The origin of animals: Can molecular clocks and the fossil record be reconciled?

John A. Cunningham[1)2)], Alexander G. Liu[1)†], Stefan Bengtson[2)] and Philip C. J. Donoghue[1)]*

The evolutionary emergence of animals is one of the most significant episodes in the history of life, but its timing remains poorly constrained. Molecular clocks estimate that animals originated and began diversifying over 100 million years before the first definitive metazoan fossil evidence in the Cambrian. However, closer inspection reveals that clock estimates and the fossil record are less divergent than is often claimed. Modern clock analyses do not predict the presence of the crown-representatives of most animal phyla in the Neoproterozoic. Furthermore, despite challenges provided by incomplete preservation, a paucity of phylogenetically informative characters, and uncertain expectations of the anatomy of early animals, a number of Neoproterozoic fossils can reasonably be interpreted as metazoans. A considerable discrepancy remains, but much of this can be explained by the limited preservation potential of early metazoans and the difficulties associated with their identification in the fossil record. Critical assessment of both records may permit better resolution of the tempo and mode of early animal evolution.

Keywords:
Cambrian explosion; ediacaran; metazoa; molecular clocks; neoproterozoic; trace fossils

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[1)] School of Earth Sciences, University of Bristol, Bristol, UK
[2)] Department of Palaeobiology and Nordic Center for Earth Evolution, Swedish Museum of Natural History, Stockholm, Sweden

***Corresponding author:**
John A. Cunningham
E-mail: john.cunningham@bristol.ac.uk

[†]Current address: Department of Earth Sciences, University of Cambridge, Downing Street, Cambridge, UK.

Introduction

The apparent absence of a fossil record prior to the appearance of trilobites in the Cambrian famously troubled Darwin. He wrote in *On the origin of species* that if his theory of evolution were true "it is indisputable that before the lowest [Cambrian] stratum was deposited ... the world swarmed with living creatures." Furthermore, he could give "no satisfactory answer" as to why older fossiliferous deposits had not been found [1]. In the intervening century and a half, a record of Precambrian fossils has been discovered extending back over three billion years (popularly summarized in [2]). Nevertheless, "Darwin's dilemma" regarding the origin and early evolution of Metazoa arguably persists, because incontrovertible fossil evidence for animals remains largely, or some might say completely, absent from Neoproterozoic rocks [3]. The conventional interpretation of the metazoan body fossil record is that it documents an abrupt (in geological terms) appearance of animals around the base of the Cambrian Period 541 million years ago (Ma) – the "Cambrian Explosion" hypothesis. By around 520 Ma, virtually all of the animal phyla with recalcitrant tissues that one might expect to become fossilized are represented in the fossil record, as revealed by Lagerstätten such as the exceptionally preserved Chengjiang biota [4] (Fig. 1 summarizes major Ediacaran and early Cambrian fossil assemblages). However, this body fossil record is at odds with molecular clock analyses (Fig. 2), which consistently indicate that Metazoa originated somewhere between 850 and 650 Ma in the Tonian or Cryogenian intervals, before diversifying through the Cryogenian and the Ediacaran [3, 5–7].

Fossil dates for the origin of a group are likely to be younger than well-calibrated molecular dates. The oldest fossil occurrence of a lineage typically reflects, at best, the time at which a population of organisms possessing a diagnostic set of morphological characters had become abundant, stable, and sufficiently geographically widespread for a number of individuals to be preserved, recovered, and identified by palaeontologists. In contrast, the molecular date represents the time at which the lineage became genetically

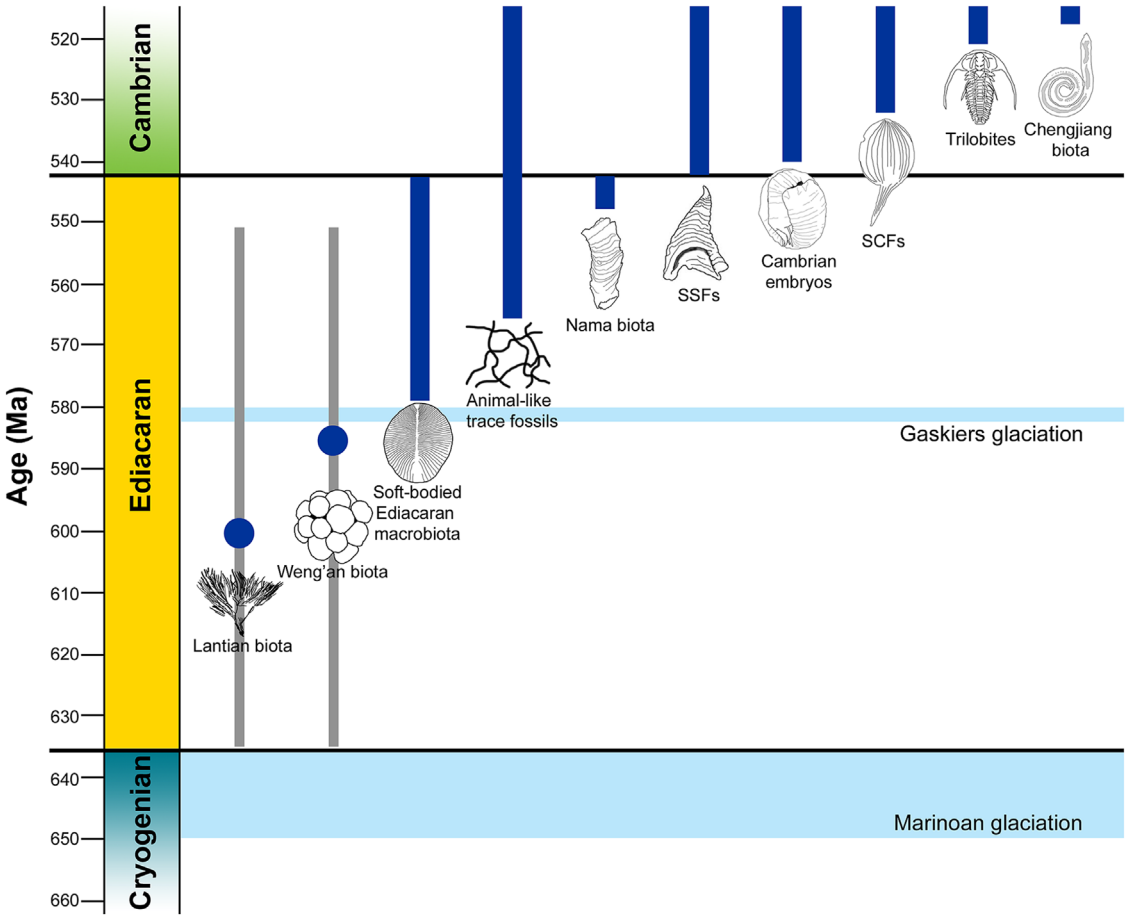

Figure 1. Summary of major Ediacaran and early Cambrian fossil assemblages. For the Lantian and Weng'an deposits blue circles represent likely ages and grey bars represent the range of uncertainty in these age estimates. SSFs, Small Shelly Fossils; SCFs, Small Carbonaceous Fossils.

