

A chelicera-bearing arthropod reveals the Cambrian origin of chelicerates

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 2:

Reviewer comments:

Referee #1

(Remarks to the Author)

I am delighted to provide a review for “Show your hand – new chelicera-bearing arthropod illuminates the Cambrian origins of chelicerates” by Rudy Lerosey-Aubril and Javier Ortega-Hernández for Nature. The manuscript describes a new genus and species of chelicerate arthropod based on a single exceptionally preserved specimen from the Cambrian Wheeler Formation of Utah and places the new taxon within a phylogenetic analysis, where it resolves as a stem chelicerate under a variety of different optimality criteria. This discovery represents the oldest unequivocal record of Chelicerata, provides important information regarding the chelicerate ground plan and as such early patterns of evolution within the group, and resolves a plethora of debates surrounding the affinities of numerous Cambrian taxa suggested to exhibit affinities to chelicerates including habeliids, sanctacariids, and mollisoniids.

Chelicerates are a diverse and important group of arthropods representing one of the major fundamental divisions within Arthropoda defined by, as the name suggests, the possession of a pair of appendages modified into pincer-like claws (later further modified into piercing fangs in some arachnids) anterior to the mouth. The oldest known members of the various chelicerate groups (either Cambrian or Silurian for pycnogonids; Ordovician for horseshoe crabs, eurypterids, and chasmataspidids; Silurian for a variety of arachnid clades) already exhibit highly derived morphologies making it impossible to accurately determine the ancestral chelicerate morphology. This uncertainty is compounded by an inability to conclusively identify the chelicerate sister-group, with researchers being divided between favouring megacheirans (which have enlarged raptorial frontal appendages) and artiopods (which possess antennae) as the closest chelicerate relatives. Not knowing the identity of the chelicerate sister-group in turn increases the difficulty of recognizing members of the chelicerate stem lineage; we essentially have no comparative framework from which to draw to know what morphologies to look for. Discoveries of exceptionally preserved specimens are needed if we have any hope of conclusively resolving the origin and early evolution of chelicerates. This manuscript presents such a specimen.

The new specimen, named *Megachelicerax cousteui*, preserves the head region and the majority of the trunk, including details of the limbs. There is no doubt that this is a chelicerate; a pair of elongate chelicerae are preserved extending anteriorly from underneath the head shield. These represent the oldest preserved unequivocal chelicerae; as noted in the manuscript, marginally older chelicerae reported from the Burgess Shale arthropod *Mollisonia* have not been universally accepted as such. It is this preservation of the chelicerae that separates this new species from other proposed stem chelicerates; neither *Sanctacaris* nor *Habelia* preserve chelicerae, and as noted the chelicerae described from *Mollisonia* are small, located far anterior to the oral region, and several researchers have expressed uncertainty regarding the identity of the structures. A chelicerate identity for *Megachelicerax* is confirmed as a chelicerate through phylogenetic analysis performed under a variety of optimality criteria, resolving the taxon as sister to all other euchelicerates under Bayesian inference and a variety of parsimony treatments. The only slight oddity in the results is the placement of pycnogonids nested within Euchelicerata, thereby rendering euchelicerates paraphyletic and making Euchelicerata and Chelicerata synonyms. The position of pycnogonids is highly unusual and contra to prevailing evidence, and it would be good for the

authors to comment on to what degree they believe this placement to be accurate. The analysis may be improved by including some more euchelicerates – particularly eurypterids and arachnids – in order to see if doing so influences the results with regard to pycnogonids and to provide a more general sampling of crown group euchelicerates beyond horseshoe crabs. It would also be beneficial to see the parsimony tree retrieved through analysis under equal weights to compare with the Bayesian and implied weights trees.

These minor suggestions regarding the phylogeny aside, the manuscript stands as an excellent description of *Megachelicerax* which resolves many outstanding issues regarding early chelicerate evolution. The presence of chelicera as a plesiomorphic trait for euchelicerates supports megacheirans as part of total group Chelicerata to the exclusion of arthropods, supporting previous analyses of exceptionally preserved neural tissues in these groups and convincingly determining the identity of the chelicerate sister-group. The morphology of the chelicera, comprising three segments – the first of which is greatly elongated – indicates that the composition of three articles is the plesiomorphic condition and that offacoliids, which possess elongate chelicera that appear to be comprised of five or six segments, possess an independently derived condition not indicative of the chelicerate ground plan. One thing of note when the authors discuss other elongated chelicera is the condition in pterygotid eurypterids, the insertion of which appears very similar to those in *Megachelicerax* and were also suggested by Kjellesvig-Waering to comprise more than three segments (but have subsequently been comprehensively shown to consist of the usual three). Similarly, the form of the prosomal and abdominal appendages also indicate that the morphology of *Mollisonia* is also not fully indicative of the ancestral condition. These insights are critical to our understanding of the early evolution of this important clade and are invaluable for identifying other stem chelicerates, including among specimens that are not exceptionally preserved.

The authors present much discussion in the supplemental material, all of which is pertinent and of import. Here they address my one major concern I had when first reading the manuscript, which was the apparent similarity between *Megachelicerax* and *Messorocaris*, another potential chelicerate relative from the same formation. In their discussion the authors present sufficient justification for the two being distinct taxa; it is worth noting that, even if the two were found to be synonyms, the conclusions of this manuscript would not change, rather the specimen would just have to be transferred to *Messorocaris* and the name *Megachelicerax* rendered a junior synonym. My only other query is regarding the morphology of the endopods, which are reconstructed as terminating in a pair of terminal claws in figure 2 but a single terminal claw in figure 3. The morphology of this termination is interesting as, if pair, it may provide a hint as to the origins of the chelate endopod limbs in offacoliids and xiphosurans. Extended figure 4n appears to clearly show paired terminal claws. This is mentioned briefly in the description of the specimen but I wonder whether the implications of this could be expanded somewhat; the authors note that these claws are non-chelate, which is itself interesting as it is then reminiscent of the tarsal claws of terrestrial arachnids.

This is an exciting and important manuscript that will be of broad interest to all arthropod researchers. I look forward to this specimen being published; its discovery is potentially transformational for our understanding of chelicerate evolution and allows for recontextualization of numerous problematic chelicerate-like fossils from the early Paleozoic.

Referee #2

(Remarks to the Author)
See Word document.

Referee #3

(Remarks to the Author)

My report is brief. This very well preserved fossil unequivocally possesses chelicerae, and indeed three-segmented ones. With a sound description and phylogenetic analysis pinning it down in the chelicerate stem-group, this “smoking gun” character is Nature-worthy. The reinterpretation of other chelicerate-allied Palaeozoic fossils in light of the new genus adds extra value to the paper.

The term “habeliid” (or “habeliid-like”) is used throughout the paper (lines 19, 45, 170, etc.) but has the potential to confuse, particular since another name, “habeliidan”, is used in the trees in Extended Data Figure 6. “Habeliid” could be read as encompassing all of Habeliida Aria and Caron, 2017 (i.e., Habeliidae + Sanctacarididae), or only Habeliidae Simonetta and Delle Cave, 1975. The former was named in the context of it having been conceived as monophyletic but in the present paper it corresponds to a grade (Extended Data Figure 6b) or an ambiguous metataxon collapsed in a polytomy (Figure 3 and Extended Data Figures 6a). Upon first usage of “habeliid” I’d prefer to see a formal taxon name, and take care to ensure that the reader knows exactly what this means in every instance.

Line 26 in the abstract probably overstates the case for megacheirans being total-group Chelicerata. “Confirms” is a strong word for a node with a posterior probability in the 0.50s that is so close to the root of the tree that alternative phylogenetic positions are not very severely tested (e.g., taxa that could pull megacheirans down into the euarthropod stem group, such as radiodonts and *Kylinxia*, are not included). For the other two groups mentioned in line 26, the case is adequate (being an extra node or two crownward). Speaking of the “0.50s” mentioned above, change all node support values in the Bayesian trees to decimals (0.59 rather 59), as they are

reported on a 0 to 1 scale rather than as percentages like bootstrap values.

In line 1, delete “Show your hand”.

In line 10, replace “acari” with “mites” for stylistic consistency with “scorpions” and “spiders”.

In Extended Data Figure 2b, there is a reddish-brown colour applied to a bilaterally symmetrical metamer structure flanking the opisthosomal midline. It doesn’t seem to be explained in the colour scheme explanation.

In the caption to Extended Data Figure 3, change all instances of “actual” to “extant”.

The artwork in Figure 2b should, in my opinion, be left for a press release to capture the public’s imagination. Really, why would we think the exopods are yellow and the endopods alternating blue and red? I am in a minority I suppose, but I’d prefer that PalaeoArt be replaced with real morphology, like details of the opisthosomal appendages that are currently relegated to Extended Data. The artwork is perfectly fine in the tree in Figure 3, but it’s taking up valuable space in Figure 2.

Character 8 in the character descriptions in Supplementary Note 7 could use a note to explain what the character states are (i.e., raw integers for the number of segments) and their range.

