiferans, as found here, remains to be determined. Future studies with increased taxon and gene sampling, and/or studies of other molecular markers (such as rare genomic changes [67] or microRNAs [68,69]), will help in addressing this issue.

The earliest fossil stem aculiferans (sachitids) and conchiferan cap-shaped shells occur in the Early Cambrian; no fossil group represents a plausible intermediate between these bodyplans (sclerites and multiple shells versus single cap-shaped shell). *Kimberella*, known from the late Ediacaran (555 Ma) [70–72], has been found associated with creeping and scraping marks suggestive of a ciliary gliding organism with a molluscan-style radula [71]. A molluscan affinity is further supported by the presence of lateral pouches of the anteriormost part of the sometimes infilled gut [71,72], resembling the distinct oesophageal pouches found in monoplacophorans, chitons and gastropods [73]. However, because *Kimberella* lacks mineralized elements, it does not provide any evidence of whether the ancestral crown mollusc was closer to a limpet-shaped conchiferan or an aculiferan, and no fossils are known that illuminate the skeletal morphology of the last common ancestor of the molluscan crown group.

5. CONCLUSIONS

The absence of Early–Mid-Cambrian chitons and aplacophorans has been problematic in the light of traditional hypotheses of molluscan evolution that regarded these groups as morphologically ancient. The growing body of evidence from the fossil record, molecular phylogenies and molecular clocks suggests that chitons and aplacophorans evolved in the Ordovician from a chiton-like ancestor with serial overlapping plates. Thus, aplacophorans evolved their vermiform appearance secondarily and do not represent a historical relict of the ancestral mollusc. If the observed relationship between cephalopods and aculiferans holds up to scrutiny, it is likely that the ancestral mollusc was a conchiferan-like form, whereas if cephalopods are indeed more closely related to the other conchiferans, then the morphology of the ancestral mollusc remains far more enigmatic.

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