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The evolutionary history and timeline of mites in ancient soils

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Acariform mites play a crucial role as primary soil decomposers, impacting the carbon cycle. However, the timing of their diversification is uncertain, with estimated dates ranging from the Precambrian (no land plants) to the Carboniferous (diverse terrestrial ecosystems). One factor affecting these time estimates is an uncertain phylogenetic position of the earliest unequivocal fossil mites from the Devonian Rhynie Chert, which have been classified in five modern families and three suborders. Here, we thoroughly examine these specimens, assign them to a single species *Protacarus crani* (family Protoacaridae, fam. nov., suborder Endeostigmata) and integrate this information into a time-calibrated phylogenetic analysis. Our phylogeny suggests a Cambrian basal divergence of Acariformes (508–486 Ma), coinciding with the land colonization by bryophytes. At this time, the mites' ecological niches were probably diversified beyond the upper soil. Our study provides temporal context, improves the accuracy of fossil dating, and underscores the importance of mites' diverse habitats and their potential roles in soil food webs.

> "Plants began the process of land colonization about 450 million years ago, accompanied of necessity by tiny mites and other organisms that they needed to break down and recycle dead organic matter on their behalf."
>
> Bill Bryson, 2003. A Short History of Nearly Everything.

The Rhynie Chert is a Devonian age deposit known for its remarkably well-preserved early freshwater and terrestrial organisms, encompassing a range of life forms, such as bacteria, algae, plants, fungi, amoebas, nematodes, and arthropods[1–8]. These ancient fossils offer valuable insights into the evolution of early terrestrial life[9–13]. Among the diverse arthropods found in the Rhynie Chert are the earliest-known acariform mites, which belong to the chelicerate order Acariformes[14–16]. This lineage is incredibly diverse, with an estimated 1–10 million extant species, although only a fraction of them has been formally described[17–19]. Acariform mites, especially oribatids, play an important role in modern soils, contributing to the global terrestrial decomposition food chain by breaking down plant litter and wood materials, thus facilitating bacterial and fungal decomposition[20]. Their role in the decomposition of organic matter in terrestrial ecosystems directly impacts the carbon cycle and the release of carbon into the atmosphere[21].

The diversification of acariform mites is thought to be connected to the development and evolution of ancient soils in early land ecosystems[22,23]. However, the exact timeframe of this diversification is still uncertain. Molecular studies have provided different estimates, ranging from the Precambrian[24], when there were no land plants and animals except for bacterial mats, to the Carboniferous[25], which was well after the establishment of diverse terrestrial ecosystems, including forests. One significant factor affecting these time estimates is the inclusion, exclusion, or misapplication of the Rhynie Chert mites as fossil calibration points in molecular phylogenies[24–30].

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Unfortunately, the classification of these fossils is unclear. The Rhynie Chert mites were described by Stanley Hirst in 1923 as a single species, *Protacarus crani*, in the extant family Eupodidae[14]. Subsequent researchers interpreted these fossils as multiple species and classified them in five modern families and three suborders[31–34]. One view even suggested that these mites are not true fossils but rather secondary inclusions of modern mites[35]. Despite these disagreements, the classification, which separates these fossils into different species and genera across extant families and suborders, is currently accepted[16,36]. This not only implies the existence of a long-lasting, diverse soil fauna of "modern" acariform mites in the Early Devonian but also extends the acariform mites' history further back in time with the implication for the accuracy of calibrating molecular phylogenies.

To address the uncertainties regarding the early terrestrial mites found in the Rhynie Chert, our study (i) re-examines the Rhynie Chert mite specimens housed at the Natural History Museum, London, UK using advanced microscopy to provide their detailed morphological descriptions; (ii) infers their phylogenetic relationships within the acariform tree of life and conducts a time-calibrated phylogenetic analysis; and (iii) assesses the accuracy of previous molecular clock studies given our refined understanding of the phylogenetic relationships of these important fossils. Then we examine the diversification timeline of acariform mites, focusing on two modern megadiverse lineages—Oribatida (soil mites) and Eriophyoidea (plant-feeding mites)—which originated from early acariform ancestors. Finally, we place our findings in the context of the evolution of terrestrial plants.

Results

Systematic palaeontology
Superorder ACARIFORMES Zachvatkin, 1952
Order SARCOPTIFORMES Reuter, 1909
Suborder ENDEOSTIGMATA Reuter, 1909
Family PROTACARIDAE Klimov and Kolesnikov, **fam. nov.**
urn:lsid:zoobank.org:act:EFC0435A-E85F-4A42-9588-3A0AE829D8D0
Type genus: *Protacarus* Hirst, 1923
(Figs. 1, 2, 3 and 4, Supplementary Information 1: S1–10; extended description and historical account: Supplementary Information 1: Notes 1–3).

Diagnosis

Female/tritonymph (specimens 2, 3, 4, 5, 6). Body length, 360–470 µm, elongated. Cuticle transparent, smooth but may be finely plicate. Subcapitulum elongated. Rutella elongated and widened. Palp 5-segmented, palp femur not divided, palp tarsus rounded and slightly widened. Palp chaetotaxy: 0–2–1–3–12. Chelicerae elongated, wide; cheliceral trochanter present. Prodorsum with a large, elongated and rounded naso and 5 pairs of setae; setae with widened tips. Setae *le* longer than other prodorsal setae, bent in middle; single unpaired rostral seta *ro* present; Gastronotum smooth, divided (Figs. 3, 6). Dorsal gastronotic setae (at least 15 pairs) widened and trunctate at tips (cuneiform), serrate, different in size. Epimeral, genital and anal setae filiform. All epimera close to each other; epimeral chaetotaxy 2(or 3)–2–?–2. Genital valves with at least 7 pairs of setae. Ovipositor large, with 3 pairs of genital papillae. Anal opening extends to dorsal hysterosoma. Anal valves with at least 6 pairs of setae. Legs IV distinctly longer than legs I-III and probably enable jumping. Femora I and IV divided into telo- and basifemur. Femora II and III undivided. Each tarsus with short peg-like empodium and 2 long, filiform lateral claws. Legs have large numbers of setae. Leg I: 1–4–5–6–11–24 + 1 solenidion ω, leg II 2–5–3–7–18 + 1 solenidion ω, leg III: 4–6–7–7–19, leg IV: 1–2–6–12–20–36.

Remarks

Mites representing the family Protacaridae have been variously classified in three suborders, Prostigmata (Eupodidae, Tydeidae)[14,32], Oribatida (Paleosomata)[37] or Endeostigmata (Nanorchestidae, Alycidae)[32]. We place the Protacaridae within the Endeostigmata based on the following character states: the rutellum is present; the body has constrictions reflecting primary opisthosomatic segmentation; the prodorsum is unsclerotized; the palps have 5 segments and lack a palptibial claw; the chelicerae chelate; genital shields are unsclerotized, bearing numerous genital setae, and covering 3 pairs of genital papillae; legs without trichobothria; pretarsi with an empodium (not padlike) and with lateral claws.

The family Protacaridae (composed of *Protacarus crani*) differs from other endeostigmatid families by the following character states: each tarsus with a short peg-like empodium and two long, filiform lateral claws, longer than empodium; femora I and IV are completely divided, femora II and III are undivided; the prodorsum has 5 pairs of setae (including bothridial setae) and an unpaired rostral seta; all propodosomal setae are widened at their tips; tarsus I is hypertrichous, with about 24 setae; tarsus IV is hypertrichous, with about 38 setae; legs IV are modified for jumping, longer than legs I-III; trochanter II with 2 setae (potential homoplasy); the opisthosoma is segmented (potential homoplasy).

Genus *Protacarus* Hirst, 1923

Protacarus Hirst, 1923: 456 (type species *Protacarus crani* Hirst, 1923 by monotypy)[14,31,32,37,38].