Version 3:

Reviewer comments:

Referee #1

(Remarks to the Author)

It was a delight to read this revised manuscript on an early Cambrian chelicerate. I thank the authors for addressing my queries and look forward to seeing this important contribution published. I feel that the revised manuscript presents a fairly comprehensive view of the current knowledge regarding the early evolution of chelicerates and accurately places the importance of the new find into a historical context. I only have one additional suggestion relating to lines 297-306 in the manuscript where the homology of the gill opercula and gill plates is discussed, where for completeness it may be worth stating that the gill opercula are likely homologous to the external sternites in tetrapulmonate arachnids (see Shultz 1999 [<https://doi.org/10.1111/j.1096-3642.1999.tb00608.x>] and Farley 2001 [<https://doi.org/10.1080/07924259.2001.9652719>]).

Overall I think this is an excellent paper describing an exciting discovery that clarifies the early evolution of a major arthropod group and will help recontextualize our understanding of numerous existing fossil taxa as well as providing a framework for comparison of future discoveries.

James C. Lamsdell

Referee #2

(Remarks to the Author)

I find that the manuscript has been improved by the authors. I also appreciate most of the suggestions from the other reviewers and the corresponding edits. Strictly speaking with respect to my previous review, the authors have addressed almost all of my comments, and in cases where they did not, they provided satisfactory reasoning.

I have only one remaining suggestion regarding our disagreement on the naming of the prosomal exopods. In your response, you stated:

This claim is not supported by the literature

on early chelicerates. As documented in Supplementary Note 3, no consensus has been reached over the past 25+ years on how to refer to this atypical exopodal morphotype. Terms as varied as “antenna-like” (Briggs & Collins, 1988), “antennule-like” (Aria & Caron, 2017a), “flagelliform” (Boxshall, 2004), “filiform” (Dunlop & Lamsdell, 2017), “pediform” (Legg, 2014; Dunlop & Lamsdell, 2017), and “stenopodous” (Lustri et al., 2024a) have been used, sometimes even within the same publication. There is therefore no “reasonably well-established” terminology to follow.

I believe it would be helpful to include a small table in the supplementary material summarizing the different nomenclatures that you propose to unify under the term tubuliaceous. This would clarify some of the confusion and help support the adoption of the new name you are suggesting. Note that stenopodous was first used by Aria & Caron (2017), not by Lustri et al. (2024a).

Lorenzo Lustri

Referee #3

(Remarks to the Author)

The authors have addressed all points raised in my original review to my satisfaction. I shall not weigh in on their responses to suggestions by referees 1 and 2 in detail except to say they have, in my opinion, been most conscientious in taking feedback on board. The addition of eurypterids and fossil scorpions to the taxon sampling in the phylogenetic analyses is a useful refinement, and I think they have done a good job diluting the impression that pycnogonids are resolved in an unexpected position; Extended Data Figure 6 shows that pycnogonids are labile between different analytical conditions, under some weighting schemes resolving stemward of synziphosurans.

I am happy to sign off on this version of the manuscript for publication.

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RESPONSE TO REFEREES

1. RESPONSE TO REFEREES

All changes made to the files composing our manuscript are highlighted in light cream to facilitate their identification below.

Referee 1

I am delighted to provide a review for “Show your hand – new chelicera-bearing arthropod illuminates the Cambrian origins of chelicerates” by Rudy Lerosey-Aubril and Javier Ortega-Hernández for Nature. The manuscript describes a new genus and species of chelicerate arthropod based on a single exceptionally preserved specimen from the Cambrian Wheeler Formation of Utah and places the new taxon within a phylogenetic analysis, where it resolves as a stem chelicerate under a variety of different optimality criteria. This discovery represents the oldest unequivocal record of Chelicerata, provides important information regarding the chelicerate ground plan and as such early patterns of evolution within the group, and resolves a plethora of debates surrounding the affinities of numerous Cambrian taxa suggested to exhibit affinities to chelicerates including habeliids, sanctacariids, and mollisoniids.

Chelicerates are a diverse and important group of arthropods representing one of the major fundamental divisions within Arthropoda defined by, as the name suggests, the possession of a pair of appendages modified into pincer-like claws (later further modified into piercing fangs in some arachnids) anterior to the mouth. The oldest known members of the various chelicerate groups (either Cambrian or Silurian for pycnogonids; Ordovician for horseshoe crabs, eurypterids, and chasmataspidids; Silurian for a variety of arachnid clades) already exhibit highly derived morphologies making it impossible to accurately determine the ancestral chelicerate morphology. This uncertainty is compounded by an inability to conclusively identify the chelicerate sister-group, with researchers being divided between favouring megacheirans (which have enlarged raptorial frontal appendages) and artiopods (which possess antennae) as the closest chelicerate relatives. Not knowing the identity of the chelicerate sister-group in turn increases the difficulty of recognizing members of the chelicerate stem lineage; we essentially have no comparative framework from which to draw to know what morphologies to look for. Discoveries of exceptionally preserved specimens are needed if we have any hope of conclusively resolving the origin and early evolution of chelicerates. This manuscript presents such a specimen.

The new specimen, named Megachelicerax cousteui, preserves the head region and the majority of the trunk, including details of the limbs. There is no doubt that this is a chelicerate; a pair of elongate chelicerae are preserved extending anteriorly from underneath the head shield. These represent the oldest preserved unequivocal chelicerae; as noted in the manuscript, marginally older chelicerae reported from the Burgess Shale arthropod Mollisonia have not been universally accepted as such. It is this preservation of the chelicerae that separates this new species from other proposed stem chelicerates; neither Sanctacaris nor Habelia preserve chelicerae, and as noted the chelicerae described from Mollisonia are small, located far anterior to the oral region, and several researchers have expressed uncertainty regarding the identity of the structures. A chelicerate identity for Megachelicerax is confirmed as a chelicerate through phylogenetic analysis performed under a variety of optimality criteria, resolving the taxon as sister to all other euchelicerates under Bayesian inference and a variety of parsimony treatments. The only slight oddity in the results is the placement of pycnogonids nested within Euchelicerata, thereby rendering euchelicerates

paraphyletic and making *Euchelicerata* and *Chelicerata* synonyms. **[Query 1] The position of pycnogonids is highly unusual and contra to prevailing evidence, and it would be good for the authors to comment on to what degree they believe this placement to be accurate.** **[Query 2] The analysis may be improved by including some more euchelicerates – particularly eurypterids and arachnids – in order to see if doing so influences the results with regard to pycnogonids and to provide a more general sampling of crown group euchelicerates beyond horseshoe crabs.** **[Query 3] It would also be beneficial to see the parsimony tree retrieved through analysis under equal weights to compare with the Bayesian and implied weights trees.**

These minor suggestions regarding the phylogeny aside, the manuscript stands as an excellent description of *Megachelicerax* which resolves many outstanding issues regarding early chelicerate evolution. The presence of chelicera as a plesiomorphic trait for euchelicerates supports megacheirans as part of total group *Chelicerata* to the exclusion of artiopods, supporting previous analyses of exceptionally preserved neural tissues in these groups and convincingly determining the identity of the chelicerate sister-group. The morphology of the chelicera, comprising three segments – the first of which is greatly elongated – indicates that the composition of three articles is the plesiomorphic condition and that offacoliids, which possess elongate chelicera that appear to be comprised of five or six segments, possess an independently derived condition not indicative of the chelicerate ground plan. **[Query 4] One thing of note when the authors discuss other elongated chelicera is the condition in pterygotid eurypterids, the insertion of which appears very similar to those in *Megachelicerax* and were also suggested by Kjellesvig-Waering to comprise more than three segments (but have subsequently been comprehensively shown to consist of the usual three).** Similarly, the form of the prosomal and abdominal appendages also indicate that the morphology of *Mollisonia* is also not fully indicative of the ancestral condition. These insights are critical to our understanding of the early evolution of this important clade and are invaluable for identifying other stem chelicerates, including among specimens that are not exceptionally preserved.

The authors present much discussion in the supplemental material, all of which is pertinent and of import. Here they address my one major concern I had when first reading the manuscript, which was the apparently similarity between *Megachelicerax* and *Messorocaris*, another potential chelicerate relative from the same formation. In their discussion the authors present sufficient justification for the two being distinct taxa; it is worth noting that, even if the two were found to be synonyms, the conclusions of this manuscript would not change, rather the specimen would just have to be transferred to *Messorocaris* and the name *Megachelicerax* rendered a junior synonym. **[Query 5] My only other query is regarding the morphology of the endopods, which are reconstructed as terminating in a pair of terminal claws in figure 2 but a single terminal claw in figure 3. The morphology of this termination is interesting as, if pair, it may provide a hint as to the origins of the chelate endopod limbs in offacoliids and xiphosurans. Extended figure 4n appears to clearly show paired terminal claws. This is mentioned briefly in the description of the specimen but I wonder whether the implications of this could be expanded somewhat; the authors note that these claws are non-chelate, which is itself interesting as it is then reminiscent of the tarsal claws of terrestrial arachnids.**

This is an exciting and important manuscript that will be of broad interest to all arthropod researchers. I look forward to this specimen being published; its discovery is potentially transformational for our understanding of chelicerate evolution and allows for recontextualization of numerous problematic chelicerate-like fossils from the early Paleozoic.

We are grateful to Referee 1 for their thoughtful and enthusiastic assessment of our manuscript. We are pleased that they found the description of *Megachelicerax* and its evolutionary significance compelling, and we particularly appreciate their recognition of the broader implications of this new taxon for resolving long-standing debates on early chelicerate evolution. Our replies to the five queries are presented below.

