



HAL
open science

Exceptionally preserved soft parts in fossils from the Lower Ordovician of Morocco clarify stylophoran affinities within basal deuterostomes.

Bertrand Lefebvre, Thomas E. Guensburg, Emmanuel L.O. Martin, Rich Mooi, Elise Nardin, Martina Nohejlová, Farid Saleh, Khaoula Kouraïss, Khadija El Hariri, Bruno David

► To cite this version:

Bertrand Lefebvre, Thomas E. Guensburg, Emmanuel L.O. Martin, Rich Mooi, Elise Nardin, et al.. Exceptionally preserved soft parts in fossils from the Lower Ordovician of Morocco clarify stylophoran affinities within basal deuterostomes.. *Geobios*, 2019, 52, pp.27-36. 10.1016/j.geobios.2018.11.001 . hal-02014812

HAL Id: hal-02014812

<https://hal.science/hal-02014812v1>

Submitted on 12 Nov 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Manuscript Details

Manuscript number	GEOBIO_2018_75
Title	Exceptionally preserved soft parts in fossils from the Lower Ordovician of Morocco clarify stylophoran affinities within basal deuterostomes
Article type	Research paper

Abstract

The extinct echinoderm clade Stylophora consists of some of the strangest known deuterostomes. Stylophorans are known from complete, fully articulated skeletal remains from the middle Cambrian to the Pennsylvanian, but remain difficult to interpret. Their bizarre morphology, with a single appendage extending from a main body, has spawned vigorous debate over the phylogenetic significance of stylophorans, which were long considered modified but bona fide echinoderms with a feeding appendage. More recent interpretation of this appendage as a posterior "tail-like" structure has literally turned the animal back to front, leading to consideration of stylophorans as ancestral chordates, or as hemichordate-like, early echinoderms. Until now, the data feeding the debate have been restricted to evaluations of skeletal anatomy. Here, we apply novel elemental mapping technologies to describe, for the first time, soft tissue traces in stylophorans in conjunction with skeletal molds. The single stylophoran appendage contains a longitudinal canal with perpendicular, elongate extensions projecting beyond hinged biserial plates. This pattern of soft tissues compares most favorably with the hydrocoel, including a water vascular canal and tube feet found in all typical echinoderms. Presence of both calcite stereom and now, an apparent water vascular system, support echinoderm and not hemichordate-like, affinities.

Keywords deuterostomes; echinoderms; Fezouata Lagerstätte; Morocco; phylogeny.

Corresponding Author Bertrand Lefebvre

Corresponding Author's Institution CNRS

Order of Authors Bertrand Lefebvre, Thomas E. Guensburg, Emmanuel L.O. Martin, Rich Mooi, Elise Nardin, Martina Nohejlova, Farid Saleh, Khaoula Kouraiss, Khadija El Hariri, Bruno David

Suggested reviewers Colin Sumrall, James Sprinkle, Derek Briggs

Submission Files Included in this PDF

File Name [File Type]

Lefebvre_cover letter.pdf [Cover Letter]

Lefebvre et al._MS.docx [Manuscript File]

Lefebvre et al._Fig. 1.tif [Figure]

Lefebvre et al._Fig. 2.tif [Figure]

Lefebvre et al._Fig. 3.tif [Figure]

Lefebvre et al._Fig. 4.tif [Figure]

Lefebvre et al._Fig. 5.tif [Figure]

Lefebvre et al._Fig. 6.tif [Figure]

Lefebvre et al._Fig. 7.tif [Figure]

Lefebvre et al._Fig. 8.tif [Figure]

Lefebvre et al._Fig. 9.tif [Figure]

To view all the submission files, including those not included in the PDF, click on the manuscript title on your EVISE Homepage, then click 'Download zip file'.

Research Data Related to this Submission

There are no linked research data sets for this submission. The following reason is given:
No data was used for the research described in the article

1 **Exceptionally preserved soft parts in fossils from the Lower Ordovician of**
2 **Morocco clarify stylophoran affinities within basal deuterostomes**

3

4 Bertrand Lefebvre ^{a,*}, Thomas E. Guensburg ^b, Emmanuel L.O. Martin ^a, Rich Mooi ^c, Elise
5 Nardin ^d, Martina Nohejlova ^a, Farid Saleh ^a, Khaoula Kouraïss ^e, Khadija El Hariri ^e, Bruno
6 David ^{f,g}

7 ^a UMR CNRS 5276 LGLTPE, Université Claude Bernard Lyon 1, F-69622 Villeurbanne cedex, France

8 ^b IRC, Field Museum, Chicago, IL 60605-2496, USA

9 ^c Department of Invertebrate Zoology and Geology, California Academy of Sciences, San Francisco, California
10 94118-4503, USA

11 ^d UMR CNRS-IRD-UPS 5563 Géosciences Environnement Toulouse, Observatoire Midi-Pyrénées, F-31400
12 Toulouse, France

13 ^e Département des Sciences de la Terre, Faculté des Sciences et Techniques-Guéliz, Université Cadi Ayyad, 40000
14 Marrakesh, Morocco

15 ^f UMR CNRS 6282 Biogéosciences, Université de Bourgogne Franche-Comté, F-21000 Dijon, France

16 ^g Muséum National d'Histoire naturelle, F-75005 Paris, France

17

18 *Corresponding author:

19 *E-mail address: bertrand.lefebvre@univ-lyon1.fr*

20

21 ABSTRACT

22

23 The extinct echinoderm clade Stylophora consists of some of the strangest known deuterostomes.
24 Stylophorans are known from complete, fully articulated skeletal remains from the middle
25 Cambrian to the Pennsylvanian, but remain difficult to interpret. Their bizarre morphology, with
26 a single appendage extending from a main body, has spawned vigorous debate over the
27 phylogenetic significance of stylophorans, which were long considered modified but bona fide
28 echinoderms with a feeding appendage. More recent interpretation of this appendage as a
29 posterior "tail-like" structure has literally turned the animal back to front, leading to
30 consideration of stylophorans as ancestral chordates, or as hemichordate-like, early echinoderms.
31 Until now, the data feeding the debate have been restricted to evaluations of skeletal anatomy.
32 Here, we apply novel elemental mapping technologies to describe, for the first time, soft tissue
33 traces in stylophorans in conjunction with skeletal molds. The single stylophoran appendage
34 contains a longitudinal canal with perpendicular, elongate extensions projecting beyond hinged
35 biserial plates. This pattern of soft tissues compares most favorably with the hydrocoel, including
36 a water vascular canal and tube feet found in all typical echinoderms. Presence of both calcite
37 stereom and now, an apparent water vascular system, support echinoderm and not hemichordate-
38 like, affinities.