Protospeleorchestes Dubinin, 1962: 462 (type species *Protospeleorchestes pseudoprotacarus* Dubinin, 1962, by original designation)[32], **syn. nov.**

Paraprotacarus Dubinin, 1962: 466 (type species *Paraprotacarus hirsti* Dubinin, 1962, by original designation)[32], **syn. nov.**

Palaeotydeus Dubinin, 1962: 466 (type species *Palaeotydeus devonicus* Dubinin, 1962, by original designation)[32], **syn. nov.**

Protacaris [lapsus calami][17,39–41].

sed non *Pseudoprotacarus* Dubinin, 1962[32] (incertae sedis)

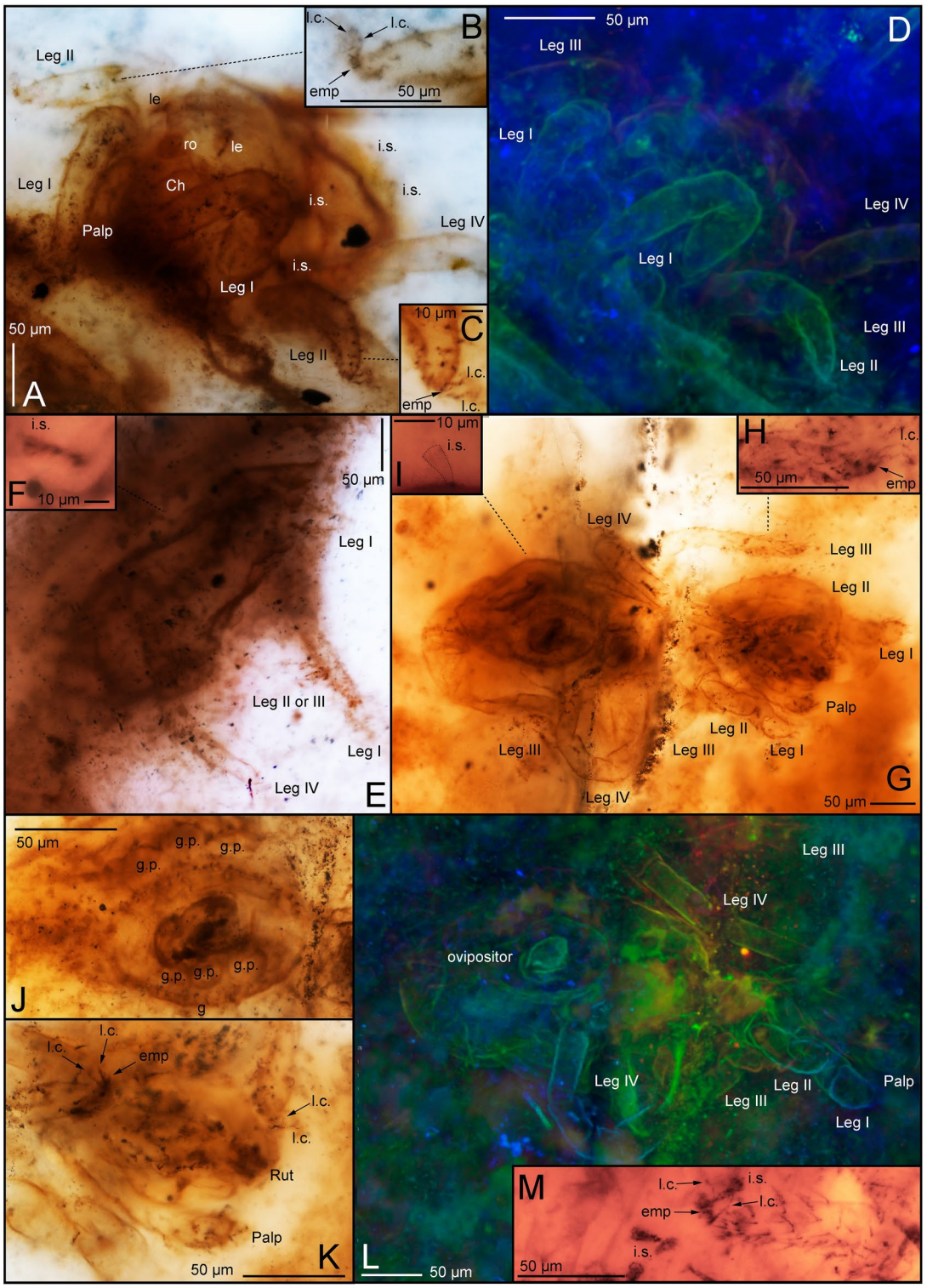

Fig. 1. *Protacarus crani* Hirst, 1923 from the Rhynie Chert (Devonian). (**a–d**) Specimen 2; (**e, f**) specimen 3; (**g–l**) specimen 4; (**m**) specimen 6; (**a, d, e, g, l**) specimen overview; (**b, c, h, m**) tarsi; (**f, i, m**) idiosomal setae; (**j**) genital area; (**k**) gnathosoma. (**a–c, e–k, m**) Differential interference contrast (DIC) photomicrographs; (**d, l**) confocal photomicrographs. Ch, chelicera; emp, empodium; g, genital seta; g.p., genital papilla; i.s, idiosoma setae; l.c., lateral claw; le, lamellar seta; ro, rostral seta; Rut, rutellum.