[Query 1] *The position of pycnogonids is highly unusual and contra to prevailing evidence, and it would be good for the authors to comment on to what degree they believe this placement to be accurate.*

Most specialists consider that pycnogonids (sea spiders) form the sister group to all other chelicerates, or Euchelicerates (i.e., Xiphosura and Arachnida). However, pycnogonids are occasionally resolved in markedly different positions within the chelicerate tree, especially when palaeontological data are included. In the morphology-based analyses of Garwood & Dunlop¹ and Garwood et al.², both of which incorporated fossil taxa, pycnogonids formed a clade with Arachnida alone, and this clade was recovered as sister to Xiphosura. In the mitochondrial-gene phylogeny of Ovchinnikov and Masta³, pycnogonids were deeply nested within Arachnida. Conversely, Aria & Caron⁴ recovered pycnogonids in the most stemward position on the chelicerate lineage – more basal even than the chelicera-lacking habeliids – although those authors also noted in their supplementary discussion that “*pycnogonids may [therefore] lie much more crownward than their “simplified” morpho-anatomy suggests.*”

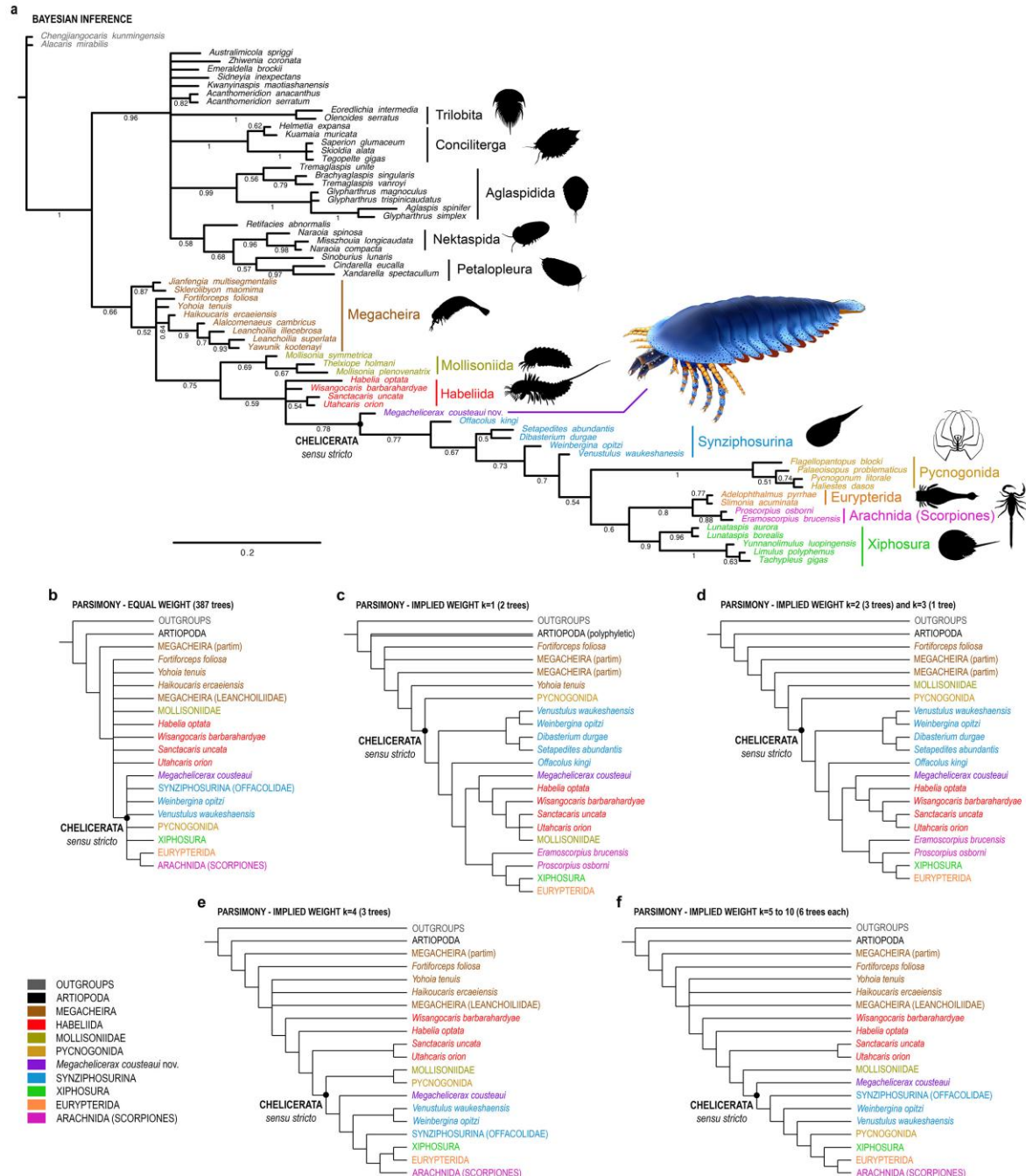
The occasional crownward placement of pycnogonids in phylogenetic analyses has been tentatively interpreted as an artefact of long branch attraction (e.g., with another fast-evolving group such as Acari)⁵. Pycnogonids indeed differ drastically from other extant and fossil chelicerates in several key anatomical features, such as the absence of a true opisthosoma in modern representatives, the segmental distribution and respiratory role of the legs, the extensions of internal organs (e.g., gut diverticulae, gonads) into those limbs, and the presence of a proboscis^{6–8}. It may be tempting to interpret these pronounced differences as the result of a long independent evolutionary history following an early split from the chelicerate lineage. However, pycnogonids also possess dorsal eyes and uniramous appendages, highly derived features known only in xiphosurans, eurypterids, and arachnids, which explains the crownward position of sea spiders in our phylogeny. In fact, the hypothesis of an early divergence of pycnogonids would not simplify the evolutionary scenario depicting the assemblage of the chelicerate body plan, as it would require independent origins of the dorsal eyes and uniramous appendages – two major anatomical traits – in pycnogonids and in derived euchelicerates.

The discovery of *Megachelicerax* significantly clarifies important aspects of the early chelicerate evolution, but not the debated issue of the phylogenetic placement of pycnogonids along the chelicerate lineage.

⇒ **Accordingly, we refrain from expressing a firm opinion on this matter in our manuscript, especially since our attempt to include additional crown-group chelicerates in our analysis failed to clarify this point (see below).**

[Query 2] *The analysis may be improved by including some more euchelicerates – particularly eurypterids and arachnids – in order to see if doing so influences the results with regard to pycnogonids and to provide a more general sampling of crown group euchelicerates beyond horseshoe crabs.*

The primary objective of our phylogenetic analysis was to determine the position of *Megachelicerax* within the chelicerate lineage relative to other (potential) early representatives, which explains our focus on the stem of this group. To assess whether our dataset could also help resolve the problematic phylogenetic placement of pycnogonids, we followed Referee 1's suggestion and added two eurypterids, two Paleozoic scorpions, and the recently described xiphosurid *Lunataspis borealis*. The resulting phylogenetic trees are presented in the revised Extended Data Figure 6:



The phylogenetic placement of pycnogonids – and, accordingly, the composition of Euchelicerata – varies markedly across analyses, with three main hypotheses emerging:

- 1) *Crownward placement*. Pycnogonida occupies a relatively crownward position within the chelicerate lineage, forming a clade with Xiphosura, Eurypterida, and Arachnida (Scorpiones), as in the trees recovered by the Bayesian inference analysis (panel a) and by implied-weights parsimony analyses with high concavity values ($k = 5\text{--}10$; panel f). A possible variant of this topology, with almost no internal resolution among chelicera-bearing taxa, appears under equal-weight parsimony (panel b).
- 2) *Moderately stemward placement*. Pycnogonida is sister to a clade uniting chelicera-bearing taxa, as recovered in the implied-weights parsimony analysis with $k = 4$ (panel e). A possible variant of this topology, with almost no internal resolution among chelicera-bearing taxa, appears under equal-weight parsimony (panel b).
- 3) *Strongly stemward placement*. Pycnogonida is recovered at the base of a clade that unites all chelicera-bearing taxa, the chelicera-lacking Habeliida, and occasionally ($k = 1$) Mollisoniidae, as in the implied-weights parsimony analyses with low concavity values ($k = 1\text{--}3$; panels c and d).

In summary, the addition of the new taxa does not resolve the position of pycnogonids relative to other total-group chelicerates. Hypothesis 2 aligns more closely with the view held by many chelicerate specialists (i.e., that pycnogonids represent the earliest-diverging members of Chelicerata sensu stricto), but within our analyses it is recovered with internal resolution only under specific conditions (implied-weights parsimony with $k = 4$). Hypothesis 3 appears least plausible. A crownward position of the chelicera-lacking Habeliida relative to the chelicera-bearing Synziphosurina would require multiple major evolutionary reversals at the origin of habeliids (e.g., loss of chelicerae; acquisition of biramous opisthosomal appendages with lobate exopods and marginal setae). We favour Hypothesis 1, as it is: 1) consistently obtained under implied-weights parsimony using sufficiently high concavity values ($k > 4$); 2) compatible with the poorly resolved tree obtained under equal-weight parsimony; 3) supported by the more demanding Bayesian inference analysis.