39

40 *Keywords:* deuterostomes, echinoderms, Fezouata Lagerstätte, Morocco, phylogeny

41

42

43 **1. Introduction**

44

45 Phylogenetic relationships among the main clades of deuterostomes (Swalla and Smith, 2008;
46 Erwin et al., 2011; David and Mooi, 2014) and in particular, the earliest chordates (including the
47 vertebrates) (Jefferies, 1986; Holland et al., 2015; Janvier, 2015) represent long-debated issues.
48 In recent years, however, the combination of molecular, embryological, and anatomical data has
49 made it possible to establish a robust phylogeny for extant deuterostomes. With the
50 Hemichordata (acorn worms), the echinoderms (sea lilies, starfish, sea urchins, and related
51 forms) are now considered to belong to a clade, the Ambulacraria, as the sister-group of
52 chordates (cephalochordates, tunicates and vertebrates) (Bottjer et al., 2006; Swalla and Smith,
53 2008; David and Mooi, 2014; Holland et al., 2015; Janvier, 2015; Lowe et al., 2015). This
54 phylogenetic scenario provides insights not only into the distinctive morphological features
55 (apomorphies) acquired within each clade of deuterostomes, but also into the primitive
56 characters (plesiomorphies) inherited from their common ancestors (Bottjer et al., 2006; Swalla
57 and Smith, 2008; David and Mooi, 2014; Lowe et al., 2015). For example, the occurrence of gill
58 slits in both extant hemichordates and chordates strongly supports the view that these structures
59 were very likely present in earliest deuterostomes (Smith, 2005; Bottjer et al., 2006; Swalla and
60 Smith, 2008; Lowe, 2015). Their absence in echinoderms is most parsimoniously regarded as a
61 secondary loss during the course of echinoderm evolution (Smith, 2005; Bottjer et al., 2006;
62 Swalla and Smith, 2008; Zamora and Rahman, 2014), or perhaps at the origin of the phylum
63 itself (Mooi and David, 1998, 2008).

64 Integrating fossil data into the phylogeny of deuterostomes offers the opportunity to document
65 the sequential acquisition of key characters through time and to provide minimum dates for
66 lineage divergences. The interpretation of the earliest known deuterostomes (Shu et al., 2001,
67 2004; Caron et al., 2010, 2013) (Cambro-Ordovician, 541–443 Myr) is particularly challenging

68 because their morphologies are often bizarrely unlike those of modern forms, with unexpected
69 combinations of anatomical features. Along with cambroernids (Caron et al., 2010), conodonts
70 (Briggs, 1992), vetulicolians (Shu et al., 2001) and vetulicystids (Shu et al., 2004), stylophorans
71 belong to the bestiary of extinct, enigmatic early deuterostomes.

72 Stylophorans (cornutes and mitrates), a group of unusual middle Cambrian–Pennsylvanian
73 (509–300 Myr) fossils, are small (typically one or two centimeters in length), bipartite organisms
74 consisting of a single, articulated appendage inserted into a flattened, asymmetric to bilaterally
75 symmetric body made of a multiplated, internal skeleton, or test (Ubaghs, 1968; Clausen and
76 Smith, 2005; Smith, 2005; David et al., 2000; Dominguez et al., 2002; Lefebvre, 2003) (Fig. 1A,
77 C). The appendage comprises two distinct parts: a highly flexible proximal region (partially
78 inserted into the test) of telescopic, imbricate rings; and a relatively stiff distal region consisting
79 of opposing, delicate biserial platelets mounted on massive, uniserial elements (ossicles). The
80 two platelet biseries form a roof over a longitudinal median groove running along the upper
81 surfaces of the ossicles. Some interpretations suggest that the biserial platelets are fixed (Fig.
82 1F), but others show them as hinged, movable elements, much like cover plates in modern sea
83 lilies and their kin (crinoids) (Fig. 1C). In life, the test likely contained the viscera, and in some
84 taxa, bears a series of pores interpreted either as respiratory (Ubaghs, 1968; David et al., 2000;
85 Lefebvre, 2003) or pharyngeal structures (Jefferies, 1986; Dominguez et al., 2002; Shu et al.,
86 2004; Clausen and Smith, 2005; Smith, 2005; Bottjer et al., 2006) (Fig. 1A, C). Both the
87 appendage and the test consist of numerous calcitic skeletal elements possessing the same
88 microstructure (called stereom) found in all echinoderms (Ubaghs, 1968; Jefferies, 1986; David
89 et al., 2000; Dominguez et al., 2002; Lefebvre, 2003; Clausen and Smith, 2005; Smith, 2005;
90 Bottjer et al., 2006; Zamora and Rahman, 2014).

91 The phylogenetic position of stylophorans within deuterostomes has been a contentious matter
92 for decades. In the early 1960s, the stylophoran appendage was interpreted as a feeding arm,
93 comparable in morphology to the feeding structures of various extant echinoderms (Ubaghs,
94 1968; David et al., 2000; Lefebvre, 2003). In this hypothesis, stylophorans are considered bona
95 fide echinoderms, with their appendage bearing a single ambulacral ray of the water vascular
96 system (Figs. 1A–C, 2). In the late 1960s, the stylophoran appendage was reinterpreted as a
97 chordate-like tail, containing a notochord and serially arranged muscle blocks (Jefferies, 1986;
98 Dominguez et al., 2002). Thereby, stylophorans were designated calcichordates, ancestral
99 members of all three modern chordate lineages. This interpretation implies that the stereom
100 skeleton of extant echinoderms was an apomorphy of all deuterostomes and therefore
101 secondarily and independently lost in all lineages with the exception of echinoderms. Most
102 recently, the stylophoran appendage has been interpreted as a muscular, hemichordate-like stalk
103 (Shu et al., 2004; Clausen and Smith, 2005; Smith, 2005; Bottjer et al., 2006; Swalla and Smith,
104 2008; Caron et al., 2010) (Figs. 1D–F, 2). In this interpretation, stylophorans remained
105 echinoderms (because of the presence of the distinctive stereom), but are among the earliest
106 members of the phylum, because they retain plesiomorphic features lost in all more derived taxa
107 (e.g., locomotory stalk), and lack apomorphies present in more derived taxa (e.g., water vascular
108 system).

109 Two basic assumptions of the calcichordate interpretation have previously been falsified.
110 First, the purported sister-group relationship between echinoderms and chordates was not
111 confirmed by molecular phylogenetics, which indicated that echinoderms are more closely
112 related to hemichordates than they are to chordates (Bottjer et al., 2006; Swalla and Smith, 2008;
113 Erwin et al., 2011; Lowe et al., 2015). Second, available evidence supports the view that stereom

114 is an echinoderm synapomorphy that first appeared in the early Cambrian (Bottjer et al., 2006;
115 Kouchinsky et al., 2012; Zamora and Rahman, 2014). The last two remaining, current
116 hypotheses both agree that stylophorans are echinoderms (Fig. 2), but differ markedly in the
117 interpretation of their appendage: feeding arm constructed like a typical echinoderm ray [H1]
118 versus tail constructed like a hemichordate-like stalk [H2].

119 Here we report the first evidence for soft part preservation in stylophorans. These data make it
120 possible to test, for the first time, soft anatomies predicted by H1 and H2. The feeding arm
121 hypothesis (H1) requires that: (1) the longitudinal median groove contained a water vascular
122 canal, with lateral tube feet; (2) biserial platelets are cover plates that opened to expose the tube
123 feet; (3) the mouth was located at the proximal end of the food groove; and (4) the fore-gut
124 (esophagus) was located within the proximal rings. The locomotory stalk scenario (H2) implies
125 that: (1) the longitudinal median groove contained a peduncular nerve; and (2) biserial platelets
126 are integrated, contiguous body wall elements sutured to each other so as to enclose a coelom.