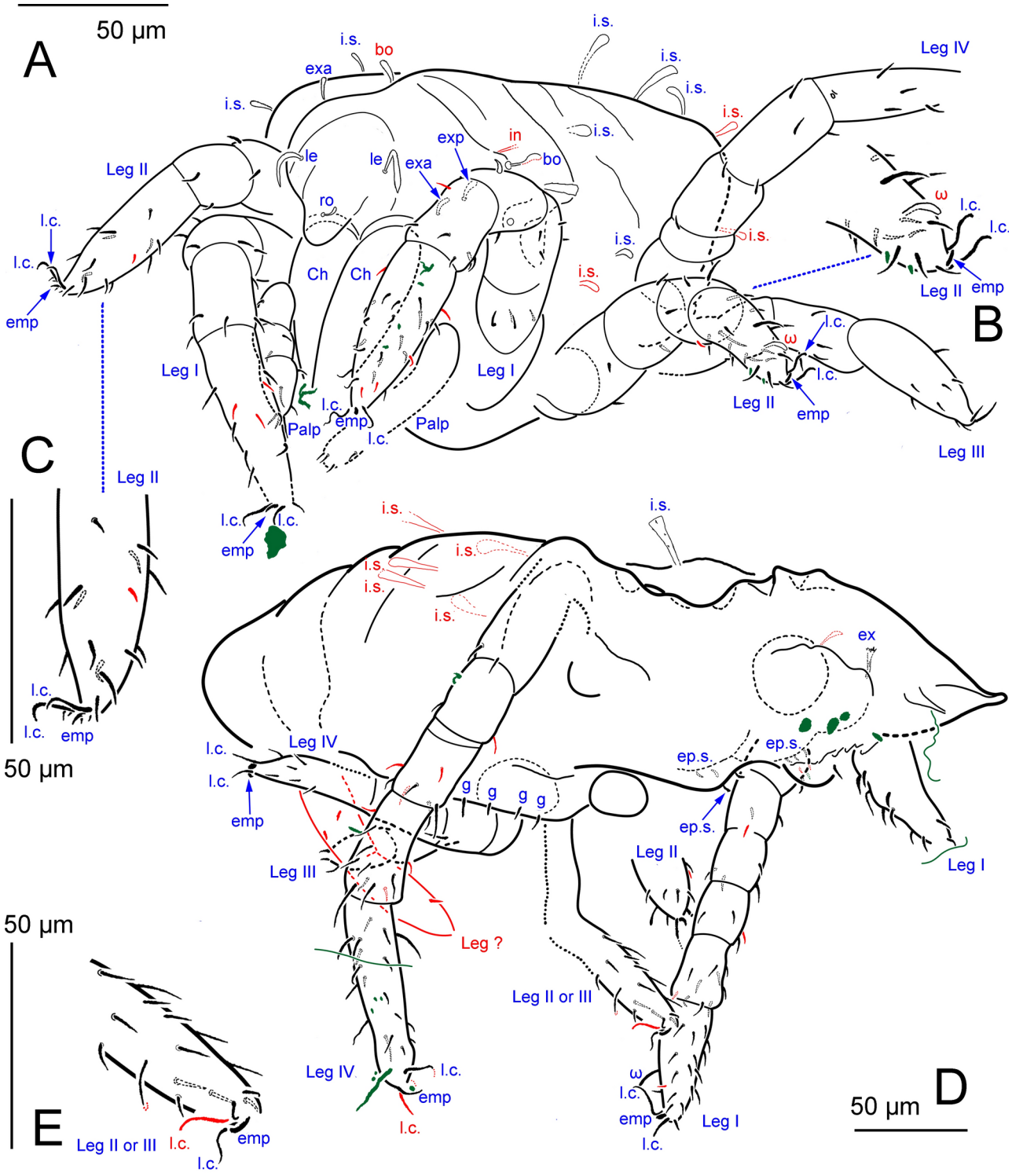

Fig. 2. *Protacarus crani* Hirst, 1923 from the Rhynie Chert (Devonian), line drawing. **(a–c)** Specimen 2; **(d, e)** specimen 3; (**a**) frontal view; (**d**) ventrolateral view; (**b, c, e**) tarsi. bo, bothridial seta; Ch, chelicera; emp, empodium; ep.s., epimeral seta; ex, exobothridial seta; exa, anterior exobothridial seta; exp, posterior exobothridial seta; g, genital seta; i.s, idiosoma setae; in, interlamellar seta; l.c., lateral claw; le, lamellar seta; ro, rostral seta; ω, tarsal solenidion.

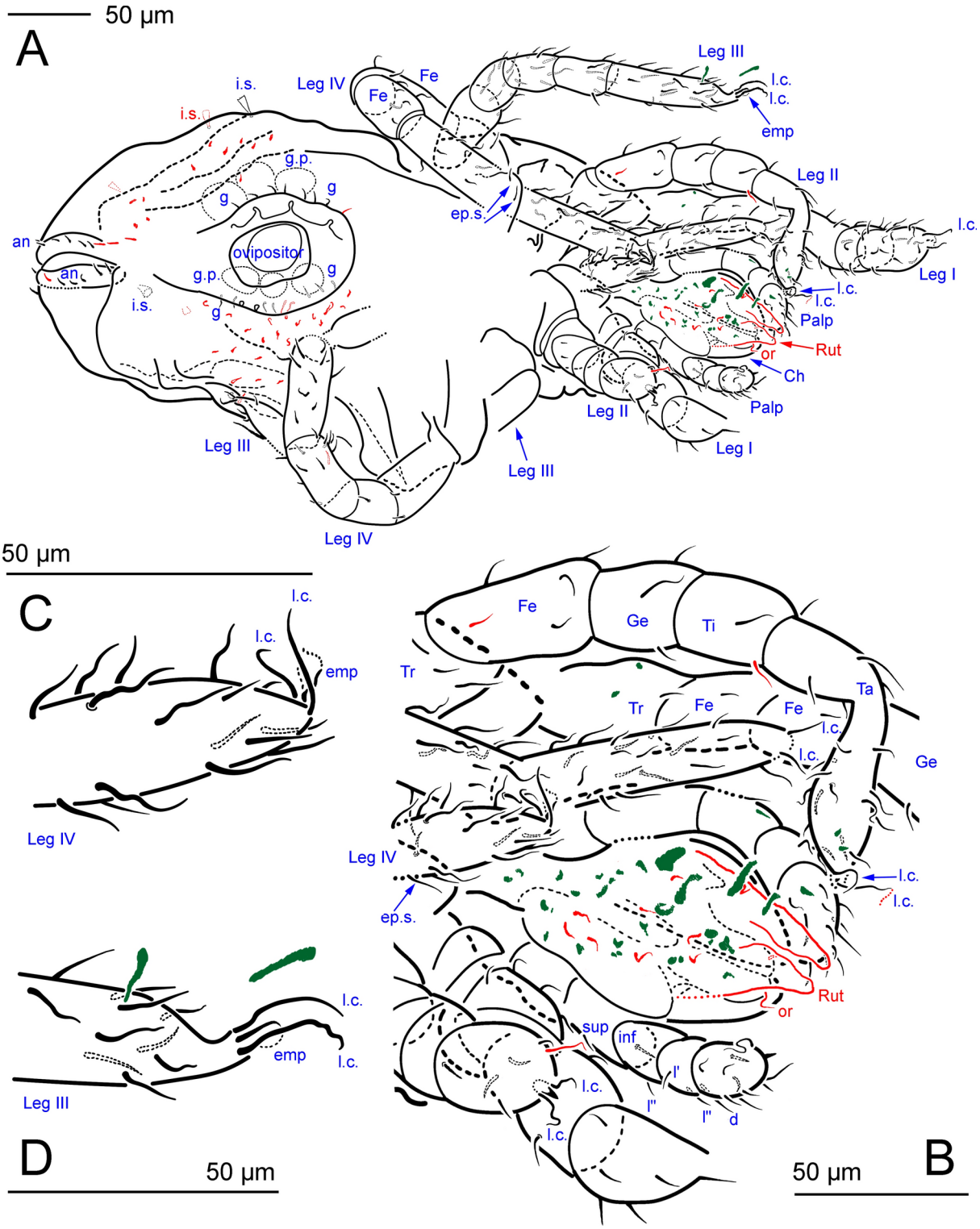

Fig. 3. *Protacarus crani* Hirst, 1923, from the Rhynie Chert (Devonian), line drawing. **(a–d)** Specimen 4; (**a**) ventral view; (**b**) anterior part, ventral view; (**c, d**) tarsi. Abbreviations: an, anal seta; Ch, chelicera; d, dorsal palpal seta; emp, empodium; ep.s., epimeral seta; Fe, femur; g, genital seta; g.p., genital papilla; Ge, genua; i.s, idiosoma setae; inf, inferior palpal seta; l, lateral palpal setae; l.c., lateral claw; or, adoral seta; Rut, rutellum; sup, superior palpal seta; Ta, tarsus; Ti, tibia; Tr, trochanter.