⇒ **Minor adjustments were made to the main text** to acknowledge the variability of these results: “Phylogenetic (Bayesian and parsimony) analysis recovers a total-group Chelicerata, comprising megacheirans, mollisoniids, habeliids, and all chelicerae-bearing arthropods (including crown-group chelicerates), as the sister clade to the antennae-bearing Artiopoda (Fig. 3, Extended Data Fig. 6). **Arachnida, Eurypterida, and Xiphosura form a clade at the crown of the chelicerate lineage, but the position of Pycnogonida – and, with it, the composition of Euchelicerata – varies markedly across analyses.** Megachelicerax is **typically** resolved on the stem of the chelicerate lineage in a position crownward of the Cambrian habeliids and mollisoniids, but stemward relative to the post-Cambrian synziphosurines, which is congruent with its intermediate morphology combining a habeliid-like dorsal exoskeleton and a synziphosurine-like appendicular anatomy. These results **shed important new light on the long-standing** debate on the closest Cambrian relatives of chelicerates (Supplementary Information).”

⇒ **Extended Data Figure 6 has been modified** to illustrate a broader range of parsimony outcomes.

⇒ **Figure 3 has been modified** according to the new results of the Bayesian inference analysis.

[Query 3] *It would also be beneficial to see the parsimony tree retrieved through analysis under equal weights to compare with the Bayesian and implied weights trees.*

⇒ **Extended Data Figure 6 has been modified** to illustrate the trees obtained using Bayesian inference, equal-weight parsimony, and implied-weights parsimony (k=1 to 10).

[Query 4] *One thing of note when the authors discuss other elongated chelicera is the condition in pterygotid eurypterids, the insertion of which appears very similar to those in Megachelicerax and were also suggested by Kjellesvig-Waering to comprise more than three segments (but have subsequently been comprehensively shown to consist of the usual three).*

We thank Referee 1 for pointing out this omission in Supplementary Note 5.

⇒ **The following sentence was added to the second part of this discussion** (“Multisegmented chelicerae in fossil taxa”):

“For completeness, it is worth noting the hypothesis that eurypterids possessed four-segmented chelicerae, a view championed by Kjellesvig-Waering (1964, 1979) but one that has lost all support in modern literature (Lamsdell, 2022, 2025). Similarly, the Jurassic scorpion Liassoscorpionides was originally described...”

[Query 5] *My only other query is regarding the morphology of the endopods, which are reconstructed as terminating in a pair of terminal claws in figure 2 but a single terminal claw in figure 3. The morphology of this termination is interesting as, if pair, it may provide a hint as to the origins of the chelate endopod limbs in offacoliids and xiphosurans. Extended figure 4n appears to clearly show paired terminal claws. This is mentioned briefly in the description of the specimen but I wonder whether the implications of this could be expanded somewhat; the authors note that these claws are non-chelate, which is itself interesting as it is then reminiscent of the tarsal claws of terrestrial arachnids.*

We agree with Referee 1 that KUMIP.314091 convincingly demonstrates the presence of a pair of claws at the tips of En3 and En 4 in the new taxon. In the main text, this feature is acknowledged in the description – “En3 comprises three (partially) exposed, relatively short podomeres and bears two, apparently non-chelal distal claws. En4 (four exposed podomeres) is similar, but longer than en3” – and discussion – “while the absence of clustered stout setae and the presence of a pair of distal spines evoke similarities with synziphosurines”. It is also illustrated in the anatomical and artistic reconstructions in Figure 2. The omission of these paired structures in Figure 3 was an oversight, which has now been corrected.

⇒ **Given the consistent distal morphology of the cephalic endopodal rami in habeliids and offacolid synziphosurines, a pair of robust terminal claws has been added to each cephalic endopodal ramus in the revised Figure 3**, despite the lack of information concerning En2, En 5, En6.

Whether these endopodal distal claws form proper chelae cannot be determined on the sole available specimen. We explicitly express this uncertainty in our main text – “*En3... bears two, apparently non-chelal distal claws*” and “*the distal spines do not appear to form functional chelae, but they could still represent their precursors*” – and Supplementary Note 3 – “*they apparently bear two stout spines distally (only one in habeliids), which may or may not form a chela*”. Finding chelate cephalic exopodal rami in *Megachelicerax* would represent yet another demonstration of the highly derived appendicular anatomy of this taxon, as it is a trait characteristic of all extant and fossil aquatic representatives of Chelicerata sensu stricto. However, we have opted for a conservative interpretation because the outlines and relative sizes of these terminal claws do not suggest that they opposed each other to form a functional pincer.

We believe that we have struck an appropriate balance in our manuscript between avoiding overinterpretation of an incompletely known anatomical feature and acknowledging both the similarity and the potential precursor relationship between these terminal claws and the exopodal chelae of more derived chelicerates (e.g., synziphosurines).

⇒ Accordingly, **we refrain from including a more extensive discussion of this anatomical trait in our text, and especially from drawing comparisons with terrestrial arachnids**, in which the lack of a chelal function for the exopodal claws is not an inherited condition but a secondary locomotory adaptation (e.g., walking, gripping a surface) associated with life outside water.

Referee 2

This study describes a new species of chelicerate, Megachelicerax cousteau, from the Wheeler Formation (lower Drumian, Cambrian Series 3). The fossils presented in this paper provide a comprehensive overview of the new species' anatomy, its phylogenetic position, and its relevance for understanding the origin of the chelicerate body plan in the Cambrian. The major findings are:

- 1) Modern-like chelicerae. Well-developed, three-segmented chelicerae were already present in the Cambrian. This is significant because the main evidence for this character in mollisonid arthropods, such as Mollisonia plenovenatrix, has not been accepted by several researchers. It also establishes the early specialization of the group toward a predatory lifestyle.*
- 2) Opisthosomal appendages. In my opinion, an even more important finding is the presence of opisthosomal appendages that already lacked endopods and likely consisted of sclerotized opercula bearing a large number (35) of gill lamellae.*
- 3) Other relevant findings described and discussed in the manuscript include: the possible presence of a lymph canal, poorly documented in fossil chelicerates and therefore of high relevance, the presence of pseudobiramous prosomal appendages with exopods similar to those of some other cheliceromorpha such as mollisonids and the presence of gnathobases.*

The authors present a coherent evolutionary scenario for early chelicerates based on these important anatomical characters and their phylogenetic analyses.

The phylogenetic analysis itself is a major contribution to the Paleozoic arthropod research community, particularly to those studying the origins of Chelicerata. Rather than inserting their new taxon into a pre-existing matrix, the authors designed a new matrix specifically to recover Megachelicerax

cousteau. The matrix has an appropriate number of characters and taxa and is well constructed for its purpose, avoiding adding too many characters, which in my opinion, often results in analytical noise. Each character chosen has important evolutionary information worthy to be coded. Furthermore, the fossil described here is the outcome of meticulous mechanical preparation. Both the preparation and the analytical methodology have been carried out extremely well, adding value to the paper beyond the exceptional nature of the fossil itself. For these reasons, I strongly recommend the publication of this paper in *Nature*. It presents highly significant new material, thoroughly studied and discussed, that informs the early origins of one of the three major branches of modern arthropods. The findings will be of great interest not only to specialists working on Cambrian arthropods but also to ecologists, as the early establishment of chelicerae in the group has important implications for the development of modern food webs.

I recommend a minor revision, mostly concerning the supplementary material and a few small technical issues on which I disagree with the authors. I have divided my comments into two categories: general comments and "Possibly in conflict of interest comments". I would be happy for both the authors and the editor to treat the latter as entirely optional for the authors to address.

We sincerely thank the referee for their highly positive evaluation of our work, and for recognizing both the significance of the fossil material and the care invested in its preparation and analysis. We appreciate that they essentially concur with our anatomical reconstruction of the new taxon and with the evolutionary scenario it supports regarding the origin of Chelicerata.

General minor comments

*-Line 24-26: I would not emphasize the relationship with megacheiran as a major finding of the paper. The authors discussed the case for Cheliceromorpha with a remarkable review of the related literature and the most important species in a supplementary discussion. However, due to the exceptional derived morphology of *Megachelicerax cousteau* it does not add much to the discussion on the evolution from megacheirans bodyplan to chelicerates body plan.*

⇒ **The sentence was modified as follows:**

*"This discovery provides the first unequivocal evidence of large predatory chelicerates in the Cambrian, illuminates their body plan's origin, and **confirms habeliids, mollisoniids, and probably megacheirans** as members of total-group Chelicerata."*

Elsewhere in the text, megacheirans are mentioned primarily as a group that may illustrate the plesiomorphic conditions of the anatomical traits under discussion, an approach that remains valid whether Megacheira is regarded as part of the chelicerate lineage or as lying immediately stemward to the chelicerate-antennate split (the alternative position favoured by some authors).

-Line 43: I would add “possible” before pycnogonid larvae as well.

⇒ The sentence was modified as follows:

“However, the Cambrian body fossil record of chelicerates is particularly elusive, consisting of Furongian three-dimensionally phosphatized larvae possibly belonging to Pycnogonida and probably *Mollisonia plenovenatrix* from the Burgess Shale.”

⇒ **Supplementary Note 1 now cites Sharma & Gavish-Regev (2025)**, a recent publication challenging the pycnogonid affinities of these fossils.