127

128

129 **2. Material and methods**

130

131 All figured specimens are housed in the collections of Cadi-Ayyad University (Faculté des
132 Sciences et Techniques, Guéliz), Marrakesh, Morocco. Locality details are indicated on
133 specimen labels, and can be provided on request. Specimens were prepared with scalpels and
134 needles under high magnification using a Zeiss SteREO Discovery.V8 stereomicroscope.
135 Interpretative drawings were made using a camera lucida attached to a Zeiss SteREO
136 Discovery.V8 stereomicroscope. Photographs were made with a Zeiss AxioCam MRc5 digital

137 camera mounted on a Zeiss SteREO Discovery.V8 stereomicroscope. Digital photographs were
138 processed in Adobe Photoshop Elements 9. Figs. 3A–B and Figs. S2A, S4A–B are composite
139 images stitched together using Adobe Photoshop Elements 9. Elemental composition was
140 analysed in a FEI Quanta 250 scanning electron microscope (SEM) equipped with an energy X-
141 ray analyser (EDX) at the Centre Technologique des Microstructures, plateforme de l'Université
142 Claude Bernard Lyon 1.

143

144

145 **3. Results**

146

147 Stylophorans with soft parts were collected in the Lower Ordovician Fezouata Konservat-
148 Lagerstätte, in a small excavation along the western flank of Bou Izargane hill, approximately 18
149 km north of Zagora (central Anti-Atlas, Morocco; Fig. 3). Here, the Fezouata Shale corresponds
150 to a 1,000 m-thick, repetitive succession of argillaceous siltstones, unconformable over the
151 middle Cambrian sandstones of the Tabanite Group (Destombes et al., 1985; Martin et al., 2016).
152 The excavation is approximately 270 m above the base of the Ordovician series and within the
153 lower stratigraphic interval (about 80 m thick) yielding soft-bodied faunas typical of the
154 Fezouata Biota (Vinther et al., 2008, 2017; Van Roy et al., 2010, 2015; Van Roy and Briggs,
155 2011; Lefebvre et al., 2016; Martin et al., 2016) (Fig. 3). Nearby graptolites indicate a late
156 Tremadocian age (*A. murrayi* Zone) (Gutiérrez-Marco and Martin, 2016; Lefebvre et al., 2016,
157 2018; Martin et al., 2016).

158 All specimens were collected from a single, small, 3 cm-thick lens of dark blue mudstones.
159 Three fossiliferous intervals occur within the lens: a lower layer with many densely packed,

160 complete, and fully articulated marrellomorphs (*Furca* sp.; Fig. 4A), an intermediate horizon
161 with a low-diversity assemblage of trilobites (*Anacheirurus* sp., *Bavarilla* sp.) with soft parts
162 (Fig. 4B–C), and an upper, low-diversity assemblage comprising almost exclusively cornute
163 stylophorans (more than 300 specimens belonging to two taxa, *Hanusia* n. sp. and *Thoralicystis*
164 n. sp.; Figs 5–9). Deposition is interpreted as rapid burial of autochthonous communities by
165 distal storm deposits in relatively shallow environmental conditions (Martin et al., 2016; Vaucher
166 et al., 2016). In this level, arthropods and echinoderms are typically preserved as faint, flattened
167 impressions on the dark rock. As in the Burgess Shale, echinoderm skeletons are indicated as
168 impressions without preservation of actual stereom. In contrast, their soft tissues appear as
169 colorful structures, resulting from the oxidation of original pyrite into yellow, red to purple iron
170 oxide pseudomorphs (Figs. 5A, 6A, 7A, 8A, C). This type of preservation is not unique to the
171 Bou Izargane cornutes (Vinther et al., 2008, 2017; Van Roy et al., 2010, 2015; Lefebvre et al.,
172 2016). Similarly preserved soft parts (e.g. guts) have been described in arthropods (Gutiérrez-
173 Marco et al., 2017), hyolithids (Marti Mus, 2016), and machaeridian annelids (Vinther et al.,
174 2008) occurring in the same stratigraphic interval of the Fezouata Shale. In the cornutes, the
175 identification and precise distribution of Fe-rich soft tissues were obtained by chemical analyses
176 and elemental mapping under SEM (Figs. 5B, E, 6).

177 At least one specimen of *Thoralicystis* n. sp. shows clear evidence of soft-bodied structures in
178 the distal part of its appendage (Figs. 5–6). Preserved soft tissues consist of: (1) two
179 superimposed longitudinal canals housed in the continuous, median groove on the upper surface
180 of ossicles, and extending from the stylocone cavity (proximally) to the distal end of the
181 appendage; (2) numerous small, lateral tube-like extensions branching regularly from the
182 external longitudinal canal and protruding outward from between widely open, hinged plates.

183 Several specimens of *Thoralicystis* n. sp. and *Hanusia* n. sp. exhibit strong evidence of soft
184 tissues preserved within the proximal part of their appendage (Figs. 7–8). All these specimens
185 display a spindle-shaped, elongate cavity extending throughout the proximal rings of the
186 appendage from the stylocone cavity (distally), to the main body cavity (proximally). All these
187 soft-bodied structures are described for the first time in stylophorans.

188

189

190 **4. Discussion**

191

192 *4.1. The stylophoran appendage is a feeding arm*

193

194 On the basis of this new evidence, it is possible to evaluate critically the two current
195 interpretations of the stylophoran appendage. In the stalk model (H2), the outermost of the two
196 longitudinal canals could be interpreted as the peduncular nerve housed in a fully enclosed body
197 cavity, or coelom. However, H2 must be rejected because of the presence of open plates that
198 precludes such a coelom, and the presence of lateral extensions that branch regularly from the
199 external longitudinal canal and protrude externally in between the open plates. This organisation
200 is incompatible with that of a stalk-, or tail-like structure, but fits requisites of the feeding-arm
201 model (H1). The evidence strongly supports H1, in which the external longitudinal canal is
202 interpreted as an ambulacral canal of the water vascular system, and the lateral, tube-like
203 structures as ambulacral tube feet (Figs. 5–6, 9), the morphology of which is strikingly similar to
204 that of exceptionally preserved tube feet described from Paleozoic arm-bearing forms (Glass and
205 Blake, 2004; Sutton et al., 2005; Glass, 2006; Clark et al., 2017). As a consequence, the mouth of

206 stylophorans is most parsimoniously located at the proximal end of this ambulacral groove (Fig.
207 5C), the spindle-shaped cavity extending from the mouth (distally) and connected to the main
208 body cavity (proximally) representing the anterior part of the gut (Figs. 5, 7–8). Comparison with
209 the situation in the arms and pinnules of modern crinoids suggests that the internal longitudinal
210 canal internal to the space above the water vascular system is a coelomic canal (Figs. 5–6). There
211 is no evidence that what are interpreted here as tube feet represent some sort of musculature,
212 because they project as free-standing structures extending from the longitudinal canal, and
213 protrude outside of the body wall into the external medium.