isolated, and is necessarily earlier [8]. Early attempts at clock analyses were often based on poorly justified single calibration points that failed to accommodate phylogenetic and dating uncertainty, and produced divergence dates that were consistently too old [9]. However, more recent relaxed clock methods [3, 6] yield divergence estimates that are in closer agreement with fossil constraints on clade age [10, 11]. Importantly, these same methods consistently provide estimates for the origin of animals that are over one hundred million years before the oldest widely accepted animal fossils. This raises questions regarding the extent to which the early fossil record of animals is incomplete or has been misinterpreted, and the extent to which methodological issues prevent molecular clocks from resolving the timing of early animal evolution.

Claims of animals from the Neoproterozoic are plentiful, some perhaps made in response to molecular clock predictions that animals were present prior to the Cambrian. Reports of Neoproterozoic crown-group animals (members of the group that contains all the descendants of the last common ancestor of living animals) include members of crown-bilaterian phyla [12, 13], bilaterians [14–16], ctenophores [17], cnidarians [18–21], eumetazoans [18, 22], and sponges [23–25]. Stem-group animals (organisms that are not in the animal crown-group, but are more closely related to it than to the nearest living relatives, the choanoflagellates) have also been reported [26, 27]. Here we critically evaluate these claims, and assess whether the fossil evidence and molecular clock estimates can be reconciled.

Is there a Neoproterozoic fossil record of animal evolution?

A number of records have the potential to inform on animal evolution in the Neoproterozoic interval during which metazoans are suggested to have diversified by molecular clocks. We consider the merits of the most widely cited claims in reverse chronological order, extending from the latest Ediacaran back through the Neoproterozoic. Where appropriate, we consider the body fossils within Lagerstätten or biotic assemblages that represent exceptionally preserved marine communities of similar age and/or depositional environment. We consider the case for assigning fossils to stem-, crown-, and total-group definitions of clades. A crown-clade is comprised of its living members, their last common ancestor and all fossil descendants of that ancestor, while the stem is comprised of all fossil species outside the crown that are more

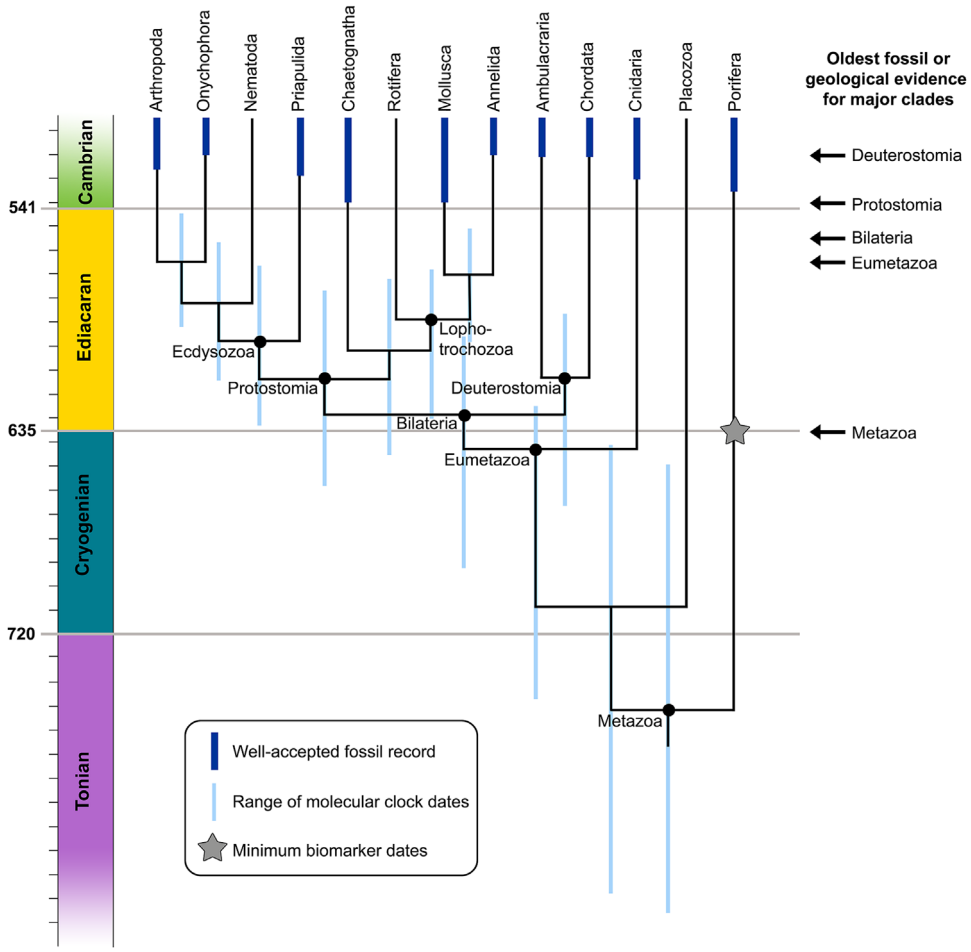

Figure 2. The mismatch between the fossil and molecular clock records of early animal evolution. The phylogeny follows [6]; note that ctenophores, the phylogenetic position of which is contentious, were not included. Dark blue bars represent well-accepted reports of fossils that can be assigned to extant animal phyla, which are limited to the Cambrian; ranges mainly follow [3]. Pale blue bars represent the range of molecular clock estimates for the origins of major clades obtained in [6]; note that the origin of eumetazoans is always inferred to predate the Ediacaran and the origins of bilaterians, protostomes, deuterostomes, ecdysozoans and lophotrochozoans are always inferred to predate the Cambrian. The righthand column shows the first evidence, as interpreted here, for major clades in the geological record: Metazoa = 635 Ma, possible biomarker evidence, alternatively 565 Ma eumetazoan trace fossils; Eumetazoa = 565 Ma, trace fossils; Bilateria = 555 Ma, trace fossils; Protostomia = 540 Ma, helcionellids, protoconodonts; Deuterostomia = 530 Ma, echinoderm plates.

closely related to this crown clade than to any other; the total-group is comprised of the crown plus the stem, and assignment to this more universal clade is usually employed to reflect ignorance as to whether a fossil species belongs to the stem or crown-clade [28].