-Line 69: I usually understand the use of a higher taxonomic group to imply that all the characteristic features of that group are present in the new taxon (e.g., “mollisonids with...”), and the diagnosis focuses on what is different. In this context, however, I believe this phrasing creates more confusion than clarity. Furthermore, our knowledge of the dorsal anatomy of *Megachelicerax cousteau* (armature, spines, tuberculation) is limited, so I would avoid using the phrase “Habeliid-like dorsal exoskeleton” (even if, of course, the overall body shape is similar).

Three reasons explain why the diagnosis we propose may seem slightly longer than what is commonly provided for Cambrian soft-bodied arthropod genera:

- 1) The remarkable preservation of KUMIP.314091 allows an unusually complete description of the anatomy of *Megachelicerax* and, by the same token, the recognition of numerous diagnostic characters.
- 2) As explained in the section “Affinity” of our main text, the new genus cannot be assigned to any known family, order, or class of chelicerates; such an assignment would have allowed us to restrict the diagnosis to characters discriminating *Megachelicerax* from the other members of the higher ranked taxon to which it would have belonged.
- 3) In many aspects of its anatomy, *Megachelicerax* is intermediate between Habeliida and Synziphosurina. This affects its diagnosis, which consists of a unique combination of characters otherwise known from one or the other of these groups, rather than a list of traits unique to the new genus.

It remains that our diagnosis was carefully drafted to capture the essence of our thorough comparisons with other stem-group chelicerates (Supplementary Note 3).

⇒ **Accordingly, we do not believe that this diagnosis should be shortened.**

Otherwise, we agree with Referee 2 that using the expression “habeliid-like” in the diagnosis of a taxon not assigned to Habeliida may be confusing. This expression refers to the strong arching of the axial region compared to the subhorizontal pleural regions typically observed in habeliids.

⇒ **The first sentence of the diagnosis has been revised**, both in the main text and in the Supplementary Note, as follows:

“Dorsal exoskeleton with a strongly arched axial region and composed of a semi-circular prosomal shield with short genal spines and at least nine opisthosomal tergites, most with well-developed, widely spaced, acute tergopleurae.”

-Line 95-96: *“The pleural regions of T1 are noticeably more slender and shorter”. I would like to see some discussion in the supplementary text “tagma” specifically regarding its potential relationship, possibly biomechanical rather than a full homology carried throughout chelicerates since it may have evolved independently more than once, to the reduced seventh tergite observed in other chelicerates.*

⇒ **The following paragraph was added to the Dorsal Exoskeleton section of Supplementary Note 3:**

“Lastly, a reduced first opisthosomal tergite (T1) is a character repeatedly observed in chelicerates with uniramous prosomal limbs (i.e., clade Prosomapoda), where it may be associated with appendicular differentiation (Lamsdell, 2013; Dunlop & Lamsdell, 2017). However, a similar reduction of T1 is also common in early Palaeozoic stem-group chelicerates, having been inferred or documented in some habeliids (e.g., Habelia; Aria & Caron, 2017), habeliid-like arthropods (e.g., Dicranocaris; Briggs et al. 2008), mollisoniids (e.g., Thelxiope; Lerosey-Aubril et al. 2020), and offacolid synziphosurines (e.g., Bunaia, Dibasterium; Briggs et al. 2012; Lustri et al. 2024b). This sporadic distribution within the chelicerate lineage suggests that the feature evolved convergently in these taxa, most likely as an adaptation facilitating flexion at the prosomal–opisthosomal boundary. Accordingly, the presence of a reduced T1 in Megachelicerax is not regarded as phylogenetically significant.”

-Line 102-105: *change to “likely independently inserting on the body wall”. You do not have direct evidence of the absence of a common basipod and inferred this from the position of both rami. I agree with your inferred interpretation, but we can not observe it so add “likely”. Otherwise, if I missed something in the text and you can refer to a figure to show this, please do.*

⇒ **We made the following changes to the main text:**

Description section: *“Each of these post-chelicerai appendages comprises an endopodal ramus and an exopodal ramus **seemingly** independently inserting on the body wall, a condition we refer to hereafter as pseudobiramicity.”*

Affinity section: *“However, the new taxon differs from all modern chelicerates by lacking dorsal eyes, and **likely** possessing pseudobiramous, rather than uniramous post-chelicerai prosomal appendages.”*

⇒ **We made the following changes to Supplementary Note 2:**

*“The term pseudobiramicity is introduced to describe the association of an exopodal ramus and an endopodal ramus with close, but **apparently** distinct insertion sites on the body, rather than connected to one another via a protopodite (biramicity sensu stricto).”*

-Line 170-171: *remove “e.g.” or somewhere else in the text write the other characters to which it resembles, I noticed only those two.*

⇒ **“e.g.” was removed.**

-All along the text and related paragraph in the supplementary material regarding the term “tubuliaceous”. I am not sure it is necessary to change what is, at this point, a reasonably well-established nomenclature for referring to this type of exopod. It is true that the term was originally co-opted from a context in which the appendages are uniramous, but it is also true that “stenopodus” in early chelicerates is always used alongside “exopods,” which are of course absent in uniramous stenopodus appendages, so there is essentially no possibility of confusion. To me, this is comparable to the use of “furcae” for cheloniellid appendicular derivatives: the term is borrowed from other arthropod groups and does not reflect homology, but rather a similarity in shape. Not ideal, but that’s often how language works, and we don’t always need a de-novo name. In this sense, even if “stenopodus” is not a unique term, it has been consistently used to refer to the condition in habeliids, mollisonids, and offacolids. If you introduce the term tubuliaceous, we will end up with two different ways of referring to this same condition in the literature, which, in my opinion, will add confusion rather than reduce it. However, this is not a strong objection, but rather, it is an invitation to reconsider whether introducing a new name for the shape of these exopods is truly necessary.

We respectfully disagree with Referee 2 and note that Referees 1 and 3 expressed no concerns regarding our introduction of the new term “tubuliaceous.” We explained the rationale for this term in detail in the terminology section of Supplementary Notes 2 and 3, but we feel compelled to address Referee 2’s arguments point by point:

- 1) *“a reasonably well-established nomenclature for referring to this type of exopod” and “In this sense, even if “stenopodous” is not a unique term, it has been consistently used to refer to the condition in habeliids, mollisonids, and offacolids.”* This claim is not supported by the literature on early chelicerates. As documented in Supplementary Note 3, no consensus has been reached over the past 25+ years on how to refer to this atypical exopodal morphotype. Terms as varied as “antenna-like” (Briggs & Collins, 1988), “antennule-like” (Aria & Caron, 2017a), “flagelliform” (Boxshall, 2004), “filiform” (Dunlop & Lamsdell, 2017), “pediform” (Legg, 2014; Dunlop & Lamsdell, 2017), and “stenopodous” (Lustri et al., 2024a) have been used, sometimes even within the same publication. There is therefore no “reasonably well-established” terminology to follow.
- 2) *“it is also true that “stenopodous” in early chelicerates is always used alongside “exopods,” which are of course absent in uniramous stenopodous appendages, so there is essentially no possibility of confusion.”* We respectfully disagree again and believe that using the same term for distinct structures is undesirable, even when the authors assume confusion is unlikely. In the present case, because one structure (the exopodal ramus) can form part of the other (a biramous appendage), the likelihood of confusion is actually increased. Whenever possible, distinct structures should be referred to with distinct terms.
- 3) *“If you introduce the term tubuliaceous, we will end up with two different ways of referring to this same condition in the literature, which, in my opinion, will add confusion rather than reduce it.”* As noted in point 1, the terminology is already fragmented: there are not two but at least six different terms currently in use. Confusion is therefore already pervasive, which underscores the need for a unique, clear, and appropriate term. Moreover, applying “stenopodous” to an exopod is misleading, since “stenopodous” refers to an entire appendage that has undergone a specific evolutionary transformation, the complete reduction of the exopodal ramus (the ramus we want to refer to here!) in relation to functional specialisation for walking. None of the characteristics

associated with stenopodous appendages (composition, evolutionary history, function) apply to the early chelicerate exopods we discuss.

The term we propose is not merely a morphological adjective; it designates a distinct exopodal condition that contrasts with the foliaceous type. It is defined by three features – multi-segmented (typically >4 podomeres), elongate, and cylindrical – as correctly recognised in Boxshall's (2013) review, where it was considered “a second basic exopodite type in Palaeozoic arthropods.” Outside the chelicerate lineage, this condition is so far known only in the cephalic appendages of the Silurian marrellomorph *Xylokorys*, but increased use of tomography may reveal additional occurrences in Early Palaeozoic arthropods.

Finally, only time will tell whether colleagues adopt the term “tubulaceous” for what we consider a clearly distinct exopodal condition, but we believe that our text provides ample justification for its introduction.

⇒ **Accordingly, we retain the new term “tubulaceous” in both the main text or the supplementary notes, which remain unchanged with respect to this terminological issue.**

-Supp. 226: Why they were limited?

This comment refers to our text: *“This size and morphology may indicate that the chela was used to hold a prey/carcass, and perhaps to pick up small pieces from it; however, its crushing and cutting abilities were probably limited, differing in this respect from both the spine- or tooth-bearing chelicerae of some pycnogonids and many arachnids (Extended Data Fig. 3d, k–o) and the scissor-like chelicerae of harvestmen and horseshoe crabs (Extended Data Fig. 3h, i).”*

Note that we used the term “probably” as these functional inferences are entirely based on morphology and therefore somewhat speculative. The explanations for why we believe the chelae of *Megachelicerax* had limited crushing and cutting abilities are in the second part of our sentence: they lack the marginal spines or teeth typically characterizing crushing chelae, or the elongate morphology and important length of chelae used for cutting.