214 Labyrinthic meshwork has been described in the proximal cavity of the stylocone (large,
215 cone-shaped, uniserial ossicle located at the proximal end of the distal part of the appendage) of
216 the primitive stylophoran *Ceratocystis* (middle Cambrian) (Clausen and Smith, 2005). Similar
217 observations in extant echinoderms suggest that this labyrinthic microstructure was associated
218 with muscle insertion, implying that the stylocone cavity once housed musculature with
219 functionality similar to that in hemichordates. However, living echinoderms express muscles as
220 well, so this trait is compatible with both H1 (Fig. 1B) and H2 (Fig. 1E), and cannot be used to
221 provide evidence for or against either.

222 Finally, the putative locomotory function of the stylophoran appendage has been frequently
223 considered as incompatible with its interpretation as a feeding arm and the presence of a water-
224 vascular canal (Rahman et al., 2009; Rozhnov and Parsley, 2017). As this is the case in many
225 extant arm-bearing echinoderms (e.g. asteroids, ophiuroids, comatulids), it is very likely that
226 stylophorans used their appendage not only for nutrition, but also locomotion (Lefebvre, 2003).

227

228 *4.2. Implications for echinoderm phylogeny*

229

230 Preserved soft tissues in cornutes from the Fezouata Shale provide strong evidence suggesting
231 the existence of ambulacral structures in the stylophoran appendage. The evidence supports the
232 view that this extinct group of ambulacrarians possessed at least two of the major apomorphies
233 supporting recognition of the phylum Echinodermata: (1) a multi-element endoskeleton, made of
234 calcitic plates displaying the typical, three-dimensional, stereomic microstructure; and (2) a
235 water vascular system. However, the precise phylogenetic position of stylophorans within
236 echinoderms remains so far unresolved because they do not display any evidence of the radial,
237 five-fold symmetry generally considered a synapomorphy of the phylum (Shu et al., 2004;
238 Smith, 2005; Bottjer et al., 2006; Swalla and Smith, 2008; Zamora and Rahman, 2014). If this
239 absence is original, stylophorans would then represent a relatively early branch of pre-radial
240 echinoderms (Ubaghs, 1968, 1975; Shu et al., 2004; Smith, 2005; Bottjer et al., 2006; Swalla and
241 Smith, 2008; Zamora and Rahman, 2014). Such an interpretation requires that anatomically
242 similar, arm-like, feeding structures were acquired independently in stylophorans in the middle
243 Cambrian, and again, in later forms such as asteroids and crinoids, in the Early Ordovician
244 (Ubaghs, 1968, 1975).

245 Alternatively, the late appearance of stylophorans in the fossil record 10-12 m.y. after radial
246 taxa (e.g., eocrinoids, helicoplacoids, and edrioasteroid-like forms such as stromatocystitoids)
247 (Kouchinsky et al., 2012; Zamora et al., 2013) and their unusual, flattened morphology more
248 parsimoniously suggest that stylophorans are relatively derived echinoderms descended from
249 pentaradial ancestors (David et al., 2000). This interpretation is supported by embryological data,
250 which provide no evidence suggesting that 5-part radial symmetry is preceded by stages with
251 fewer rays (Mooi and David, 2008). In this context, the possession of arm-like, ambulacral

252 structures containing coelomic extensions other than the hydrocoel could represent an
253 apomorphy uniting stylophorans with forms such as crinoids (David et al., 2000; Lefebvre,
254 2003).

255

256 *4.3. Implications for early deuterostome phylogeny*

257

258 The exceptionally preserved cornute specimens from Morocco bring revolutionary new
259 evidence to consider. That evidence actually reverses the anterior-posterior axis suggested by
260 some very recent interpretations seeking to revive a modified version of the calcichordate
261 interpretation (Shu et al., 2001, 2004; Clausen and Smith, 2005; Smith, 2005; Bottjer et al., 2006;
262 Swalla and Smith, 2008; Zamora and Rahman, 2014). Since the early 2000s, molecular
263 phylogenies have strongly supported an ambulacrarian clade that unites hemichordates and
264 echinoderms as sister-group to the chordates (Bottjer et al., 2006; Swalla and Smith, 2008; David
265 and Mooi, 2014; Holland et al., 2015; Janvier, 2015; Lower et al., 2015). As a consequence of
266 this new phylogenetic framework, several authors reinterpreted stylophorans as early members of
267 the Echinodermata, morphologically extremely close to the last common ancestor of both
268 hemichordates and echinoderms (Shu et al., 2001, 2004; Clausen and Smith, 2005; Smith, 2005;
269 Bottjer et al., 2006; Swalla and Smith, 2008). In this interpretation, stylophorans would possess a
270 single echinoderm apomorphy: the calcite, stereom skeleton. They would not yet have acquired
271 two other significant echinoderm apomorphies: the water-vascular system and the radial, five-
272 fold symmetry.

273 In a surprising parallel to the calcichordate hypothesis, stylophorans would share with other
274 (early) ambulacrarians and deuterostomes (1) a bipartite body organization, with a head and a
275 muscular, locomotory tail (or stalk); and (2) the presence (plesiomorphic retention) of gill slits
276 (Shu et al., 2001, 2004; Conway Morris, 2003; Clausen and Smith, 2005; Smith, 2005; Bottjer et
277 al., 2006; Swalla and Smith, 2008; Caron et al., 2010; Conway Morris and Caron, 2012; Zamora
278 and Rahman, 2014; Conway Morris et al., 2015). As a consequence, this interpretation of
279 stylophorans as hemichordate-like, early echinoderms with a bipartite organization (anterior head
280 and posterior, tail-like appendage) has in the last 15 years caused many comparisons of early
281 deuterostomes to stylophorans, i.e. *Herpetogaster* and cambroernids (Caron et al., 2010), *Pikaia*
282 and other putative early chordates (Donoghue et al., 2003; Gee, 2006; Conway Morris and
283 Caron, 2012), vetulicolians (Shu et al., 2001; Conway Morris, 2003; Vinther et al., 2011; Ou et
284 al., 2012), and vetulicystids (Shu et al., 2004; Conway Morris et al., 2015).

285 Our study demonstrates that: (1) stylophorans had a water-vascular system; and (2) that their
286 appendage was not a locomotory, muscular tail. By bringing new, unequivocal evidence that the
287 stylophoran appendage is an echinoderm feeding arm (comparable in morphology to a crinoid
288 feeding arm) and not a hemichordate-like tail (or stalk), our discovery shows that the
289 interpretation of these fossils as early echinoderms retaining features of basal ambulacrarians
290 and/or hemichordates can be definitively rejected. In fact, any suggestion that stylophorans can
291 tell us something about common ancestry of echinoderms with other deuterostomes is seriously
292 compromised by the solid evidence that such conclusions rely on a reconstruction that has the
293 animal the wrong way around. The bipartite body organization of stylophorans (theca and
294 feeding arm) is not homologous to the bipartite body organization of *Herpetogaster*,
295 vetulicolians, vetulicystids, early chordates and/or hemichordates (head and post-anal tail).

296 Whatever the precise phylogenetic position of stylophorans within the Echinodermata, the
297 significance of the Fezouata fossils lies in the picture they give of the remarkable diversity
298 within the phylum throughout its evolution, and not of the supposed origins of the phylum itself.