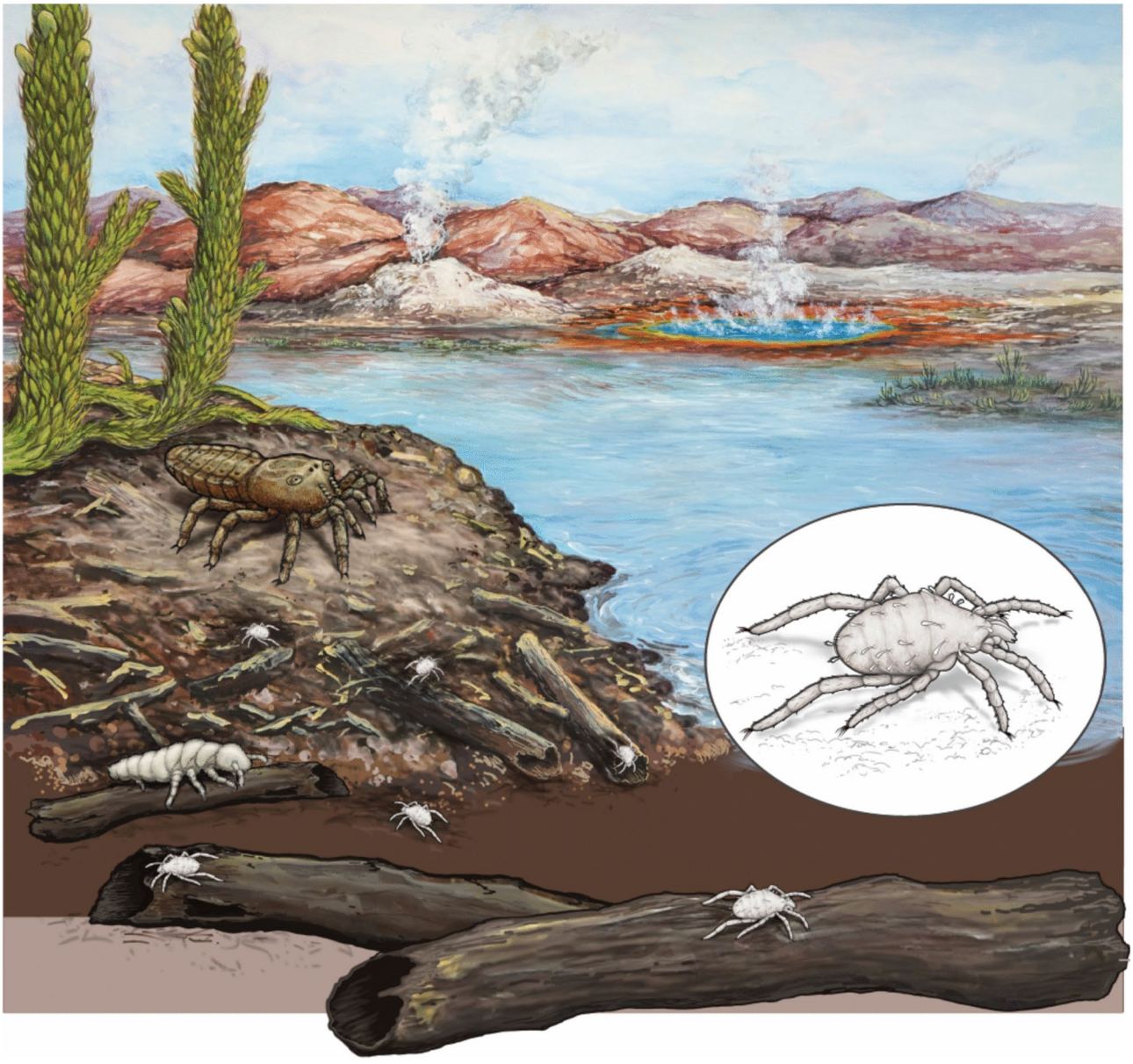

Fig. 4. Reconstruction of *Protacarus crani* and its habitat in the Rhynie Chert ecosystem (Early Devonian). Artist: Gabriela Sincich (Purdue University).

Type species: *Protacarus crani* Hirst, 1923, by monotypy.

(Figs. 1, 2, 3 and 4, Supplementary Information 1: Figs S1-10; extended description and historical account: Supplementary Information 1: Notes 1–3).

Diagnosis

Belongs to the monotypic family, see diagnosis above.

Protacarus crani Hirst, 1923

Protacarus crani Hirst, 1923: 456, Text-figs. 1, 2, Plate XIa,b (holotype: **specimen 2** (Pl. XIb) located in Area 1 (NHMUK PI IA 24665), "Old Red Sandstone, Rhynie Chert Bed, Aberdeenshire. Cran Coll No 58". Paratypes: **specimen 1** (Pl. XIa) (lost**), specimens 3** (text-fig. 1) (NHMUK PI IA 24666), **specimen 4** (text-fig. 2) (NHMUK PI IA 24667), **specimen 5** in Area 0 (text- fig. 1a) (NHMUK PI IA 24668), **specimen 6** (text- fig. 1b) (NHMUK PI IA 24669) ["Eupodidae ?"][14], *sed non* specimen 1= *Pseudoprotacarus scoticus* Dubinin, 1962 [Alicorhagiidae] (lost, considered as a species *incertae sedis* here)[31,32,38,42–45].

Protospeleorchestes pseudoprotacarus Dubinin, 1962: 462 [Nanorchestidae][32] (nom. nov. pro *Protacarus crani* Hirst, 1923, specimen 6; redrawn and re-interpreted from Hirst, specimens not studied by Dubinin), Fig. 1340, **syn. nov.**

Paraprotacarus hirsti Dubinin, 1962: 466, Fig. 1347 [Tydeidae][32] (nom. nov. pro *Protacarus crani* Hirst, 1923, specimen 4; redrawn and re-interpreted from Hirst, specimens not studied by Dubinin), **syn. nov.**

Palaeotydeus devonicus Dubinin, 1962: 466, Fig. 1346 [Tydeidae][32] (nom. nov. pro *Protacarus crani* Hirst, 1923, specimen 3; specimen 5: Area 0 was also included: Fig 1346A; redrawn and re-interpreted from Hirst, specimens not studied by Dubinin), **syn. nov.**

(Figs. 1, 2, 3 and 4, Supplementary Information 1: Figs S1-10; extended description and historical account: Supplementary Information 1: Notes 1-3)

Diagnosis

Cuticle transparent, plicate. Rutella (40 µm), adoral setae *or* (7 µm) filform. Palps (80–90 µm), palpal setae filiform. Chelicerae (60–80 µm) elongated and widened. Prodorsum separated from opisthosoma by sejugal furrow. Naso large, elongated and rounded; with single unpaired seta *ro* (5 µm), widened at tip, bent, situated at middle of naso. Lamellar setae *le* long (15–22 µm), lanceolate, bent at middle. Anterior exobothridial setae *exa* (7–9 µm) and posterior exobothridial setae *exp* (8 µm) shorter than other setae, widened at tips. Interlamellar setae *in* weakly widened at tips. Faint transverse furrow posterior to bothridial setae. Opisthosoma segmented, lacks post-pedal constrictions. Gastronotic setae (12–25 µm) widened and truncated at tips, serrate, 29 setae observed. Genital setae 2–8 µm long. Setae of ovipositor filiform (5 µm). Three pairs of large genital papillae. Anal valves have 6–9 filiform setae (4–8 µm). Epimera I–IV close to each other, almost touching. All epimeral setae filiform, 5–10 µm. Legs smooth.

Remarks

We assign all six Rhynie Chert specimens studied here to a single species based on the following shared character states: (1) all tarsi with peg-like empodia and two long, filiform claws (longer than empodium); (2) leg setae are similar in shape and length; (3) legs IV are longer than other legs; (4) femora I and IV are divided, femora II-III are undivided; (5) dorsal idiosomal setae are widened and truncated at their tips, serrate; (6) propodosomal setae are widened; (7) chelicerae are widened; (8) opisthosoma is segmented; (9) naso is present; (10) there are 3 pairs of genital papillae.

Phylogenetic analysis and divergence time estimates

We studied six museum specimens of acariform mites from the Rhynie Chert and suggest that they are conspecific: *Protacarus crani* Hirst, 1923 (= *Protospeleorchestes pseudoprotacarus* **syn. nov.**, *Paraprotacarus hirsti* **syn. nov**., *Palaeotydeus devonicus* **syn. nov**.). We also show that *Protacarus crani* belongs to a new family, Protacaridae, **fam. nov.** in Endeostigmata. Our work resolves the great uncertainty associated with these fossils as they were previously classified into four different species, five modern families (Nanorchestidae, Alycidae, Tydeidae, Eupodidae, Ctenacaridae) and three suborders (Endeostigmata, Trombidiformes, Sarcoptiformes). The Rhynie Chert specimen representing *Pseudoprotacarus scoticus* was not found in the Natural History Museum, London, and we consider it as a species *incertae sedis*. We scored character states of *Protacarus crani* and entered them into a morphological character matrix, allowing us to do a comprehensive time-calibrated phylogenetic analysis.

Our time-informed phylogenetic inference (Fig. 5) recovered the superorder Acariformes as a basal dichotomy, with the orders Trombidiformes and Sarcoptiformes (including Endeostigmata) diverging from each other at 503 Ma (484–514) (median, 95% HPD). The ages of crown groups Trombidiformes and Sarcoptiformes were 486 (439–511) and 493 Ma (471–511), respectively. The suborder Endeostigmata was paraphyletic with respect to the suborder Oribatida, which is arguably the most diverse and common mite lineage in modern soils. Our phylogeny dated the origin of Oribatida to 411 Ma (384–450). The two Devonian mite species, *Protacarus crani* and *Archaeacarus dubinini*, were inferred within the Endeostigmata. *Protacarus crani* (family Protacaridae) was sister to the modern family Alycidae, suborder Endeostigmata, split 467 Ma (430–500). *Archaeacarus dubinini*, which originates from a younger Devonian site (uppermost Panther Mountain strata, Gilboa; 383–384 Ma)[15], was sister to the genus *Alicorhagia* within the modern family Alicorhagiidae. The superfamily Eriophyoidea (plant-feeding mites) and the soil/sand-inhabiting Nematalycidae split 376 Ma (314–441); while the diversification of crown-group Eriophyoidea occurred 305 Ma (260–364) (Fig. 5).