⇒ **We have clarified our views as follows:**

*“This **small** size and **simple** morphology may indicate that the chela was used to hold **prey or carcasses**, and perhaps to pick **off** small pieces, **while having limited crushing and cutting ability. Indeed, it lacks the robust construction and the spines or teeth characteristic of the chelae of pycnogonids and arachnids used to crush prey** (Extended Data Fig. 3d, k–o), **and the scissor-like morphology associated with cutting in harvestmen and horseshoe crabs** (Extended Data Fig. 3h, i).”*

Possibly in conflict of interest comments

-Line 102-105: Offacolids have been united in Lustri et al. 2024 Royal Society Open Science. You should cite it here.

Our numbered-line version of the text shows no mention of offacolids in lines 102–105. Instead, the group is first referenced in line 172 (second paragraph of the Affinity section): “In contrast, *chelicerae* are well-documented in synziphosurines, even in their oldest representatives – the Ordovician–Silurian Offacoliidae – but the body shape of these animals (e.g., rounded prosomal shield, reduced tergopleurae) differs from the habeliid-like morphology of the new taxon^{20,25,26}.” Their next mention is in line 242 (end of third paragraph of the Discussion section): “...and 2) offacolids other than *Offacolus* possess only six pairs of prosomal appendages^{20,26,20,26} (Extended Data Fig. 7).”

⇒ **Lustri et al. 2024 (<https://doi.org/10.1098/rsos.240499>) is now cited** at the end of this second sentence.

-Supp. 239-234: I generally agree with the described scenario. However, I believe it should be said somewhere that among all the species listed, *Setapedites* is the only one showing direct evidence of “The rami of the biramous appendage attach to a protopodite (basipodite) of rectangular shape (bs in Figs. 2E and 2F, Supplementary Fig. 2G and 2I).” Of course, the author can then express their disagreement with this interpretation in *Setapedites*, but I do not recall any habeliids or mollisoniids for which the pseudobiramicity is known from direct fossil evidence, and this is the case also for *M. cousteau* if I did not miss a figure showing the presence of two different insertion points in the body of the two branches, clearly excluding the presence of a common basipod.

This comment refers to this sentence in the Discussion section of the main text: “However, this claim overlooks previous reports of pseudobiramous prosomal limbs in Cambrian habeliids and mollisoniids, an older origin for this atypical trait confirmed by *M. cousteau*.” It also concerns the following part of Supplementary Note 3: “*Setapedites*, the oldest (Early Ordovician) synziphosurine described to date, was recently reconstructed as having truly biramous post-cheliceral prosomal appendages (Lustri et al. 2024a). This observation was considered by the latter authors as supporting evidence to Briggs et al.’s (2012) hypothesis, but they omitted in their evolutionary scenario the fact that pseudobiramicity had also been inferred in earlier diverging stem-group chelicerates, such as habeliids and mollisoniids. As confirmed by *M. cousteau*, pseudobiramicity likely predates the evolution of synziphosurines.”]

We agree with Referee 2 that pseudobiramicity in Cambrian stem-group chelicerates has thus far been inferred only indirectly, based on the seemingly independent distribution and orientation of the endopodal and exopodal rami. No specimen unequivocally shows independent insertion sites for these two types of rami on the body.

⇒ To better acknowledge this uncertainty and to clarify the situation of *Setapedites*, **the sentences cited above were modified as follows:**

- In the Main text: “However, **the apparent pseudobiramicity of the** prosomal limbs in habeliids, mollisoniids, **and** *M. cousteau* **suggests a Cambrian** origin for this atypical trait.”
- In Supplementary Note 3: “*Setapedites*, the oldest (Early Ordovician) synziphosurine described to date, was recently reconstructed as having **basipodites and therefore**, truly biramous post-cheliceral prosomal appendages (Lustri et al. 2024a). This observation was considered by the latter authors as supporting evidence to Briggs et al.’s (2012) hypothesis. **However, the seemingly distinct insertions on the body of the endopodal and exopodal rami** in earlier-diverging stem-

*group chelicerates – such as habeliids, mollisoniids, and now M. cousteau – suggests that pseudobiramicity **may** predate the evolution of synziphosurines.”*

-Supp. 259-269: I invite the author to reconsider this paragraph after re-reading what's stated in Lustri et al 2024 Nature communications. “Exopodial setal brushes are also present in *Habelia optata*. However, they are short and confined to the inner margin of the exopod in *Habelia optata*, whereas they are long and radially arranged from the inner to the outer margins in Offacolidae, clearly differentiating these features. *Mollisonia plenovenatrix* also shows a single exopod in one specimen with a termination made of setae, but their precise arrangement is unknown. Possessing exopodial setal brushes may be homologous amongst these taxa, but we interpret the precise morphology of stenopodous exopods 2–5 ending in a brush-like group of long and radially arranged setae to be an autapomorphy of Offacolidae,”

It seems to us that Referee 2 disagrees with these two sentences of our Supplementary Note 3: “Lustri et al. (2024a) recently regarded the presence of a ‘brush-like group of long and radially arranged setae on the distalmost podomere’ as a diagnostic character for the synziphosurine family Offacolidae. None of the exopodal rami of *M. cousteau* display a brush-like tip (Fig. 1, Extended Data Fig. 4a–l), but this feature is observed in some cephalic rami of habeliids (Legg, 2014, fig. 2b; Extended Data Fig. 4o) and mollisoniids (Aria & Caron, 2019, fig. 2g, extended data fig. 3f), which indicates that it is plesiomorphic in synziphosurines.”

⇒ To address Referee 2's concern, **we revise and shorten our text as follows:**

“Lustri et al. (2024a) recently regarded the presence of a ‘brush-like group of long and radially arranged setae on the distalmost podomere’ as a diagnostic character for the synziphosurine family Offacolidae. None of the exopodal rami of *M. cousteau* display a brush-like tip (Fig. 1, Extended Data Fig. 4a–l), but ***somewhat similar features are*** observed in some cephalic rami of habeliids (Legg, 2014, fig. 2b; Extended Data Fig. 4o) and mollisoniids (Aria & Caron, 2019, fig. 2g, extended data fig. 3f).”

Looking forward to seeing this manuscript published.

We thank again Referee 2 for these kind words.

Referee 3

My report is brief. This very well preserved fossil unequivocally possesses chelicerae, and indeed three-segmented ones. With a sound description and phylogenetic analysis pinning it down in the chelicerate stem-group, this “smoking gun” character is Nature-worthy. The reinterpretation of other chelicerate-allied Palaeozoic fossils in light of the new genus adds extra value to the paper.

We thank Referee 3 for their succinct and positive evaluation, and for suggesting that the resulting manuscript presents findings likely to appeal to the Nature readership.

The term “habeliid” (or “habeliid-like”) is used throughout the paper (lines 19, 45, 170, etc.) but has the potential to confuse, particularly since another name, “habeliidan”, is used in the trees in Extended Data

Figure 6. “Habeliid” could be read as encompassing all of Habeliida Aria and Caron, 2017 (i.e., Habeliidae + Sanctacariidae), or only Habeliidae Simonetta and Delle Cave, 1975. The former was named in the context of it having been conceived as monophyletic but in the present paper it corresponds to a grade (Extended Data Figure 6b) or an ambiguous metataxon collapsed in a polytomy (Figure 3 and Extended Data Figures 6a). Upon first usage of “habeliid” I’d prefer to see a formal taxon name, and take care to ensure that the reader knows exactly what this means in every instance.

⇒ For the sake of conciseness and simplicity, we did not explicitly clarify our use of the term “habeliid” in the summary paragraph, but **we define it at its first occurrence in every section of our manuscript where it appears:**

- in the introduction of the main text: “The complex yet partially unresolved appendicular anatomy of *M. plenovenatrix* has bolstered the hypothesis that chelicerates evolved from members of the order Habeliida (*‘habeliids’ hereafter*) during the early Cambrian.”
- in the Terminology section of Supplementary Note 2: “For members of the order Habeliida (*‘habeliids’ hereafter*), whose deutocerebral appendages are either non-chelate or of uncertain morphology...”
- in the Dorsal Exoskeleton section of Supplementary Note 3: “..., characters shared with most representatives of the order Habeliida (*‘habeliids’ hereafter*; Fig. 1a, c, f; Extended Data Figs. 1, 2).”
- in the introductory paragraph of Supplementary Note 4: “Three groups of marine arthropods present in the Cambrian have been repeatedly regarded as the most likely candidates: artiopods, habeliids (Order Habeliida), and megacheirans (e.g., Dunlop, 2005).” The scientific names, including ‘Habeliida’, now replace the vernacular names in the section headings as well.
- in the section dedicated to fossil taxa in Supplementary Note 5 (one and only mention): “The non-chelate deutocerebral appendages of Habelia – the only member of the order Habeliida in which these appendages are known – are also short (Aria & Caron, 2017a).”
- in the Remarks section of Character 36 in Supplementary Note 7: “... regardless of whether it is fused to the appendage-bearing segments (e.g., artiopods, megacheirans, members of the order Habeliida or ‘habeliids’) or not (e.g. fuxianhuiids).”