299

300

301 **Acknowledgements**

302

303 This paper is a contribution of the ANR (Agence Nationale de la Recherche) research project
304 entitled "The Rise of Animal Life (Cambrian-Ordovician): organisation and tempo" (grant
305 number ANR-11-BS56-0025), the CNRS-CNRST cooperation project VALORIZ (grant number
306 52943) and the CNRS-INSU TelluS-INTERRVIE 2018 project TAPHO FEZOUATA. Abel
307 Prieur (Lyon 1 University, Villeurbanne) is recognized for discovering the level with exceptional
308 preservation in the field, Jean Vannier (Lyon 1 University, Villeurbanne), for technical
309 assistance with SEM, and both Bernard Pittet and Romain Vaucher (Lyon 1 University,
310 Villeurbanne) for making available detailed sedimentological information on the locality. Ronald
311 L. Parsley (Tulane University, New Orleans), Imran Rahman (Oxford University Museum of
312 Natural History), Sergei Rozhnov (Russian Academy of Sciences, Moscow), Samuel Zamora
313 (Instituto Geológico y Minero de España, Zaragoza) and five anonymous reviewers provided
314 insightful comments on several versions of the MS.

315

316

317 **References**

318

319 Bottjer, D.J., Davidson, E.H., Peterson, K.J., Cameron, R.A., 2006. Paleogenomics of
320 echinoderms. *Science* 314, 956–960.

321 Briggs, D.E.G., 1992. Conodonts - a major extinct group added to the vertebrates. *Science* 256,
322 1285–1286.

323 Caron, J.B., Conway Morris, S., Shu, D.G., 2010. Tentaculate fossils from the Cambrian of
324 Canada (British Columbia) and China (Yunnan) interpreted as primitive deuterostomes.
325 *PlosOne* 5, e9586.

326 Caron, J.B., Conway Morris, S., Cameron, C.B., 2013. Tubicolous enteropneusts from the
327 Cambrian period. *Nature* 495, 503–506.

328 Clark, E.G., Bhullar, B.A.S., Darroch, S.A.F., Briggs, D.E.G., 2017. Water vascular system
329 architecture in an Ordovician ophiuroid. *Biology Letters* 13, 20170635

330 Clausen, S., Smith, A.B., 2005. Palaeoanatomy and biological affinities of a Cambrian
331 deuterostome (Stylophora). *Nature* 438, 351–354.

332 Conway Morris, S., 2003. The Cambrian "explosion" of metazoans and molecular biology:
333 would Darwin be satisfied? *International Journal of Developmental Biology* 47, 505–515.

334 Conway Morris, S., Caron, J.B., 2012. *Pikaia gracilens* Walcott, a stem-group chordate from the
335 Middle Cambrian of British Columbia. *Biological Reviews* 87, 480–512.

336 Conway Morris, S., Halgedahl, S.L., Selden, P., Jarrard, R.D., 2015. Rare primitive
337 deuterostomes from the Cambrian (Series 3) of Utah. *Journal of Paleontology* 89, 631–636.

338 David, B., Mooi, R., 2014. How Hox genes can shed light on the place of echinoderms among
339 the deuterostomes. *EvoDevo* 5, 22.

340 David, B., Lefebvre, B., Mooi, R., Parsley, R.L., 2000. Are homalozoans echinoderms? An
341 answer from the extraxial-axial theory. *Paleobiology* 26, 529–555.

342 Destombes, J., Hollard, H., Willefert, S., 1985. Lower Palaeozoic rocks of Morocco, in:
343 Holland, C.H. (Ed.), *Lower Palaeozoic Rocks of the World Volume 4: Lower Palaeozoic of*
344 *North-Western and West-Central Africa*. John Wiley & Sons, Chichester, pp. 91–336.

345 Dominguez, P., Jacobson, A.G., Jefferies, R.P.S., 2002. Paired gill slits in a fossil with a calcite
346 skeleton. *Nature* 417, 841–844.

347 Donoghue, P.C.J., Smith, M. P., Sansom, I.J., 2003. The origin and early evolution of chordates:
348 molecular clocks and the fossil record, in: Donoghue, P.C.J., Smith, M.P. (Eds.), *Telling the*
349 *Evolutionary Time: Molecular Clocks and the Fossil Record*. Taylor & Francis, London, pp.
350 190–223.

351 Erwin, D.H., Laflamme, M., Tweedt, S.M., Sperling, E.A., Pisani, D., Peterson, K.J., 2011. The
352 Cambrian conundrum: early divergence and later ecological success in the early history of
353 animals. *Science* 334, 1091–1097.

354 Gee, H., 2006. Careful with that amphioxus. *Nature* 439, 923–924.

355 Glass, A., 2006. Pyritized tube feet in a protasterid ophiuroid from the Upper Ordovician of
356 Kentucky, U.S.A. *Acta Palaeontologica Polonica* 51, 171–184.

357 Glass, A., Blake, D.B., 2004. Preservation of tube feet in an ophiuroid (Echinodermata) from the
358 Lower Devonian Hunsrück Slate of Germany and a redescription of *Bundenbachia beneckeii*
359 and *Palaeophiomys grandis*. *Paläontologische Zeitschrift* 78, 73–95.

360 Gutiérrez-Marco, J.C., Martín, E.L.O., 2016. Biostratigraphy and palaeoecology of Lower
361 Ordovician graptolites from the Fezouata Shale (Moroccan Anti-Atlas). *Palaeogeography*
362 *Palaeoclimatology Palaeoecology* 460, 35–49.

363 Gutiérrez-Marco, J.C., Garcia-Bellido, D.C., Rábano, I., Sá, A.A., 2017. Digestive and
364 appendicular soft-parts, with behavioural implications, in a large trilobite from the Fezouata
365 Lagerstätte, Morocco. *Scientific Reports* 7, 39728.

366 Holland, N.D., Holland, L.Z., Holland, P.W.H., 2015. Scenarios for the making of vertebrates.
367 *Nature* 520, 450–455.

368 Janvier, P., 2015. Facts and fancies about early fossil chordates and vertebrates. *Nature* 520,
369 483–489.

370 Jefferies, R.P.S., 1986. *The Ancestry of the Vertebrates*. British Museum (Natural History),
371 London.

372 Kouchinsky, A., Bengtson, S., Runnegar, B., Skovsted, C., Steiner, M., Vedrasco, M., 2012.
373 Chronology of early Cambrian biomineralization. *Geological Magazine* 149, 221–251.

374 Lefebvre, B., 2003. Functional morphology of stylophoran echinoderms. *Palaeontology* 46, 511–
375 555.

376 Lefebvre, B., El Hariri, K., Lerosey-Aubril, R., Servais, T., Van Roy, P., 2016. The Fezouata
377 Shale (Lower Ordovician, Anti-Atlas, Morocco): a historical review. *Palaeogeography*
378 *Palaeoclimatology Palaeoecology* 460, 7–23.