The Nama biota

The latest Ediacaran Nama biota (550–541 Ma; [29, 30]) is best known from Namibia, but representative taxa have also been found elsewhere. It includes *Cloudina*, *Namacalathus*, *Sinotubulites*, and *Namapoikia*, all of which have been interpreted as animals with biomineralized skeletons [16, 31–36]. These organisms are often associated with tubular organisms possessing lightly biomineralized or chitinous exoskeletons such as *Corumbella*, *Vendoconularia*, and *Gaojiashania* [37–39].

Cloudina (Fig. 3A) is a millimeter-scale tubular fossil composed of a series of nested calcite funnels, which are occasionally branched [35, 40]. It shows possible evidence for flexible deformation [31], but individual funnels were sufficiently rigid in life to have seemingly been bored by other organisms [41], and to have formed reefs (e.g. [34]). *Cloudina* has been compared to polychaetes [13, 35] on the basis of similarities in overall morphology, microstructure, and evidence for asexual reproduction by budding. However, these features are also consistent with an interpretation as a cnidarian-like organism [19, 31, 42]. Evidence for budding [35] and hexagonal symmetry [36] are compatible with a total-group cnidarian and, therefore, a crown-eumetazoan affinity. However, corroborative evidence is required to confirm this suggestion, as may be provided by data on the histology of the tube wall. In the interim, it is difficult to constrain the affinity of *Cloudina* with any degree of confidence.

Namacalathus (Fig. 3B) is an enigmatic goblet-shaped organism that consists of an enclosed hollow "cup" that has

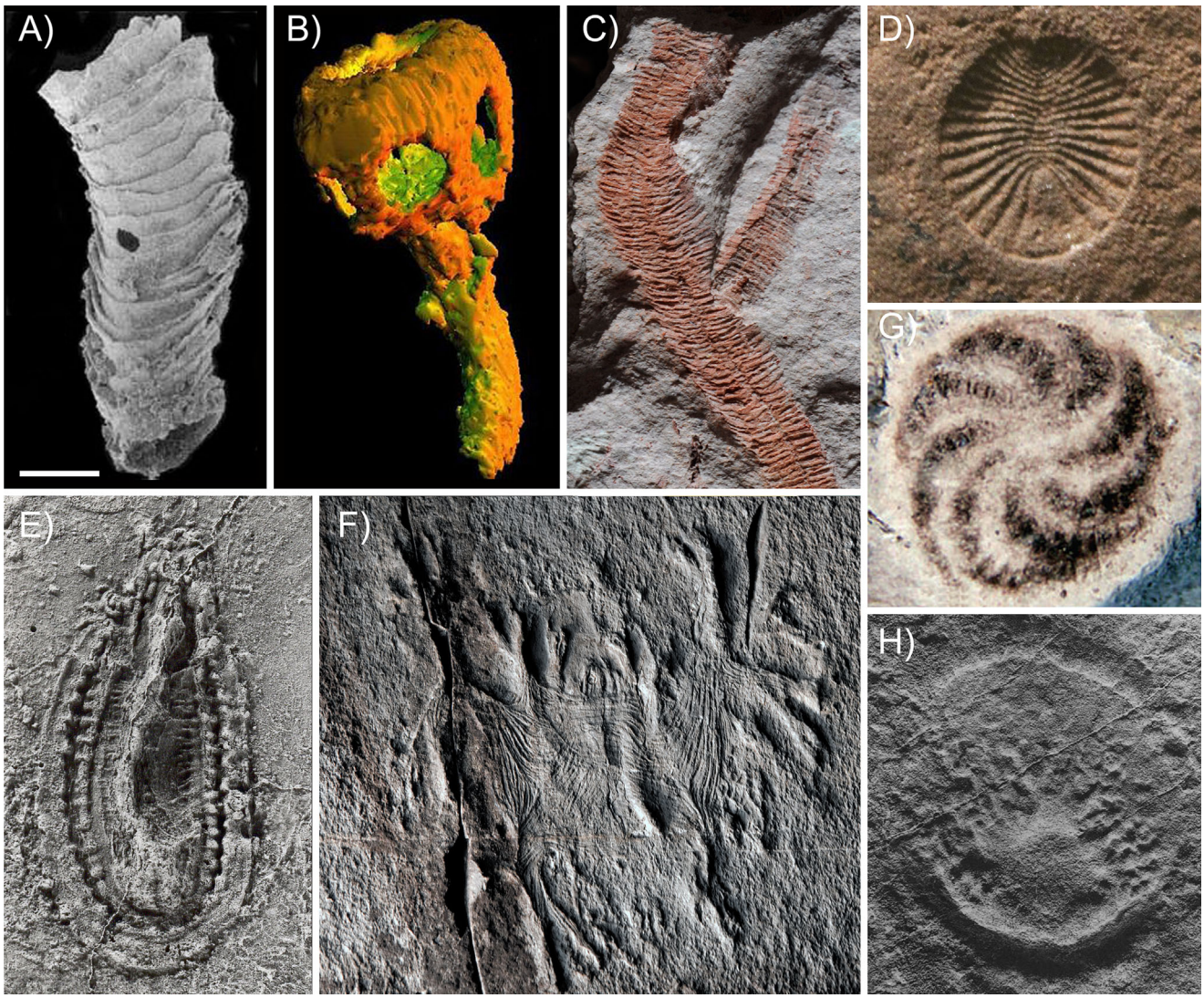

Figure 3. Putative Ediacaran animal body fossils from the Nama biota (**A–C**) and the soft-bodied Ediacaran macrobiota (**D–H**). **A**: *Cloudina*. **B**: *Namacalathus*. **C**: *Corumbella*. **D**: *Dickinsonia*. **E**: *Kimberella*. **F**: *Haootia*. **G**: *Eoandromeda*. **H**: *Palaeophragmodictya*. Image credits: Bengtson and Yue [39] (**A**); A. Knoll and W. Watters (**B**); L. Parry (**C**); A. Liu (**D**, **F**); A. Ivantsov (**E**); Tang et al. [16] (**G**); Gehling and Rigby [24] (**H**). Scale bar: (**A**) 0.3 mm; (**B**) 5.5 mm; (**C**) 3 mm; (**D**) 5 mm; (**E**) 5 mm; (**F**) 20 mm; (**G**) 4 mm; (**H**) 17 mm.