‘Habeliid(s)’ does appear in Supplementary Notes 1 and 6. It is also worth noting that unlike the order Habeliida, **the family Habeliidae is never mentioned in any components of our manuscript**, which should prevent confusion about our use of the term ‘habeliid(s)’.

Line 26 in the abstract probably overstates the case for megacheirans being total-group Chelicerata. “Confirms” is a strong word for a node with a posterior probability in the 0.50s that is so close to the root of the tree that alternative phylogenetic positions are not very severely tested (e.g., taxa that could pull megacheirans down into the euarthropod stem group, such as radiodonts and Kylinxia, are not included). For the other two groups mentioned in line 26, the case is adequate (being an extra node or two crownward).

⇒ This sentence has been modified as follows:

*“This discovery provides the first unequivocal evidence of large predatory chelicerates in the Cambrian, illuminates their body plan’s origin, and **confirms habeliids, mollisoniids, and probably megacheirans as members of total-group Chelicerata.**”*

Elsewhere in the text, megacheirans are mentioned primarily as a group that may illustrate the plesiomorphic conditions of the anatomical traits under discussion, an approach that remains valid whether Megacheira is regarded as part of the chelicerate lineage or as lying immediately stemward to the chelicerate-antennate split (the alternative position favoured by some authors).

Speaking of the “0.50s” mentioned above, change all node support values in the Bayesian trees to decimals (0.59 rather 59), as they are reported on a 0 to 1 scale rather than as percentages like bootstrap values.

- ⇒ **In Extended Data Figure 6, percentages have been replaced with decimals as requested.**
- ⇒ **In Figure 3, node support values have been removed for clarity;** readers are directed to Extended Data Figure 6 for detailed results, as noted in the caption.

In line 1, delete “Show your hand”.

- ⇒ **“Show your hand” was removed from the title.**

In line 10, replace “acari” with “mites” for stylistic consistency with “scorpions” and “spiders”.

- ⇒ **“acari” was replaced by “mites” in this sentence.**

In Extended Data Figure 2b, there is a reddish-brown colour applied to a bilaterally symmetrical metameric structure flanking the opisthosomal midline. It doesn’t seem to be explained in the colour scheme explanation.

The preserved elements of the haemolymphatic system are regarded as internal within parts of the appendages, as indicated by their differing outlines in the prosoma and opisthosoma, which mirror the shapes of the corresponding appendages. When both haemolymphatic remains and appendicular structures are visible – in most opisthosomal segments of the counterpart (Extended Data Fig. 2) – the haemolymphatic system is shown as lying beneath the cuticle of the gill plates. Its colour therefore appears intermediate between that of fully exposed haemolymphatic remains (dark brown) and that of the gill plates (medium pink).

To clarify this, Extended Data Figure 2b was revised as follows:

- ⇒ **The colour key for the haemolymphatic system has been divided into two entries,** distinguishing the colour used when it is fully exposed from that used when preserved within the gill plates.
- ⇒ **The associated text now includes the terms “exposed/underlying” in parentheses.**
- ⇒ **The caption now states: “The haemolymphatic system may be preserved either alone (‘exposed’) or within the appendages (‘underlying’).”**

In the caption to Extended Data Figure 3, change all instances of “actual” to “extant”.

⇒ “actual” was replaced by “extant” throughout this caption.

The artwork in Figure 2b should, in my opinion, be left for a press release to capture the public’s imagination. Really, why would we think the exopods are yellow and the endopods alternating blue and red? I am in a minority I suppose, but I’d prefer that PalaeoArt be replaced with real morphology, like details of the opisthosomal appendages that are currently relegated to Extended Data. The artwork is perfectly fine in the tree in Figure 3, but it’s taking up valuable space in Figure 2.

While we fully acknowledge that the colour pattern applied to the appendages of the new taxon in our reconstruction is arbitrary – given the lack of any evidence for original pigmentation in the fossil material – it should be noted that the scheme was inspired by an extant crustacean, the Blue Leg Hermit Crab (*Clibanarius tricolor*). Specifically, the colours assigned to the endopods of *Megachelicerax* correspond to those of the legs of *Clibanarius*, the colours of the exopods mirror those of the antennae, and the colours of the chelicerae are derived from those of the pincers.



[REDACTED]

Mentally reconstructing the 3D anatomy of a complex animal like *Megachelicerax* from photographs of a two-dimensionally preserved fossil may be challenging. As 3D imaging techniques (e.g., tomography) cannot be applied to this specimen, we believe that 3D artistic reconstructions offer valuable support in conveying how we interpret the anatomy of the new taxon to a broad readership. We also follow the precedent set by several studies on Cambrian organisms published in Nature, which include artistic reconstructions in the main text to convey similarly complex reconstructed anatomies.

⇒ **We are happy to defer a final decision on this matter to the handling editor**, as they are best placed to judge whether Figure 2b provides sufficient added value to warrant its inclusion in the main text or, alternatively, whether the artistic reconstructions should instead be placed elsewhere (e.g., as an Extended data figure or a Supplementary figure).

Character 8 in the character descriptions in Supplementary Note 7 could use a note to explain what the character states are (i.e., raw integers for the number of segments) and their range.

⇒ **We added the following definitions of the character states for character 8** (number of post-ocular appendage-bearing cephalic segments): “(0) zero; (1) one; (2) two; (3) three; (4) four; (5) five; (6) six; (7) seven”.

REFERENCES CITED IN THIS RESPONSE

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RESPONSE TO REFEREES

All the descriptions of changes made to the files composing our manuscript are highlighted in light cream to facilitate their identification below.

Referee 1

It was a delight to read this revised manuscript on an early Cambrian chelicerate. I thank the authors for addressing my queries and look forward to seeing this important contribution published. I feel that the revised manuscript presents a fairly comprehensive view of the current knowledge regarding the early evolution of chelicerates and accurately places the importance of the new find into a historical context. I only have one additional suggestion relating to lines 297-306 in the manuscript where the homology of the gill opercula and gill plates is discussed, where for completeness it may be worth stating that the gill opercula are likely homologous to the external sternites in tetrapulmonate arachnids (see Shultz 1999 [<https://doi.org/10.1111/j.1096-3642.1999.tb00608.x>] and Farley 2001 [<https://doi.org/10.1080/07924259.2001.9652719>]).

Overall I think this is an excellent paper describing an exciting discovery that clarifies the early evolution of a major arthropod group and will help recontextualize our understanding of numerous existing fossil taxa as well as providing a framework for comparison of future discoveries.

James C. Lamsdell

Once again, we thank our colleague for their positive evaluation and constructive comment.

- ⇒ **The relevant sentence was modified as follows:** “We hypothesize that the gill opercula of synziphosurines, eurypterids, and xiphosurids – **and most likely the external sternites of some tetrapulmonate arachnids**^{46,47} – and the gill plates of *M. cousteau* are homologous structures based on their shared position and morphology.”
- ⇒ **The two suggested references were added to the reference list.**

Referee 2

I find that the manuscript has been improved by the authors. I also appreciate most of the suggestions from the other reviewers and the corresponding edits. Strictly speaking with respect to my previous review, the authors have addressed almost all of my comments, and in cases where they did not, they provided satisfactory reasoning.

I have only one remaining suggestion regarding our disagreement on the naming of the prosomal exopods. In your response, you stated: “This claim is not supported by the literature on early chelicerates. As documented in Supplementary Note 3, no consensus has been reached over the past 25+ years on how to refer to this atypical exopodal morphotype. Terms as varied as “antenna-like” (Briggs & Collins, 1988),

“antennule-like” (Aria & Caron, 2017a), “flagelliform” (Boxshall, 2004), “filiform” (Dunlop & Lamsdell, 2017), “pediform” (Legg, 2014; Dunlop & Lamsdell, 2017), and “stenopodous” (Lustri et al., 2024a) have been used, sometimes even within the same publication. There is therefore no “reasonably well-established” terminology to follow.”

I believe it would be helpful to include a small table in the supplementary material summarizing the different nomenclatures that you propose to unify under the term tubuliaceous. This would clarify some of the confusion and help support the adoption of the new name you are suggesting. Note that stenopodous was first used by Aria & Caron (2017), not by Lustri et al. (2024a).

Lorenzo Lustri

We thank the referee for their positive assessment of the revised manuscript and our responses to their comments.

⇒ **As requested, a non-exhausting list of the terms used to refer to the post-cheliceran cephalic exopods/exopodal rami of early chelicerates is provided as a new table within Supplementary Note 3.**

Referee 3

The authors have addressed all points raised in my original review to my satisfaction. I shall not weigh in on their responses to suggestions by referees 1 and 2 in detail except to say they have, in my opinion, been most conscientious in taking feedback on board. The addition of eurypterids and fossil scorpions to the taxon sampling in the phylogenetic analyses is a useful refinement, and I think they have done a good job diluting the impression that pycnogonids are resolved in an unexpected position; Extended Data Figure 6 shows that pycnogonids are labile between different analytical conditions, under some weighting schemes resolving stemward of synziphosurans.

I am happy to sign off on this version of the manuscript for publication.

We thank the referee for their careful evaluation and are pleased that all points raised in the original review have been satisfactorily addressed.