379 Lefebvre, B., Gutiérrez-Marco, J.C., Lehnert, O., Martin, E.L.O., Nowak, H., Akodad, M., El
380 Hariri, K., Servais, T., 2018. Age calibration of the Lower Ordovician Fezouata Lagerstätte,
381 Morocco. *Lethaia* 51, 296–311.

382 Lowe, C.J., Clarke, D.N., Medeiros, D.M., Rokhsar, D.S., Gerhart, J., 2015. The deuterostome
383 context of chordate origins. *Nature* 520, 456–465.

384 Martí Mus, M., 2016. A hyolithid with preserved soft parts from the Ordovician Fezouata
385 Konservat-Lagerstätte of Morocco. *Palaeogeography Palaeoclimatology Palaeoecology* 460,
386 122–129.

387 Martin, E.L.O., Pittet, B., Gutiérrez-Marco, J.C., Vannier, J., El Hariri, K., Lerosey-Aubril, R.,
388 Masrour, M., Nowak, H., Servais, T., Vandenbroucke, T.R.A., Van Roy, P., Vaucher, R.,
389 Lefebvre, B., 2016. The Lower Ordovician Fezouata Konservat-Lagerstätte from Morocco:
390 age, environment and evolutionary perspectives. *Gondwana Research* 34, 274–283.

391 Mooi, R., David, B., 1998. Evolution within a bizarre phylum: homologies of the first
392 echinoderms. *American Zoologist* 38, 965–974.

393 Mooi, R., David, B., 2008. Radial symmetry, the anterior/posterior axis, and echinoderm Hox
394 genes. *Annual Review of Ecology Evolution, and Systematics* 39, 43–62.

395 Ou, Q., Conway Morris, S., Han, J., Zhang, J., Liu, J., Chen, A., Zhang, X., Shu, D.G., 2012.
396 Evidence for gill slits and a pharynx in Cambrian vetulicolians: implications for the early
397 evolution of deuterostomes. *BioMed Central Biology* 10, 81.

398 Rahman, I.A., Jefferies, R.P.S., Südkamp, W.H., Smith, R.D.A., 2009. Ichnological insights into
399 mitrate palaeobiology. *Palaeontology* 52, 127–138.

400 Rozhnov, S.V., Parsley, R.L., 2017. A new cornute (Homalozoa: Echinodermata) from the
401 uppermost middle Cambrian (Stage 3, Furongian) from northern Iran: its systematics and
402 functional morphology. *Paleontological Journal* 51, 500–509.

403 Shu, D.G., Conway Morris, S., Han, J., Chen, L., Zhang, X.L., Zhang, Z.F., Liu, H.Q., Li, Y.,
404 Liu, J.N., 2001. Primitive deuterostomes from the Chengjiang Lagerstätte (Lower Cambrian,
405 China). *Nature* 414, 419–424.

406 Shu, D.G., Conway Morris, S., Han, J., Zhang, Z.F., Liu, J.H., 2004. Ancestral echinoderms
407 from the Chengjiang deposits of China. *Nature* 430, 422–428.

408 Smith, A.B., 2005. The pre-radial history of echinoderms. *Geological Journal* 40, 255-280.

409 Sutton, M.D., Briggs, D.E.G., Siveter, D.J., Siveter, D.J., Gladwell, D.J., 2005. A starfish with
410 three-dimensionally preserved soft parts from the Silurian of England. *Proceedings of the*
411 *Royal Society B* 272, 1001–1006.

412 Swalla, B.J., Smith, A.B., 2008. Deciphering deuterostome phylogeny: molecular, morphological
413 and palaeontological perspectives. *Philosophical Transactions of the Royal Society B* 363,
414 1557–1568.

415 Ubahgs, G., 1968. Stylophora, in: Moore, R.C. (Ed.), *Treatise on Invertebrate Paleontology*. Part
416 S. Echinodermata 1. Geological Society of America and University of Kansas Press,
417 Lawrence, S495–S565.

418 Ubahgs, G., 1975. Early Paleozoic echinoderms. *Annual Review of Earth and Planetary Sciences*
419 3, 79–98.

420 Van Roy, P., Briggs, D.E.G., 2011. A giant Ordovician anomalocaridid. *Nature* 473, 510–513.

421 Van Roy, P., Orr, P.J., Botting, J.P., Muir, L.A., Vinther, J., Lefebvre, B., El Hariri, K., Briggs,
422 D.E.G., 2010. Ordovician faunas of Burgess Shale type. *Nature* 465, 215–218.

423 Van Roy, P., Briggs, D.E.G., Gaines, R.R., 2015. The Fezouata fossils of Morocco; an
424 extraordinary record of marine life in the Early Ordovician. *Journal of the Geological Society*
425 172, 541–549.

426 Vaucher, R., Martin, E.L.O., Hormière, H., Pittet, B., 2016. A genetic link between *Konzentrat-*
427 *and Konservat-Lagerstätten* in the Fezouata Shale (Lower Ordovician, Morocco).
428 *Palaeogeography Palaeoclimatology Palaeoecology* 460, 24–34.

429 Vinther, J., Van Roy, P., Briggs, D.E.G., 2008. Machaeridians are Palaeozoic armoured annelids.
430 Nature 451, 185–188.

431 Vinther, J., Smith, M.P., Harper, D.A.T., 2011. Vetulicolians from the Lower Cambrian Sirius
432 Passett Lagerstätte, North Greenland, and the polarity of morphological characters in basal
433 deuterostomes. Palaeontology 54, 771–719.

434 Vinther, J., Parry, L., Briggs, D.E.G., Van Roy, P., 2017. Ancestral morphology of crown-group
435 molluscs revealed by a new Ordovician stem aculiferan. Nature 542, 471–475.

436 Zamora, S., Rahman, I.A., 2014. Deciphering the early evolution of echinoderms with Cambrian
437 fossils. Palaeontology 57, 1105–1119.

438 Zamora S., Lefebvre B., Alvaro J.J., Clausen S., Elicki O., Fatka O., Jell P., Kouchinsky A., Lin
439 J.P., Nardin E., Parsley R.L., Rozhnov S., Sprinkle J., Sumrall C.D., Vizcaïno D., Smith A.B.,
440 2013. Cambrian echinoderm diversity and palaeobiogeography, in: Harper, D.A.T., Servais,
441 T. (Eds.), Early Palaeozoic Biogeography and Palaeogeography. Geological Society of
442 London Memoirs 38, 157–171.

443

444 **Figure captions**

445

446 **Fig. 1.** The two current interpretations of stylophoran morphology and their implications for soft-
447 tissue anatomy, based on the cornute *Phyllocystis blayaci* (Lower Ordovician, France). **A–C.** H1,
448 stylophorans as typical echinoderms with a single feeding arm. **D–F.** H2, stylophorans as pre-
449 radial echinoderms with a hemichordate-like stalk. **A, D.** Basic anatomical features deduced
450 from skeletal morphology. **B, E.** Reconstructions of soft tissue anatomy along a longitudinal

451 section of the stylophoran appendage. **C, F.** Three-dimensional reconstructions of the soft tissue
452 anatomy in the distal part of the stylophoran appendage.