multiple apertures and is attached to a stalk. The cup apertures include a circular opening at the top, and between five and seven symmetrically arranged openings in the facetted sides [16, 32]. *Namacalathus* has generally been interpreted as a protist [43] or as an animal possibly with cnidarian or stem-eumetazoan affinities [32, 44], but has most recently been reinterpreted as a total-group lophotrochozoan based on its three-layered wall with a foliate structure, and speculative evidence for columnar inflections in the walls and bilaterally symmetrical buds [16]. This latter interpretation is intriguing, but it is difficult to reconcile with the typically hexaradial symmetry of the cup, which is arguably more consistent with a cnidarian affinity. It is difficult to constrain the affinity of *Namacalathus* with confidence. *Namapoikia*, an encrusting organism from the same assemblage, formed mounds up to one meter in diameter. It displays similarities to chaetetid sponges or colonial cnidarians [44], but possesses no characters diagnostic of any particular eukaryotic group.

Corumbella (Fig. 3C) is known from <550 Ma Ediacaran deposits in Brazil, the USA, and Paraguay [45], and is a tubular organism whose distal regions have an approximately square cross-section and rounded corners. It is typically 20–25 mm in diameter and up to 80 mm in length, has fourfold radial symmetry with each face being annulated, and a longitudinal groove that runs down the midline of each face [37]. The tube is composed of polygonal organic plates with pores and papillae [45]. The morphology and tube construction are strikingly similar to both the extant scyphozoan cnidarian *Nausithoe*, and the extinct conulariids, which have been interpreted as scyphozoans [46]. *Paraconularia*, which occurs with *Corumbella* in Brazil, is also closely comparable to conulariids [47]. *Corumbella* and *Paraconularia* are plausibly interpreted as total-group cnidarians and, thus, Ediacaran crown-eumetazoans.

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The soft-bodied Ediacaran macrobiota

This global assemblage of soft-bodied organisms is found in sedimentary strata of ~579–541 Ma. There is currently little consensus regarding the phylogenetic affinities of many taxa within this biota, but they have often been interpreted as early animals [48], though see [49]. The biota encompasses a range of taxa with tubular, frondose, modular, and sheet-like morphologies, and it is now widely acknowledged that these are unlikely to comprise a clade [14]. Perhaps the most prominent candidate metazoan among the biota is *Kimberella* (Fig. 3E), the oldest records of which are dated to between 558 Ma ±1 Myr and 555.3 Ma ± 0.3 Myr [50]. *Kimberella* has been considered to be: a mollusc or mollusc-like bilaterian [12]; a stem-group mollusc [51]; a "trochophore animal of a pre-molluscan stage" [52]; or a mollusc-like animal [3]. Some have considered its identification as an animal to be sufficiently secure to use *Kimberella* as a calibration point for the minimum age of crown-Metazoa, crown-Protostomia, and crown-Lophotrochozoa in molecular clock analyses [53]. *Kimberella* exhibits bilateral symmetry and anterior-posterior polarity, and its overall form is indeed mollusc-like, with structures resembling both a foot and a mantle [12, 52, 54], which appear to reflect differentiation of tissues within the organism. The association of *Kimberella* with fan-shaped scratch-like markings composed of paired ridges on bedding planes (the latter interpreted as trace fossils produced by a radula; [52, 54–56]), has provided further evidence to suggest that *Kimberella* was a motile, heterotrophic, muscular organism. However, there remain unresolved questions regarding how these scratches were formed and by what kind of structure [57], and there is insufficient evidence to test whether the structure that produced them was homologous with a molluscan radula. The presence of differentiated anterior-posterior anatomy and bilateral symmetry, comparatively large size, a possible radula-like structure, and evidence of movement suggest that *Kimberella* was a total-group bilaterian. Suggestions of a more derived affinity place too much credence on the inference of a molluscan radula, and the nature of the mantle-like structure.

The iconic *Dickinsonia* (Fig. 3D) is another widely discussed possible metazoan. Known from Russia and Australia, the oldest *Dickinsonia* specimens associated with a radiometric date, from the White Sea, are slightly older than 558 Ma ± 1 Myr [50]. *Dickinsonia* is a flat, approximately elliptical organism with a morphologically differentiated growth axis. It is divided into discrete elongate "units" that cross the midline (Fig. 3D), imparting a bilateral symmetry on the organism [15]. Most large specimens of *Dickinsonia* have undergone post-mortem contraction, which may be due to muscular contraction but could alternatively result from hydrostatic processes in a fluid-filled organism [58]. Gold et al. [15] presented evidence that they interpret as showing that *Dickinsonia* grew by terminal addition, and argued, based on character state reconstruction, that this mode of growth is a synapomorphy of Bilateria. However, there are differences between terminal addition in most extant bilaterians and that seen in *Dickinsonia*. In bilaterians, somites are usually added at a subterminal position (ahead of, for example, the telson in arthropods). If the smallest units in *Dickinsonia* were added last (as assumed in [15]), then they are added in a genuinely terminal position, and *Dickinsonia* development would thus be inconsistent with that seen most frequently in extant bilaterians. A bilaterian affinity may or may not be at odds with the interpretation of *Dickinsonia* as a placozoan-grade organism [59], depending on the phylogenetic position of Placozoa [60, 61], though recent analyses have tended to place Placozoa outside Bilateria [60]. Serial *Dickinsonia* impressions named *Epibaion* are associated with some specimens [55], and have been interpreted as evidence for movement and for external digestion of the underlying microbial mat through the ventral sole of the organism. If this interpretation is correct, then *Dickinsonia* had a combination of movement and external ventral digestion that is thought to be unique to placozoans [59]. Evidence for anatomical differentiation, bilateral symmetry, achievement of large (~50 cm diameter) size, post mortem contraction, and in vivo movement are consistent with *Dickinsonia* being a metazoan, and possibly even a total-group eumetazoan. However, more derived and precise phylogenetic hypotheses are currently unsupported.