This study describes a new species of chelicerate, *Megachelicerax cousteau*, from the Wheeler Formation (lower Drumian, Cambrian Series 3). The fossils presented in this paper provide a comprehensive overview of the new species' anatomy, its phylogenetic position, and its relevance for understanding the origin of the chelicerate body plan in the Cambrian. The major findings are:

- 1) Modern-like chelicerae. Well-developed, three-segmented chelicerae were already present in the Cambrian. This is significant because the main evidence for this character in mollisonid arthropods, such as *Mollisonia plenovenatrix*, has not been accepted by several researchers. It also establishes the early specialization of the group toward a predatory lifestyle.
- 2) Opisthosomal appendages. In my opinion, an even more important finding is the presence of opisthosomal appendages that already lacked endopods and likely consisted of sclerotized opercula bearing a large number (35) of gill lamellae.
- 3) Other relevant findings described and discussed in the manuscript include: the possible presence of a lymph canal, poorly documented in fossil chelicerates and therefore of high relevance, the presence of pseudobiramous prosomal appendages with exopods similar to those of some other cheliceromorpha such as mollisonids and the presence of gnathobases.

The authors present a coherent evolutionary scenario for early chelicerates based on these important anatomical characters and their phylogenetic analyses.

The phylogenetic analysis itself is a major contribution to the Paleozoic arthropod research community, particularly to those studying the origins of Chelicerata. Rather than inserting their new taxon into a pre-existing matrix, the authors designed a new matrix specifically to recover *Megachelicerax cousteau*. The matrix has an appropriate number of characters and taxa and is well constructed for its purpose, avoiding adding too many characters, which in my opinion, often results in analytical noise. Each character chosen has important evolutionary information worthy to be coded. Furthermore, the fossil described here is the outcome of meticulous mechanical preparation. Both the preparation and the analytical methodology have been carried out extremely well, adding value to the paper beyond the exceptional nature of the fossil itself. For these reasons, I strongly recommend the publication of this paper in Nature. It presents highly significant new material, thoroughly studied and discussed, that informs the early origins of one of the three major branches of modern arthropods. The findings will be of great interest not only to specialists working on Cambrian arthropods but also to ecologists, as the early establishment of chelicerae in the group has important implications for the development of modern food webs.

I recommend a minor revision, mostly concerning the supplementary material and a few small technical issues on which I disagree with the authors. I have divided my comments into two categories: general comments and "Possibly in conflict of interest comments". I would be happy for both the authors and the editor to treat the latter as entirely optional for the authors to address.

General minor comments

-Line 24-26: I would not emphasize the relationship with megacheiran as a major finding of the paper. The authors discussed the case for Cheliceromorpha with a remarkable review of the related literature and the most important species in a supplementary discussion. However, due to

the exceptional derived morphology of *Megachelicerax cousteauui* it does not add much to the discussion on the evolution from megacheirans bodyplan to chelicerates body plan.

-Line 43: I would add “possible” before pycnogonid larvae as well.

-Line 69: I usually understand the use of a higher taxonomic group to imply that all the characteristic features of that group are present in the new taxon (e.g., “mollisonids with...”), and the diagnosis focuses on what is different. In this context, however, I believe this phrasing creates more confusion than clarity. Furthermore, our knowledge of the dorsal anatomy of *Megachelicerax cousteauui* (armature, spines, tuberculation) is limited, so I would avoid using the phrase “Habeliid-like dorsal exoskeleton” (even if, of course, the overall body shape is similar).

95-96: “The pleural regions of T1 are noticeably more slender and shorter”. I would like to see some discussion in the supplementary text “tagma” specifically regarding its potential relationship, possibly biomechanical rather than a full homology carried throughout chelicerates since it may have evolved independently more than once, to the reduced seventh tergite observed in other chelicerates.

-Line 102-105: change to “likely independently inserting on the body wall”. You do not have direct evidence of the absence of a common basipod and inferred this from the position of both rami. I agree with your inferred interpretation, but we can not observe it so add “likely”. Otherwise, if I missed something in the text and you can refer to a figure to show this, please do

-Line 170-171: remove “e.g.” or somewhere else in the text write the other characters to which it resembles, I noticed only those two.

-All along the text and related paragraph in the supplementary material regarding the term “tubuliaceous”.

I am not sure it is necessary to change what is, at this point, a reasonably well-established nomenclature for referring to this type of exopod. It is true that the term was originally co-opted from a context in which the appendages are uniramous, but it is also true that “stenopodus” in early chelicerates is always used alongside “exopods,” which are of course absent in uniramous stenopodus appendages, so there is essentially no possibility of confusion. To me, this is comparable to the use of “furcae” for cheloniellid appendicular derivatives: the term is borrowed from other arthropod groups and does not reflect homology, but rather a similarity in shape. Not ideal, but that’s often how language works, and we don’t always need a de-novo name. In this sense, even if “stenopodus” is not a unique term, it has been consistently used to refer to the condition in habeliids, mollisonids, and offacolids. If you introduce the term tubuliaceous, we will end up with two different ways of referring to this same condition in the literature, which, in my opinion, will add confusion rather than reduce it. However, this is not a strong objection, but rather, it is an invitation to reconsider whether introducing a new name for the shape of these exopods is truly necessary.

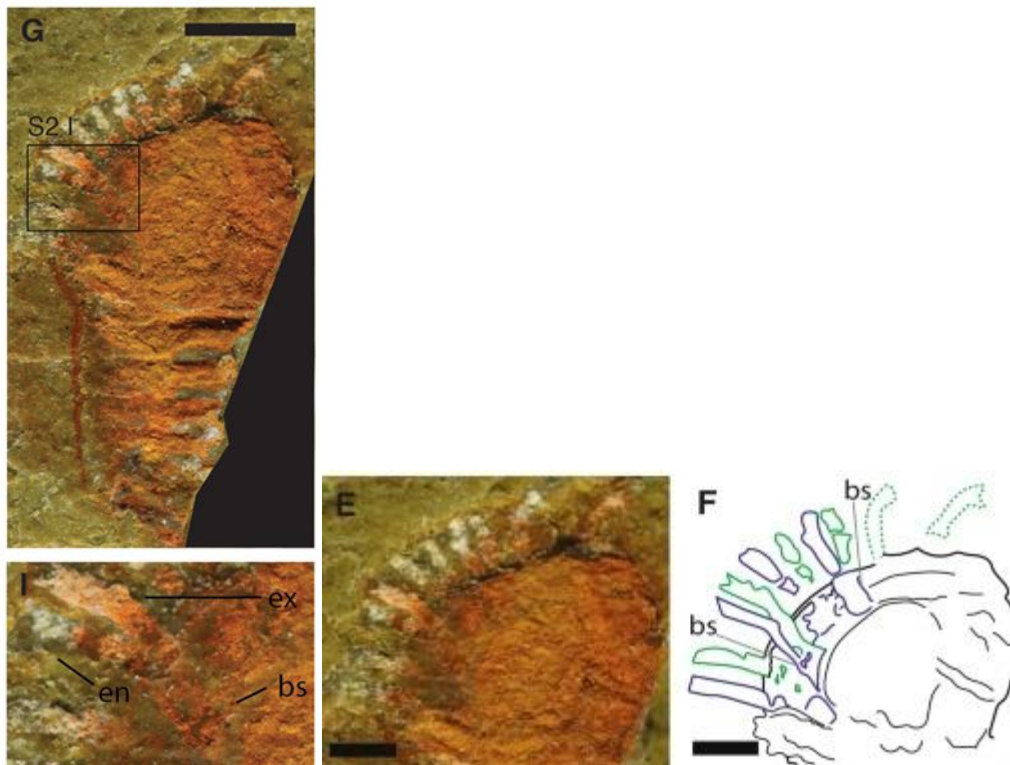
-Supp. 226: Why they were limited?

Possibly in conflict of interest comments

-Line 102-105: Offacolids have been united in Lustri et al. 2024 Royal Society Open Science. You should cite it here.

-Supp. 239-234: I generally agree with the described scenario. However, I believe it should be said somewhere that among all the species listed, *Setapedites* is the only one showing direct evidence of “The rami of the biramous appendage attach to a protopodite (basipodite) of rectangular shape (bs in Figs. 2E and 2F, Supplementary Fig. 2G and 2I).” Of course, the author can then express their disagreement with this interpretation in *Setapedites*, but I do not recall any habeliids or mollisoniids for which the pseudobiramicity is known from direct fossil evidence, and this is the

case also for *M. cousteaui* if I did not miss a figure showing the presence of two different insertion points in the body of the two branches, clearly excluding the presence of a common basipod.



-Supp. 259-269: I invite the author to reconsider this paragraph after re-reading what's stated in Lustri et al 2024 Nature communications.

"Exopodial setal brushes are also present in *Habelia optata*. However, they are short and confined to the inner margin of the exopod in *Habelia optata*, whereas they are long and radially arranged from the inner to the outer margins in Offacolidae, clearly differentiating these features. *Mollisonia plenovenatrix* also shows a single exopod in one specimen with a termination made of setae, but their precise arrangement is unknown. Possessing exopodial setal brushes may be homologous amongst these taxa, but we interpret the precise morphology of stenopodous exopods 2–5 ending in a brush-like group of long and radially arranged setae to be an autapomorphy of Offacolidae,"

Looking forward to seeing this manuscript published.