453
454 **Fig. 2.** The two current interpretations of stylophoran phylogenetic position within
455 ambulacrarian deuterostomes. H1, stylophorans as typical echinoderms with a single feeding
456 arm. H2, stylophorans as pre-radial echinoderms with a hemichordate-like stalk.

457
458 **Fig. 3.** Geographic and geologic context of Bou Izargane locality. On the maps (left), Ordovician
459 outcrops in north-western Africa and in the Zagora area are indicated in green. The red star
460 shows the location of Bou Izargane hill. On the right, partial stratigraphic column of the
461 Fezouata Shale, showing the position of the Bou Izargane excavation. The left column indicates
462 sea level changes. Numbers along the right column indicate the height (in meters) above the
463 Cambrian/Ordovician boundary. The colors used in the log correspond to those of outcropping
464 rocks.

465
466 **Fig. 4.** Arthropods with soft-tissue preservation collected in the same lens as cornute
467 stylophorans with soft parts, late Tremadocian (Lower Ordovician), Bou Izargane, Zagora area
468 (Morocco). **A.** Two associated specimens of the marrellomorph *Furca* sp. (AA.BIZ15.OI.364).
469 **B–C.** Two specimens of the trilobite *Bavarilla zemmourensis*. **B.** Arrows indicate four
470 locomotory appendages below the thorax (AA.BIZ15.OI.181). **C.** The arrow shows one frontal
471 appendage at the anterior extremity of the cephalon (AA.BIZ15.OI.16). Scale bars, 5 mm (A, C);
472 2 mm (B).

473

474 **Fig. 5.** *Thoralicystis* n. sp. from the Fezouata Shale, Lower Ordovician, Morocco
475 (AA.BIZ15.OI.259). **A–C.** Complete specimen in lateral view. **A.** Composite photographic
476 reconstruction. **B.** Composite SEM elemental map of Fe. **C.** Composite reconstruction of soft
477 part anatomy based on camera lucida drawings, SEM elemental maps (Fe) and back-scattered
478 electron micrographs. **D–F.** Magnified views of parts of the distal appendage outlined in (A–C).
479 **D.** Back-scattered electron micrograph. The arrows indicate the position of tube feet. **E.** SEM
480 elemental map showing the distribution of Fe. **F.** Reconstruction of the soft parts based on
481 camera lucida drawings, SEM elemental maps (Fe) and back-scattered electron micrographs. pr.
482 cavity, proximal cavity; pr. rings, proximal rings; styl., stylocone; wvs, water vascular system.
483 Scale bars, 5 mm (A–C); 1 mm (D–F).

484
485 **Fig. 6.** Soft-tissue preservation in the distal appendage of the cornute stylophoran *Thoralicystis*
486 n. sp. (AA.BIZ15.OI.259), late Tremadocian (Lower Ordovician), Bou Izargane, Zagora area
487 (Morocco). **A.** Photographic view of the magnified area. **B.** Back-scattered electron micrograph
488 showing the water vascular system (ambulacral canal, tube feet) and the second, more internal,
489 longitudinal canal. Skeletal elements (biserial platelets, ossicles) are not visible. **C–J.** SEM
490 elemental maps of Al (**C**), C (**D**), Fe (**E**), K (**F**), Mg (**G**), Na (**H**), O (**I**) and Si (**J**). Scale bars, 1
491 mm.

492
493 **Fig. 7.** Soft-tissue preservation in the test and proximal appendage of the cornute stylophoran
494 *Hanusia* n. sp. (AA.BIZ15.OI.110), late Tremadocian (Lower Ordovician), Bou Izargane, Zagora
495 area (Morocco). **A.** Composite photographic reconstruction of the specimen in lateral view. **B.**
496 Composite reconstruction based on back-scattered electron micrographs. **C.** Reconstruction of

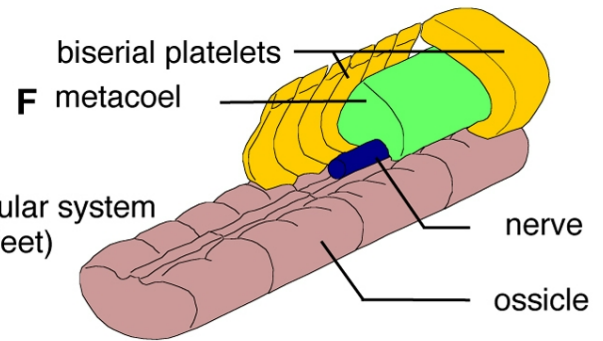
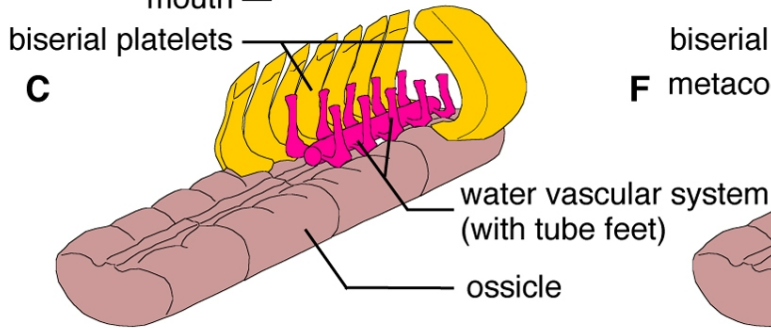
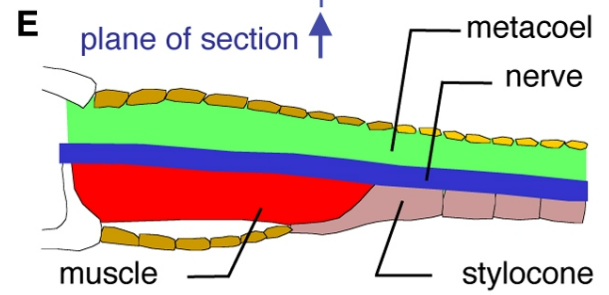
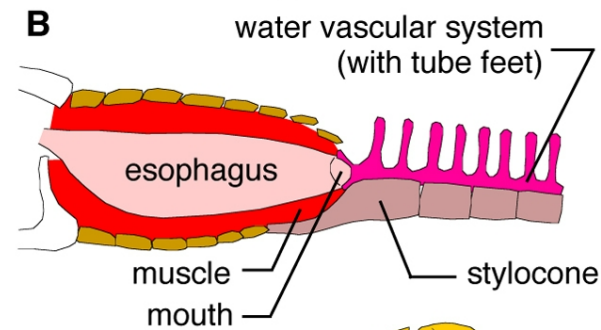
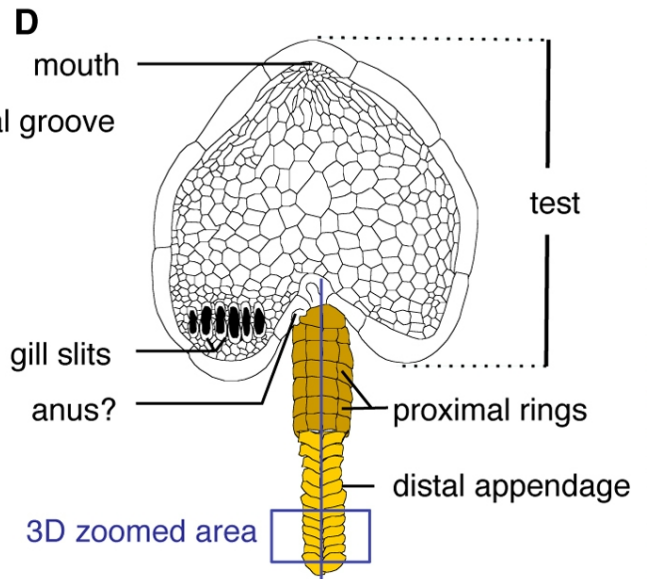
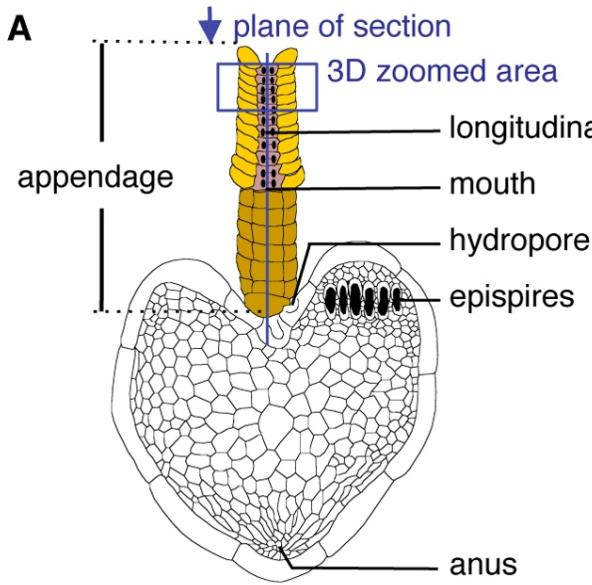
497 the soft parts based on camera lucida drawings and back-scattered electron micrographs. bis.
498 platelets, biserial platelets. Scale bars, 5 mm.