Once we refined the phylogenetic placement of the Rhynie Chert mite *Protacarus crani*, we used our phylogenetic framework to assess the accuracy of previous time-calibrated inferences[23–29,46] (Fig. 6). Among the nine studies, three papers[25,27,30] inferred stem group Acariformes to be younger than the age of *Protacarus crani*, rendering these molecular clock estimates inconsistent with the fossil evidence (Fig. 6). Two of these studies[25,30] also produced stem group divergence time estimates that were incompatible with the fossil evidence (Fig. 6).

Discussion

Having thoroughly examined the Rhynie Chert mite specimens, we assigned them to a single species, *Protacarus crani* (Protoacaridae), and incorporated this species into a time-calibrated phylogenetic analysis (Fig. 5). This phylogeny suggests that stem-group Acariformes and the two major lineages, crown Trombidiformes and Sarcoptiformes, emerged within a relatively short time span (median estimates: 508–493 Ma) during the Cambrian. These estimates nearly coincide with the time of land colonization by non-vascular rootless plants (bryophytes) 515–470 Ma[47] and predate the establishment of complex soil profiles at 440 Ma[48] (Fig. 5).

The timing of the acariform mite origin and their ancestral habitats have been subjects of debate in the literature (Fig. 6). One hypothesis suggests a Precambrian origin, dating back to 571 Ma, with mite colonization of land occurring through interstitial habitats[24]. On the other hand, an alternative hypothesis proposes a Cambrian to Silurian origin of crown Acariformes (417–508 million years ago), linked to soils formed by early land ecosystems[23]. The former hypothesis relies on the assumption of a marine ancestor for mites, but multiple

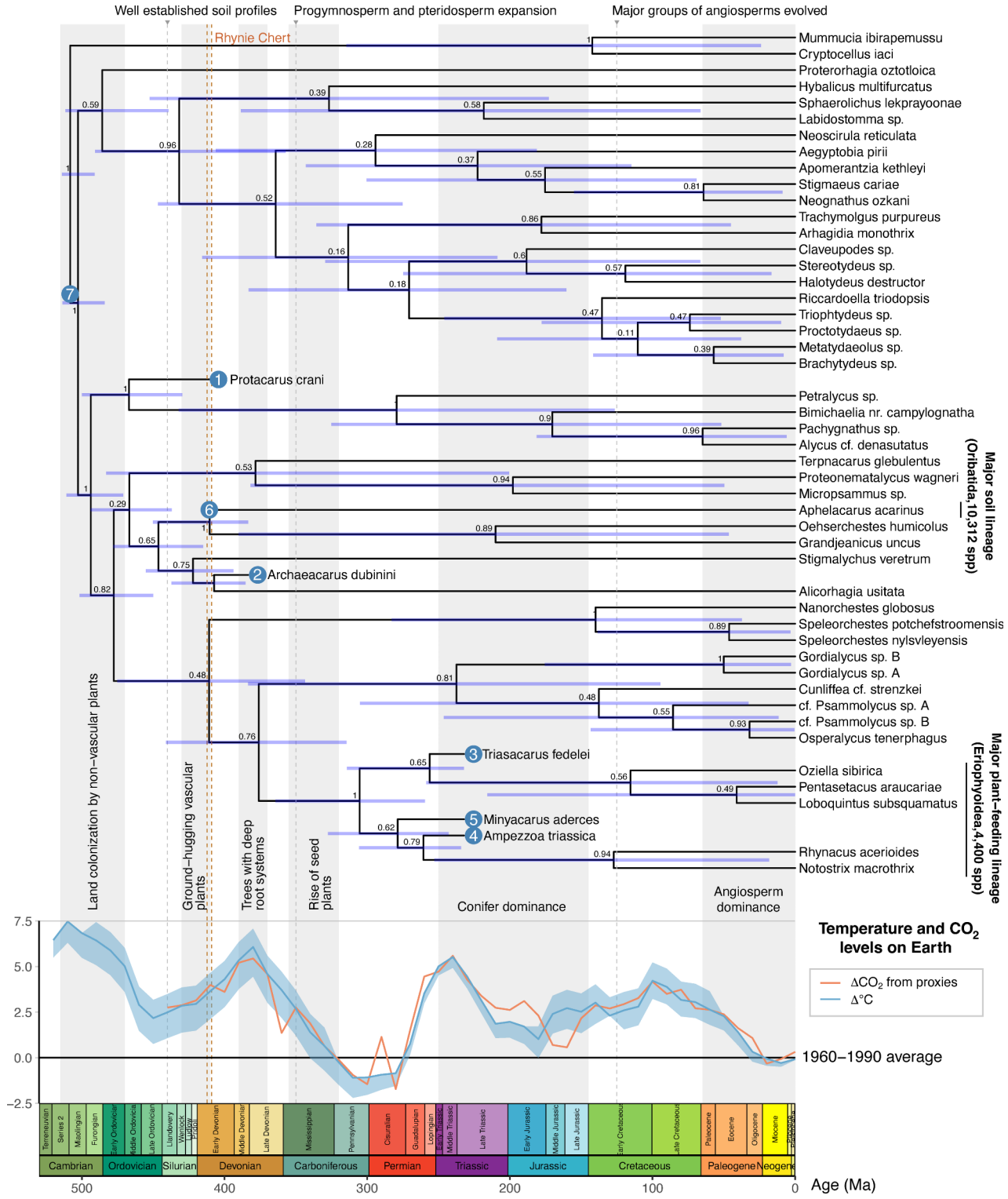

Fig. 5. Bayesian time-calibrated, maximum clade credibility phylogeny of Acariformes inferred by BEAST using morphology. Bars at nodes represent 95% HPD (highest posterior density) for node ages. Fossil calibration points (1–7) are listed in Table 1. Major events in Earth history are highlighted (see Supplementary Information 1: Supplementary Table 1 for source data and references). Adjusted temperature and carbon dioxide levels[79] are shown (see Source Data). Ages are assigned after the Internal Commission on Stratigraphy ICS2013[80]. °C, degree Celsius (temperature); CO_2, carbon dioxide; Δ, difference; sp., unidentified species.