Eoandromeda (Fig. 3G) is a centimeter-scale organism with eight spiral arms radiating from a globular central structure [17, 62, 63]. Specimens from black shales in China (~551 Ma) preserved as carbonaceous films show that the arms bore transverse structures arguably homologous to the comb plates of ctenophores [17]. In combination with a lack of crown-group ctenophore characters such as tentacles, statoliths, polar fields, and biradial symmetry, this feature led to the suggestion that *Eoandromeda* is a stem-group ctenophore [17]. There is a lack of direct evidence that the proposed comb plates were composed of cilia (which would in any case have a limited chance of preservation), but their spacing (closely comparable to ctenophore comb plates), as well as the presence of eightfold radial symmetry, indicates that the ctenophores are the most morphologically similar extant group. On the basis of current evidence, we consider *Eoandromeda* to be a plausible stem-group ctenophore and a reasonable candidate for a total-group animal (potentially more informative, depending on the placement of ctenophores within Metazoa; see [64]).

Haootia (Fig. 3F) from ~560 Ma Ediacaran strata in Newfoundland, Canada, has been interpreted as a possible cnidarian [20, 65], and is preserved as sediment moulds of twisted fibrous bundles that extend into four distinct bifurcating branches. Its fibrous construction is distinct from that of all contemporaneous macrofossils currently known within the Ediacaran macrobiota, and has been tentatively interpreted as reflecting impressions of muscle fibers comparable to those of extant staurozoan cnidarians [20]. The interpreted arrangement of muscle fibers has been contested [66] but the polyp gestalt has not, leaving *Haootia* as a credible candidate total-group cnidarian. Corroborative evidence of theca symmetry and mesentery supports would add credence to this claim of an Ediacaran cnidarian.

Palaeophragmodictya (Fig. 3H) from ~555 Ma rocks in Australia is perhaps the most widely recognized candidate for a sponge within the Ediacaran macrobiota [25]. Its overall form and the presence of radial structures interpreted as a spiculate mesh have seen it compared to hexactinellid sponges, but the evidence for mineralized spicules is equivocal [67, 68] and the

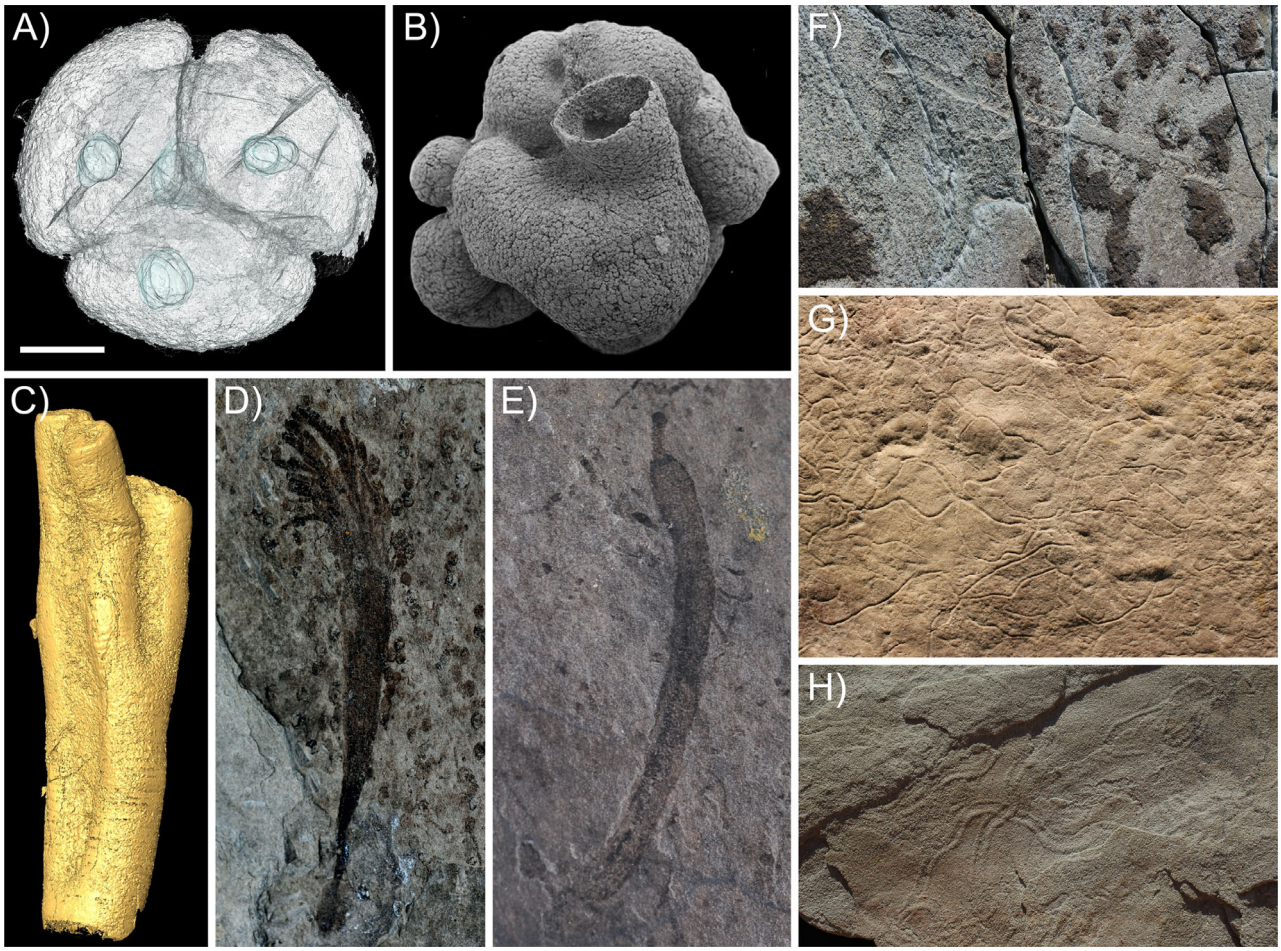

Figure 4. Putative animal body fossils from the Weng'an biota, Doushantuo Formation (**A–C**) and Lantian biota (**D–E**), and Ediacaran trace fossils interpreted to have been produced by animals (**F–H**). A: Embryo-like fossil *Tianzhushania*. **B**: *Eocyathispongia*. **C**: *Ramitubus*. **D**: *Lantiella*. **E**: *Xiuningella*. **F**: Putative eumetazoan trace fossil from Mistaken Point, Newfoundland. **G**: *Helminthoidichnites*, a putative bilaterian trace fossil. **H**: *Archaeonassa*, a putative bilaterian trace fossil. Image credits: J. Cunningham (**A**, **C**); Z. Yin (**B**); S. Xiao (**D**, **E**); A. Liu (**F**, **G**); S. Jensen (**H**). (**A**) 135 μm; (**B**) 250 μm; (**C**) 170 μm; (**D**) 5.5 mm; (**E**) 3 mm; (**F**) 50 mm; (**G**) 55 mm; (**H**) 20 mm.

taxon may be more readily interpreted as decayed attachment discs from an organism of uncertain affinity [68, 69]. *Coronacollina*, also from Australia, is reconstructed as a millimetric truncated cone, associated with putative "spicules" up to 370 mm in length. It has been compared to the Cambrian to Lower Ordovician sponge *Choia* [70], but few specimens exhibit a consistent relationship between the cone and the linear spicules. As such, neither *Palaeophragmodictya* nor *Coronacollina* are considered to reflect poriferans, or even metazoans, on the basis of current evidence.

Weng'an biota

The Weng'an biota of the Doushantuo Formation, South China, is likely to predate the classical Ediacaran macrobiota [71]. The Doushantuo Formation can be dated to 635–551 Ma, and although the age of the fossiliferous units is poorly constrained within this interval, they are most likely ~570–600 Ma [71]. Algal fossils, acritarchs, and three-dimensionally phosphatised microfossils are abundant, and the latter include specimens preserved at a subcellular level (Fig. 4A) that have been interpreted as embryos of animals [72] or stem-group animals [26]. These microfossils exhibit a variety of morphotypes and are among the most intensively studied of any Precambrian fossils. None of the characters used to support an animal interpretation, such as Y-shaped junctions between cells or palintomic cell division, are unique to animals [73]. Though a stem-group animal interpretation cannot yet be ruled out, affinities with other eukaryotic lineages, including algal and protist clades, are at least equally likely and require further investigation [27, 71, 73, 74].

The recently described *Eocyathispongia* (Fig 4B) from Weng'an [23] is three-dimensionally preserved and has a cup-shaped morphology comparable to extant sponges. It represents the most plausible report of a sponge from the Precambrian. However, more analyses and specimens are needed to test this hypothesis. In particular, high-resolution tomographic analysis of the walls of the specimen could reveal whether pores are present, and therefore whether *Eocyathispongia* could have functioned as a sponge.

Additional tubular microfossils (Fig. 4C) from Weng'an have been interpreted as eumetazoans or stem-eumetazoans based on comparisons to tabulate corals [18, 22, 71, 75].

However, the presence of likely biological structures preserved in the spaces between the cross walls is incompatible with a tabulate-like body plan, because in extant corals these spaces are the empty former living positions of the polyp [76]. There is thus currently no justification for interpreting these fossils as animals.

Lantian biota

The Lantian biota is a macrofossil assemblage of algae and putative animals preserved as carbonaceous compressions [77, 78]. The age of the biota is poorly constrained. The Lantian Formation has been correlated with the Doushantuo Formation (551–635 Ma), and the fossiliferous horizons could be as old as 590–635 Ma [78]. However, this is the oldest possible age, and the unit can only be constrained to >551 Ma if more conservative correlations are followed. The putative animal fossils include *Lantianella* (Fig. 4D), which has similarities to scyphozoan cnidarians [21, 77, 78], and *Xiuningella* (Fig. 4E), which has a gut-like axial trace and an anterior region that resembles a scalidophoran introvert [77, 78]. However, these comparisons are based mainly on broad similarities in overall body shape and in each case a macroalgal interpretation cannot yet be ruled out [78].

Trace fossils

Perhaps the best fossil evidence for total-group bilaterians in the Ediacaran comes from trace fossils. In addition to the traces associated with *Dickinsonia* and *Kimberella*, a range of surface traces and burrows are recognized in late Ediacaran marine settings. The oldest simple surface traces (Fig. 4F) occur in a deep-marine deposit at ~565 Ma and were produced by an organism with a circular basal surface, likely a muscular non-bilaterian eumetazoan employing a mode of locomotion similar to some extant cnidarians [79, 80]. These traces are followed ~555 Ma by abundant meandering, shallow horizontal burrows of a few millimeters width, created on or just beneath the sediment-water interface in marine siliciclastic sediments worldwide (e.g. *Helminthoidichnites*; Fig. 4G–H; [57, 81]). Ediacaran ichnofossil assemblages are of low diversity and exhibit only limited behavioural complexity [81, 82]. Although non-metazoans (e.g. [83]), and the action of currents on microbial aggregates [84] can produce surface impressions, several Ediacaran traces exhibit characters that are consistent with production by bi laterian animals [85–87]. Trends identified within Ediacaran ichnofossil assemblages include a gradual increase in maximum size of traces toward the Cambrian, and at the end of the interval, the first (very shallow) burrow systems (e.g. *Treptichnus*; [81]) and possible bioturbation [88]. A marked increase in ichnofossil diversity, size, and complexity from the Fortunian onwards clearly documents a Cambrian radiation of bilaterian clades and behaviours, most notably those associated with arthropods [81]. However, the Ediacaran trace fossil record suggests that total-group eumetazoans are likely to have been present from 565 Ma onwards, with total-group bilaterian traces (and thus bilaterian trace-makers; see [81]) from ~555 Ma.

Biomarkers

Sponges are a basal metazoan group expected to have high preservation potential given the presence of mineralized spicules in most extant sponges, including members of the earliest branching groups [89]. However, the abundant record of Cambrian sponge spicules [90] and body fossils [91] does not extend into the Neoproterozoic – Porifera is the animal phylum with perhaps the least convincing pre-Cambrian fossil record [68] – despite the observation that molecular clocks predict crown-sponges to have been present in the Cryogenian [3]. Possible evidence for sponges comes from molecular biomarkers in rocks from Oman, which suggest that demosponges were present by 635 Ma [92–94]. The sterane 24-isopropylcholesterol (24-ipc) is only known from demosponges and pelagophyte algae [94]. However, the production of 24-ipc in pelagophytes can be linked to a gene duplication event that is not thought to have occurred until the Phanerozoic [94]. Demosponges have, therefore, been considered the most likely source for the Precambrian 24-ipc record. Cryostane, a sterane that has been recovered from rocks older than 740 Ma, has also been tentatively suggested to have been produced by sponges, but requires further investigation [95].