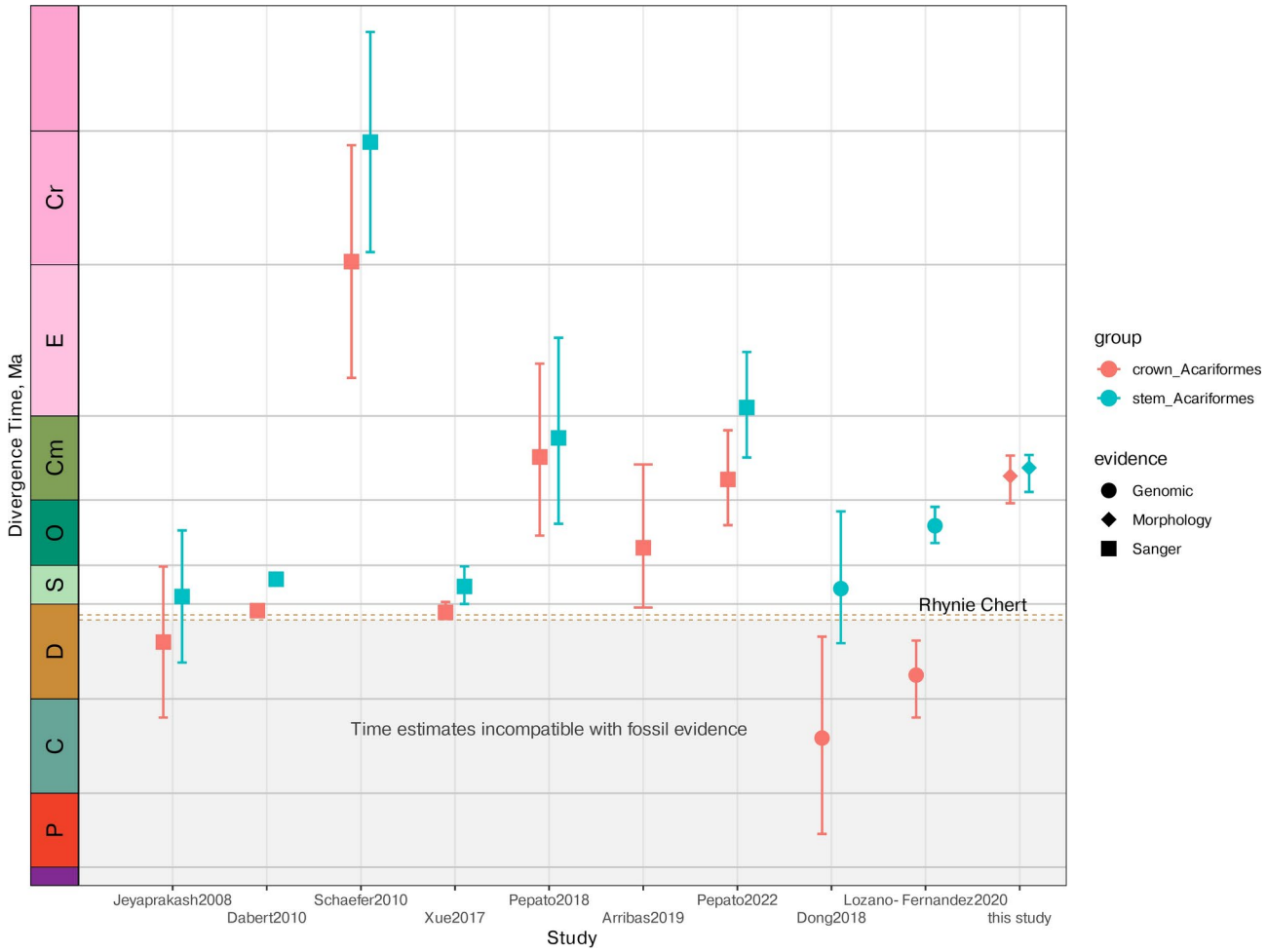

Fig. 6. Divergence time of the crown and stem-group Acariformes estimated by different studies. Estimates incompatible with respect to fossil evidence (*Protacarus crani*, Rhynie Chert) are in the grey area. For the data used to build the plot, see Supplementary Information 1: Supplementary Table 2. C, Carboniferous; Cm, Cambrian; Cr, Cryogenian; D, Devonian; E, Ediacaran; Ma, million years ago; O, Ordovician; P, Permian; S, Silurian.

studies have consistently indicated that mites are more closely related to terrestrial arachnid lineages, such as solifugids[30,49–51]. The divergence timing estimated by the latter hypothesis[23] broadly aligns with our estimates. However, the connection between mites and early upper soils as proposed by these authors is nuanced.

The Cambrian origin of crown Acariformes along with the relatively advanced morphology of the Rhynie Chert mite collectively indicate that a substantial diversity of acariform mites existed before the Early Devonian. While the Rhynie Chert represents a specialized hot spring environment, our divergence time estimates for early acariform mites incorporate multiple fossil calibration points, suggesting that mites were already adapted to diverse terrestrial habitats by this time. Although our time estimates imply that major basal acariform diversification coincided temporally with the land colonization by bryophytes (liverworts, hornworts, and mosses), it does not necessarily mean that mites were exclusive to upper soils, as suggested by Arribas et al.[23]. Based on observations of extant mites, a diverse mite fauna can be found in other habitats. Early derivative lineages within the Endeostigmata are common inhabitants of mosses (e.g., *Alicorhagia*, Alycidae, Nanorchestidae). Endeostigmata are also found in freshwater and marine interstitial habitats (Nanorchestidae, Nematalycidae) as well as in deep soils (e.g., *Stigmalychus*, *Alicorhagia*, Nematalycidae). Some of these deep soil nematalycid mites are likely to be microbial feeders rather than decomposers[52]. In this context, these mites could be integrated into soil food webs that rely, at least in part, on chemoautotrophic bacteria[53,54] rather than on photosynthetic plants. Fossil soils that could be potentially dominated by chemoautotrophic bacteria that utilize inorganic chemical compounds as their energy sources are known in the Precambrian[55]. The ecological communities dependent on chemoautotrophic prokaryotic producers were available for potential mite colonization in the Precambrian, much like they are today (although it is unlikely that mites existed at that time). Consequently, the ecological roles of acariform mites in early terrestrial ecosystems may be multifaceted and extend beyond the conventional perspective of mites functioning as primary decomposers in upper soil communities based on autotrophic (photosynthetic) producers. This aligns with a recent hypothesis that apulmonate arachnids, including mites, colonized land earlier and more rapidly than arachnopulmonates (e.g., scorpions, spiders),

potentially explaining their long branches[56] and supporting the idea that mites adapted to diverse terrestrial habitats before the rise of vascular plants.

Our study also presents a temporal framework that sheds light on the evolutionary history of the Eriophyoidea, a major group of plant-feeding mites that originated within the Endeostigmata. Eriophyoids are notable for their exceptional diversity, with approximately 4,400 described species[57]. To understand the evolution of these mites it is essential to unravel their ancient origins[58–60]. Fossil stem-group eriophyoids have been found in Triassic amber[58,61], dating back to approximately 227-237 Ma. These ancient mites are believed to have thrived on gymnosperm hosts, as angiosperms (flowering plants) had not yet emerged during that period[61]. This insight into their historical host associations is important for investigation into eriophyoids' evolutionary timeline and historical ecology. Our study estimated the divergence time of the crown group of Eriophyoidea to 305 Ma (260–364) Ma. Remarkably, this divergence nearly aligns with the emergence of early conifers, such as *Swillingtonia denticulata*, which appeared around 310 Ma[62]. This temporal congruence suggests a potential link between ancestral crown Eriophyoidea and early conifers, suggesting that stem-group Eriophyoidea (314–441 Ma) might have their hosts among progymnosperms and seed ferns (pteridosperms). Notably, in the case of progymnosperms, this hypothesis opens the door to the possibility of coevolution between ancestral eriophyoid mites and their plant hosts, as progymnosperms were the evolutionary lineage that led to seed plants[63]. Furthermore, our phylogenetic analysis offers an intriguing hypothesis regarding the shift in habitat during the evolution of stem-group eriophyoids. The majority of these mites are known for feeding on above-ground plant organs, yet their origins can be traced back to deep-soil, vermiform nematalycid ancestors[58–60]. This raises the question of how they transitioned from a subterranean existence to a surface-dwelling one. We propose that this transition may have occurred through an intermediate stage that involved feeding on plant root systems. This suggestion is particularly compelling given that the nematalycid-eriophyoid divergence (376 Ma) aligns temporally with the appearance of early arborescent plants that had well-developed deep root systems 390–370 Ma[47] (Fig. 5). It is remarkable that major milestones in the evolution of eriophyoids were correlated to the periods of warming in the Middle Devonian, Triassic, and Cretaceous, corresponding to the origin and diversification of conifers and angiosperms, respectively. These events are likely to be linked to a burst in the host plant diversification mediated by a warm climate and high carbon dioxide concentrations (Fig. 1).