The credibility of the biomarker records suffers from similar problems to that of bioclasts recovered from Cryogenian Trezona Formation limestones in South Australia and interpreted as sponge-grade animals by Maloof et al. [24]. The case for a sponge affinity for those structures is based solely on the presence of ~1 mm diameter circular canals that are compared to sponge ostia. However, in the absence of corroborative evidence of sponge characters (and the extraordinary variability of size and morphology amongst the Trezona assemblage), these features are insufficient to substantiate a sponge or, indeed, an animal affinity. Similarly, the biomarkers constitute a single character and, in the absence of corroborative evidence [96], cannot be considered sufficient to conclusively demonstrate the presence of Cryogenian sponges and, therefore, animals [97–99].

Summary of the Neoproterozoic metazoan body fossil record

We consider that there is good evidence from trace fossils for the presence of animals by around 565 Ma, and bilaterians by around 555 Ma. This timeline is supported by plausible reports of animal body fossils including the possible bilaterian *Kimberella* from the Ediacaran macrobiota, also around 555 Ma. If *Dickinsonia* is accepted as a eumetazoan, White Sea records give evidence of both Metazoa and Eumetazoa at ~558 Ma. The probable cnidarian *Corumbella* constrains the minimum age of crown-Cnidaria, ~543 Ma, while *Haootia* and *Eoandromeda*, respectively, offer plausible stem representatives of Cnidaria and Ctenophora at ~560 and 555 Ma. Older claims for Ediacaran animal fossils do not withstand close scrutiny, and on the basis of currently available data we do not consider any reports of animal fossils from the Cryogenian to be credible. However, biomarkers from Cryogenian rocks provide some evidence to suggest that sponges may have been present by ~635 Ma.

Many of these records are not sufficiently well dated or phylogenetically constrained to be used effectively in molecular clock calibration. Nevertheless, this assessment demonstrates that not only has the gap between the fossil record and molecular clock estimates narrowed in recent years, but that the fossil record appears to reveal the broadly expected pattern of appearance of first Metazoa (~635 Ma), then Eumetazoa (~565–560 Ma), and later the Bilateria (555 Ma). We emphasize that these dates represent minimum ages for the appearance of these groups, and that there are older, albeit weaker, claims suggestive of even greater antiquity. However, there remains a genuine mismatch with molecular clock estimates of the divergence times of these clades, even in analyses using the most conservative calibration strategies ([6]; Figure 2). We now explore the possible reasons for this apparent discrepancy.

Rationalizing mismatch between rocks and clocks

The rock record does not preserve a uniform record of time and environments

The non-uniform nature of the fossil record is a potential barrier to the recovery of early animal fossils. There is a correlation between the areal extent of rock available for sampling fossils and the diversity of fossils recovered, per unit time, through the Phanerozoic [100]. Although quantitative studies have yet to be undertaken in the Precambrian, it is clear that the rock record from the critical interval around the Precambrian-Cambrian boundary is less complete than we might like. In many localities around the world, shallow-marine Cambrian or Ordovician rocks lie unconformably above Precambrian rocks [101]. This "Great Unconformity" records a prolonged period of continental denudation in the latest Neoproterozoic followed by a major marine transgression, suggesting that many latest Ediacaran shallow-marine rock sequences – reflecting environments that may be expected to contain the highest diversity of animal life – have been eroded away. Moreover, plate tectonic reconstructions suggest that much of the remaining sediment deposited during the latest Precambrian and Early Cambrian would have been subducted or accreted and destroyed at the Delamerian-Ross subduction zone, which initiated in the Cambrian [102]. The rock record, and therefore the fossil record, is probably particularly poor during the precise interval over which the first animals are predicted to have diversified and radiated, despite the apparently excellent potential for soft tissue preservation at this time [103].

Difficulty in inferring the biology of ancestral animals makes their fossil remains concomitantly difficult to identify

We must also consider which criteria are required for us to be able to identify a Precambrian fossil as an animal, and whether these criteria are likely to be met. In the case of crown-group members of extant animal phyla, identification should be relatively straightforward, notwithstanding the usual biases and difficulties associated with interpreting the fossilized remains of soft-bodied organisms [28, 104]. The preservation of the combination of biological characters used to identify these phyla today and in the Phanerozoic fossil record are sufficient. However, recent molecular clock analyses estimate that the crown-groups of most animal phyla did not originate until the Cambrian [3, 6]. The presence of crown members of most animal phyla in the Ediacaran is therefore not an expectation of most molecular clock studies. Palaeontologists should therefore not necessarily expect to be able to assign Ediacaran and Cryogenian fossils to extant phyla. Rather, molecular clock estimates predict the existence of stem members of extant phyla in the late Ediacaran, and stem representatives of aggregate clades of phyla before this time. If this is the case, identifying where taxa should lie within such broader groups will be difficult, since stem representatives of a given animal phylum will, by definition, lack some or all of its diagnostic characters, and may also possess derived characters that are absent from the crown group members [105].

Identifying stem representatives of more inclusive animal clades, from the stems of superphyla, to stem-Metazoa itself, must also assume the presence of some but not all of the characters diagnostic of the crown-group. In practice, such animals are likely to be very hard to identify, not least because of the expectation that fewer characters will unite early branching animal groups, and a current lack of agreement as to which characters are actually diagnostic of these major animal clades. Different interpretations of, for instance, the monophyly [60, 106] versus paraphyly [3, 89, 107] of sponges, the lack of consensus regarding lophotrochozoan interrelationships [108], and the phylogenetic position of placozoans [60, 61] and ctenophores [64, 109, 110], result in different predictions of character distribution among early animal lineages. For example, if sponges are paraphyletic, the last common ancestor of Metazoa would have been a sponge-like animal. Following this inference, early fossil stem- and crown-metazoans would be anticipated to be animals with a sponge-like body plan having characters such as a water canal system. If, on the other hand, sponges are monophyletic then such characters might be limited to the sponges and would not necessarily be expected in other early metazoans, which may have been morphologically more complex [111]. It is clear that until the phylogenetic relationships between extant clades are resolved, the character sets diagnostic of phylum level total-groups will remain uncertain. Thus, it will not be possible to determine whether critical fossils should be assigned to the stems of phyla, or to the stems of more universal clades. Debate over the affinity of tommotiids [112], *Odontogriphus* [113], and *Wiwaxia* [114] bear witness to this challenge. The problem may have been further complicated by the use of parsimony-based phylogenetic methods that can resolve the positions of fossil taxa with false precision [115].

Comparative molecular developmental biology affords another approach to inferring the nature of ancient members of universal clades. For example, some consider that the ancestral bilaterian possessed the features regulated by the

genes shared between protostomes and deuterostomes – eyes, appendages, a heart, metamerism, etc., – or at least the evolutionary rudiments of these structures [116, 117], though others argue that the urbilaterian was a much simpler organism [118, 119]. As evolutionary developmental biology has matured as a discipline, expectations of one-to-one genotype-phenotype mapping now appear naïve, and the recent finding that Xenacoelomorpha (xenoturbellids plus acoelomorph flatworms) is likely to be the sister group of the remaining bilaterians [119, 120] provides some support for this view because it suggests that the urbilaterian was probably a simple animal.

Uncertainties regarding the inferred biology of early animals are further compounded by the fact that the characters present in the first animals are predicted to be simple, open to multiple interpretations when encountered in fossils, and perhaps subject to convergent evolution. Moreover, it is not possible to identify most metazoan synapomorphies in the fossil record because they have no potential for fossilization (Box 1). Palaeontologists must therefore consider not only the primitive morphologies of early animals, but also how to discriminate them from other eukaryotic clades, when attempting to interpret problematic fossils from the Neoproterozoic.

Box 1

Identifying early metazoans

It is informative to consider the characters that the first animals would have possessed, and their potential for preservation in the geological record and identification by palaeontologists. Ax [132] listed the autapomorphies of metazoans as: a gonochoric organism (i.e. one with only one sex per individual) with diploid body cells, haploid gametes and a diploid zygote; oogenesis with one fertilizable egg and three abortive polar bodies; spermatogenesis with four identical sperm developing from one spermatocyte; sperm with a head containing the nucleus, a middle piece with four mitochondria and two centrioles at right angles, and a terminal filament with one cilium; impermeable cell-cell connections; radial cleavage; extracellular matrix with collagen fibrils; and a flat crawling blastaea in the adult stage with somatic differentiation into outer ciliate cells and inner aciliate cells. If we adopt this definition of a metazoan, we can infer that the urmetazoan would likely have had few fossilizable characters. Though there are exceptional instances of some such characters being preserved [133, 134], the only one with a reasonable chance of preservation is radial cleavage, given the peculiar record of embryo-like fossils from the Ediacaran [26, 72]. Moreover, the urmetazoan was probably a simple organism, and even if it were preserved and recovered, there would be great difficulty in identifying it as an animal over any other multicellular eukaryote, of which there are many. This point is particularly pertinent given that fossil and molecular evidence reveals that other extant (and perhaps some extinct) multicellular eukaryotic clades were also evolving and diversifying rapidly during the Neoproterozoic (reviewed in [135]).

The precision of molecular clocks and fossil calibrations are closely correlated

We have so far focused on problems with interpreting the fossil record, and the issues that must be considered when dealing with enigmatic fossils of possible animals. Doubtless, disagreement between the rock and clock records stem also from the molecular clock methods used to resolve early animal divergences. The molecular clock struggles fundamentally to resolve early animal divergences because of phylogenetic uncertainty, difficulties in selecting an appropriate rate model, and sequence saturation. The manner in which the fossil record is interpreted to derive probabilistic calibrations on clade ages is of particular significance since researchers differ in which fossil evidence they accept, and have different views on how the remaining data constrain the possible ages of clades [6]. However, integrating the impact of all of these factors on divergence estimates [6] shows that while they conspire to yield increasingly imprecise estimates (i.e. increasingly broad probabilistic estimates of clade age), they do not alter the conclusion that metazoans originated by the Cryogenian and diversified prior to the Cambrian. Even if the rate of evolution was elevated during the early diversification of animals [121, 122], or if the evolution of higher taxa such as Panarthropoda was to be limited to the Phanerozoic [121], a deep Neoproterozoic history is required to account for the radiation of early animal clades [123].

Future advances may improve molecular clock dates, but there is a limit to how much precision can be achieved through the addition of sequence data, and this asymptote has already been reached [6]. While increasing the volume of sequence data has beneficial statistical effects in estimating rate, it can also lead to increasingly precise but inaccurate divergence time estimates (i.e. the span of the estimate narrows but does not include the true clade age) when combined with incorrect fossil calibrations [124]. Therefore, further precision can only be achieved, while maintaining accuracy, by increasing the precision with which fossil evidence informs clade age [124]. Alternative approaches to calibration, like the fossilized birth-death model [125], total evidence dating [126], and their combination [127, 128], may overcome uncertainty in the phylogenetic affinity of both living and fossil lineages, but these methods are currently experimental, some yielding clade ages that vastly exceed those based on conventional node-calibration [129].

Conclusions and prospects

A number practical measures can be identified to overcome the interconnected limitations of the fossil and molecular records. Palaeontologists must improve understanding of known fossils and refine methods for interpreting stem-animal

taxa and distinguishing them from other multicellular eukaryotes. Neoproterozoic stratigraphy requires greater resolution and, within this framework, time intervals and environments that are under-represented by fossils must be targeted for sampling. The resulting advances in our understanding of the fossil record will improve calibration points for molecular clock analyses. Much effort has been expended in attempting to resolve the early branching order in metazoan phylogeny, but the relationships within groups such as the Lophotrochozoa and Ecdysozoa remain largely unresolved (though the situation in Lophotrochozoa is beginning to improve [130, 131]).

Nevertheless, though there is a discrepancy between molecular clock analyses and the rock record regarding the origin of animals, it is not as great as generally perceived. The latest molecular clock analyses estimate that animals evolved by ~650 Ma and, while Neoproterozoic fossils cannot generally be assigned to extant metazoan phyla, there is biomarker evidence suggesting that animals may have been present by ~635 Ma, and reasonably convincing fossil evidence from 565 Ma onwards. Both records confirm that animals were present prior to the Cambrian, and therefore that the origin and early evolution of animals in the "Cambrian Explosion" was neither Cambrian nor explosive. It is likely that the mismatch between fossil-based interpretations and molecular clock estimates result from all of the factors discussed above. Indeed, the problems associated with preserving and identifying early animals effectively predict a significant mismatch between molecular clock estimates and the fossil record. Thus, accepting their limitations, both molecular clocks and the fossil record provide an accurate, if not precise, timescale for animal evolutionary history. The research directions we outline will fundamentally improve our knowledge of Neoproterozoic animal evolution, facilitating tests of hypotheses on the causes and consequences of this formative event.

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