When conducting a phylogenetic time-calibrated analysis, various factors can affect the precision of divergence time estimates. These factors include having a robust dataset that provides sufficient phylogenetic signal, representative taxon sampling, analytical methods, well-fitting clock, tree and substitution models, a sufficient set of accurately examined fossils used for time calibration purposes, and robust approaches to use fossil evidence to calibrate and constrain ages[64]. However, all else being equal, the accuracy of the fossil record directly impacts divergence time estimates. The placement of the earliest known acariform mites on the acariform tree of life has been ambiguous since the first description of *Protacarus crani* one hundred years ago by Stanley Hirst[14]. Our study presents a comprehensive re-evaluation of these records using modern technology and places them on the acariform tree through a robust phylogenetic analysis. We identified these fossils as sister taxon to the modern family Alycidae. Another fossil mite, *Archaeacarus dubinini*, a Middle Devonian endeostigmatid from the Gilboa locality of North America, was inferred here as sister taxon to the modern endeostigmatid genus *Alicorhagia*, refining its systematic placement beyond the initial assignment to Alicorhagiidae by Kethley et al.[15]. As extant species of Alycidae and *Alicorhagia* can be sequenced, this information may significantly enhance the precision of divergence time estimates in future molecular phylogenies. While this task still remains to be accomplished, here we utilize the fossils and our time-calibrated analysis to assess the accuracy of previous analyses. There are nine major studies, each using different sets of fossil calibration points and data types, such as Sanger sequenced genes, phylogenomics, or phylotranscriptomics (see Fig. 6). Inconsistencies between the estimated divergence date for stem-group Acariformes and the fossil evidence are noted above, including estimates younger than the age of *Protacarus crani* (Fig. 6). Our findings revise the known fossil record of several mite families. Specifically, the families Alycidae and Tydeidae now lack any confirmed fossil representatives; Alicorhagiidae lack Rhynie Chert records but retain the Devonian *Archaeacarus dubinini* as sister of *Alicorhagia*; while Nanorchestidae has no verified fossil record apart from *'Sarcoptes' kutchensis* from Middle Eocene Kutch amber, which was a misidentification and may also result from sample contamination.

Finally, a word of caution should be issued regarding the timing of acariform mite divergence. Our study is based on the earliest known Devonian mites. In Euramerica, from which most body fossil records are derived, there is a bias in terrestrial sediments being rare before the late Silurian. This contrasts with the extended microfossil record, which has a higher chance of preservation[12]. Given this preservation bias and the fact that our study suggests the presence of diverse mite lineages before the Early Devonian, the discovery of older mite fossils is anticipated. Therefore, it is possible that our time estimates may change when older fossils are found. In this context, the reported occurrence of an Ordovician brachypyline oribatid[65] is intriguing. However, the presence of modern mite lineages, such as Brachypylina, in the Ordovician is unexpected and the putative fossil mite was later recognized as a contaminant by Bernini (as cited in[66]). We therefore follow the general consensus and regard the Ordovician brachypyline mite as an artifact.

In conclusion, our study elucidates the taxonomic classification of the Rhynie Chert mites, enhances the precision of estimating the divergence times of early acariform mites, and provides insights into their evolutionary timeline in relation to the formation of early soils and associated communities of soil-dwelling decomposers. We resolve a long-standing question regarding the placement of earliest known mites from the Rhynie Chert as sister taxon to endeostigmatan family Alycidae and provide a comprehensive temporal framework for understanding the evolution of acariform mites. The Cambrian diversification of crown-group Acariformes temporally coincides with the time of land colonization by bryophytes. However, it is likely that ecological niches of these mites were more diversified and extended beyond upper soil in contrast to what is sometimes assumed in the literature. For example, early mite communities may trophically rely, fully or partially, on chemoautotrophic bacteria occurring

in deep soil. While early acariform mites probably lived among non-vascular rootless land plants, the modern mega-diverse soil lineage, Oribatida, originated in the Early Devonian, when land was dominated by ground hugging vascular plants. We highlight the ancient origin of the stem-group eriophyoids (plant-feeding mites) as they transitioned from deep soil-dwelling ancestors to above-ground plant feeders. Our study provides temporal context, improves the accuracy of fossil dating, and underscores the importance of mites' diverse habitats and their potential roles in soil food webs.

Material and methods

Imaging

Specimens were imaged in transmitted light using an Olympus BX63 microscope equipped with 10x, 20x (dry) and 40 × and 60x (water immersion) objectives. We took a series of stacked images (Z-axis) and a series of overlapping stacks (X, Y axes) using the automatic functionality of the microscope. Image stacks were assembled in Helicon Focus v7.5. Line drawings were prepared in Adobe Photoshop using the stacked images as a background.

Confocal images were captured using a Nikon A1Si confocal laser scanning microscope fitted to a Nikon Eclipse E upright microscope. Initial trials used the microscope's spectral detector to determine the fluorescence response from the samples at each laser excitation line. Fluorescence excitation was obtained over a range of 670-750 nm using 561 and 639 nm lasers. Once the optimal combination of laser excitation and fluorescence response from the sample was determined, high resolution data were then collected. Data for the figures were collected with either a 20 × Plan Apo lens with a 0.75numerical aperture or a 40 × Pan Fluor lens (0.75n.a.) using a 561 nm laser (Coherent Inc., USA) and two photo multiplier detector channels set at 570-620 nm (corresponding to the software preset for Texas Red) and 663-738 nm (corresponding to the software preset for Alexa 647). The pinhole was set to 43µm and between 82 and 132 slices were collected at a resolution of 0.22µm per pixel in the X/Y dimension and 0.72–0.8µm in the z-dimension. Frames were averaged over 4 or 8 frames to improve the signal to noise ratio in the final image. The lower magnification lens was used to provide a larger field of view.

Images were processed using the Nikon NIS-Elements software and data were collected as Maximum Intensity Profile (MIP) images (where the brightest pixel is selected at any X/Y position within the z stack) or as re-orientated views (by tilting the Z-stack) and displayed as either MIP or height coded images where the depth position of the pixel in the z-stack is encoded using a rainbow colour code with blue tones corresponding to regions closest to the sample surface and red tones corresponding to regions furthest from the surface.

Specimen label information

Hirst's slide label information (see below) is arranged as follows:

(1) Specimen number—original Hirst's specimen designation (Hirst, 1923, p. 459) and figure reference; (2) NHMUK PI IA 2466[#] – museum accession (Natural History Museum, London, UK); *Binomial name*—name used by Dubinin[32]; (3) Label Left/Right—Label information. Where AMNH(9)XII (and similar abbreviations) refers to the original publication, Annals & Magazine of Natural History. Ser.9,Vol.12; (4) Areas—areas mapped on the Rhynie Chert chip photograph (provided here) to show different specimens inside in the same chip; (5) Specimen/areas on chip examined here (a statement).

Specimen 1, Pl. XIa. *Pseudoprotacarus scoticus*. Not in NHMUK, originally stated that specimen is in Cran collection[14]. The repository is likely to be NHMUK or National Museum of Scotland[67]. However, according to S. F. Morris, the specimen was unfortunately lost by Hirst before registration in the BM(NH) collections (currently NHMUK)[68]. Specimen not found in NHMUK or National Museum of Scotland, not examined.

Specimen 2: XIb. NHMUK PI IA 24665 *Protacarus crani*. Left labels (3): "Type", "In. 24665" (yellow label), no text (green circular label). Right label: *Protacarus crani* Hirst HOLOTYPE, Old Red Sandstone, Rhynie Chert Bed, Aberdeenshire fig., Pl XI. $AMNH_9$ XII, Cran Coll No 58. Areas 1, 2, 3 (designated here, Supplementary Fig. 3a). Area 1 = Hirst' specimen 2, Fig. XIb, and slide labels refer to Area 1. Areas 2, 3 were not examined previously. Areas 1–3 examined here.

Specimen 3: text-fig. 1. NHMUK PI IA 24666 *Palaeotydeus devonicus*. Left labels (2): "In. 24666" (yellow label), no text (green circular label). Right Label: P.T.O., Old Red Sandstone, Rhynie Chert Bed, Aberdeenshire, no 3, $AMNH_{(9)}$ XII, p.456, text fig. 1 [illegible]. Examined.

Specimen 4: text-fig. 2. NHMUK PI IA 24667 *Paraprotacarus hirsti*. Left labels (3): "Rhynie Chert. slide 65, mite 4, 20.6.21, W. Cran", "In. 24667" (yellow label), no text (green circular label). Right label:, Old Red Sandstone, Rhynie Chert Bed, Aberdeenshire, no. 4, $AMNH_9$ XII, Cran Coll, text fig. 2. Examined.

Specimen 5: text-fig. 1a. NHMUK PI IA 24668. *Palaeotydeus devonicus*. Left labels (2): "In. 24668" (yellow label), no text (green circular label). Right label: No. 5. *Protacarus*, fragment, Old Red Sandstone, Rhynie Chert Bed, Aberdeenshire, Ann. Mag. N.H. (9)XII, $p.45_7$, Fig. 1a, Cran Coll. Examined. Area 0 = single leg described by Hirst in text-fig. 1a, not found on slide (may be overlooked by us); Area 1 = fragments new species, not described here because incomplete. Area 0 not examined; Area 1 examined.

Specimen 6: text-fig. 1b. NHMUK PI IA 24669 *Protospeleorchestes pseudoprotacarus*. Left labels (3): "Specimen No. 6, text-fig. 1b, Ann. Mag. Nat. Hist. 9 XII.1924", "In. 24669" (yellow label), no text (green circular label). Right label: P.T.O. Hirst., fan shaped hairs, Old Red Sandstone, Rhynie Chert Bed, Aberdeenshire, Cran Coll, No 42. Hirst reported only dorsal setae (text fig. 1b), however, many other mite fragments are present in this sample. Examined (whole sample).

Additional Rhynie Chert specimens

Two additional mite specimens were collected by a palaeobotanical group from the University of Münster, and they are only known from microphotographs[4]. This team reported (pers. comm.) that they are in the process of cataloging and organizing their specimens, and these mites cannot be easily located at the moment.

id	fossil	age (My)	Calibration type	Reference
1	*Protacarus crani*	410.7	Tip	Rhynie Chert: 412.3—409.1[67]
2	*Archaeacarus dubinini*[15]	383.5	Tip	Uppermost Panther Mountain strata, Gilboa: 383—384
3	*Triasacarus fedelei*[61]	232.1	Tip	Carnian: 237.0—227.3[80]
4	*Ampezzoa triassica*[61]	232.1	Tip	Carnian: 237.0—227.3[80]
5	*Minyacarus aderces*[61]	232.1	Tip	Carnian: 237.0—227.3[80]
6	*Protochthonius gilboa*[78]	383.5–500.5	Node = Oribatida stem, prior = Uniform: lower–upper	Lower: Stem group Enarthronota 500.5[26]. Upper: Uppermost Panther Mountain strata, Gilboa: 383-384
7	Acariformes (stem)	410.7–514.0	Node = Acariformes_root, prior = Uniform: lower–upper	Lower: Opiliones (Crown) 514[26]. Upper: Rhynie Chert: 412.3—409.1[67]

Table 1. Fossil calibrations used for divergence time inferences of Acariform mites.

Terminology and notations in morphological descriptions

Measurements were taken between furthest points of a structure and are given in micrometers (μm). Formulas for leg and palp setation are given as follow: trochanter–basifemur–telofemur–genu–tibia–tarsus (if the femur is split into basifemur and telofemur) or trochanter–femur–genu–tibia–tarsus (femur is entire). Morphological terminology used in this paper follows that of Walter[69].

The following abbreviations were used: Prodorsum: *ro*, *le*, *in*, *exa*, *exp*, *bo* = rostral, lamellar, interlamellar, anterior exobothridial, posterior exobothridial and bothridial setae, respectively. Opisthosoma: *c*, *d*, *f*, *h*, *ps* = gastronotic setae. Gnathosoma: *or* = adoral seta; *sup*, *inf*, *d*, *l* = palp setae; *Rut* = rutellum; Pp = palp; Ch = chelicera; *cha*, *chb* = cheliceral setae. Epimeral and lateral podosomal regions: *ep.s.* = epimeral setae. Anogenital region: *g*, *eg*, *an*, *ad* = genital, eugenital, anal and adanal setae, respectively; *g.p.* = genital papilla. Legs: *Tr*, *Fe*, *Ge*, *Ti*, *Ta* = leg trochanter, femur, genu, tibia, and tarsus, respectively; *emp* = empodia; *l.c.* = lateral claws; ω, φ, σ = tarsal, tibial, genual solenidia, respectively; ε = famulus; *d*, *l*, *v*, *bv*, *ev*, *ft*, *p*, *u*, *a*, *s*, *m*, *it*, *tc*, *pv* = leg setae (dorsal, lateral, ventral, basiventral, external ventral, fastigial, proral, unguinal, anterolateral, subunguinal, medial, iteral, tectal and primiventral, respectively), palp. In addition, in the text we used the following notations: (+) = tentative homology. Drawings have the following colour conventions and notations: red = tentatively identified structures (may be mite or non-mite); green = apparent artifacts; blue = apparent mite structures; black-purple-orange = color codes for Z-axis offset and overlapping structures, where black = surface; purple = depth 1 (closer to surface); orange = depth 2 (deeper); arrowhead = gastronotic seta. In taxonomic redescriptions, the presence of a structure in at least one specimen is interpreted as its presence; the largest setal count on a structure is considered accurate. Tentatively identified structures (red colour in figures) are omitted. Images were processed in Adobe Photoshop 2020.

Phylogenetic inference and time calibration

We updated a previously published morphological data matrix[58] to include *Protacarus crani* (Devonian, Rhynie Chert) and *Archaeacarus dubinini* (Devonian, Gilboa)[15]. Also, four character states in the outgroups were modified. Our data matrix contained 52 taxa and 110 characters (Supplementary Information 2). We treated all characters as unordered. A maximum parsimony with parsimony ratchet analysis was done in PAUP v4a168[70] and PAUPRat (Supplementary Information 2: nexus data matrix with a character listing and Ratchet commands; Supplementary Information 3: Strict and majority-rule consensus trees). Simultaneous topology inference and divergence time estimation was done in a Bayesian framework using BEAST v2.7.0[71] (Supplementary Information 4: input file; Supplementary Information 5: Timetree). For this analysis, we used a combination of tip-dating (*Protacarus, Archaeacarus, Triasacarus*, *Ampezzoa*, *Minyacarus*) and node dating (*Protochthonius*, acariform root) (see Table 1 for more detail), the birth and death speciation model, optimized relaxed clock, and four MRCA priors enforcing monophyly (Acariformes, Sarcoptiformes, *Archaeacarus* + *Alicorhagia*, *Protacarus* + Alycidae). The use of these priors was based on the results of the maximum parsimony analysis and was necessary since the use of a Bayesian clock model artificially placed the Devonian fossils closer to the root (a conflict with the morphology). Using age constraints is strongly advised in the literature because unconstrained tip calibration analyses may be biased toward older estimates[72–75]. Our BEAST analysis was summarized in TreeAnnotator[76], using median heights. This phylogeny was plotted in R using the function coord_geo of the deeptime R package to plot the geological scale[77] along with other metadata using a custom script (Supplementary Information 6). In analyses and text, we standardized fossil dates using the Fossilworks database[67] and other sources (Table 1). In the text, ages are presented as median height and 95% highest posterior density interval.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Data availability All fossils are curated at the fossil collections of the Natural History Museum, London, UK. The data underlying this article are available in its online Supplementary Information. Source data are provided with this paper.

Code availability

The codes used for the study are provided in the Supplementary Information files 6, 7. Phylogenetic analyses were performed in PAUP v4a168 (http://phylosolutions.com/paup-test/), PAUPRat (https://people.iab.uaf.edu/derek_sikes/software2.htm) and BEAST v2.7.0 (https://github.com/CompEvol/beast2/releases/tag/v2.7.0).

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Author contributions
P.B.K. designed this study, imaged specimens using light microscopy, wrote R scripts, performed analyses, prepared Figs. 5 and 6, reviewed taxonomic descriptions and figures, and wrote and revised the manuscript; V.B.K. prepared taxonomic descriptions and figures; D.D.V. imaged specimens using light microscopy; A.D.B. imaged specimens using confocal microscopy; Q.H. wrote R scripts; all co-authors contributed to discussion and drafting the manuscript.

Competing interests
The authors declare no competing interests.

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