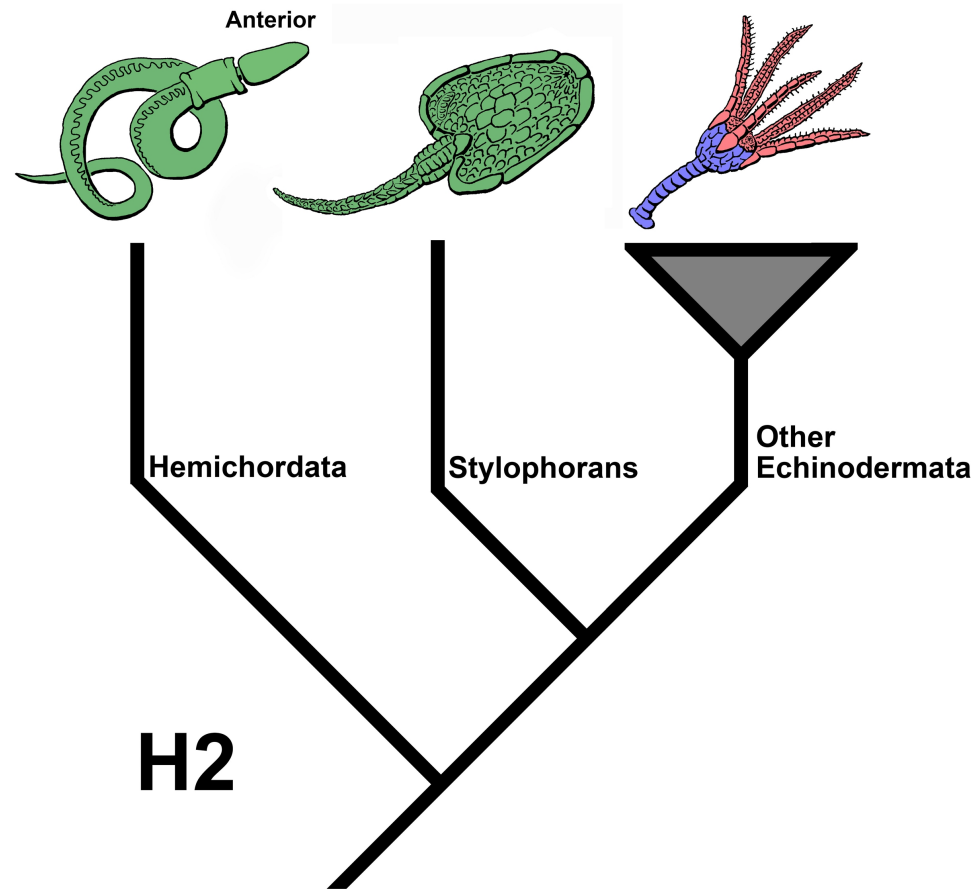
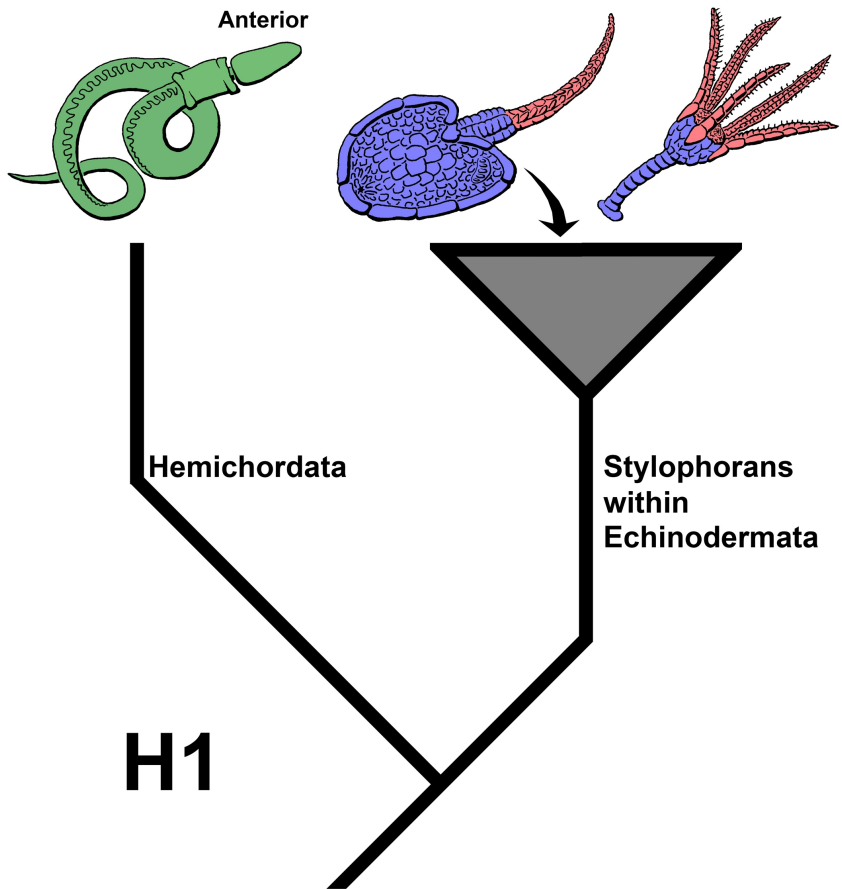
499

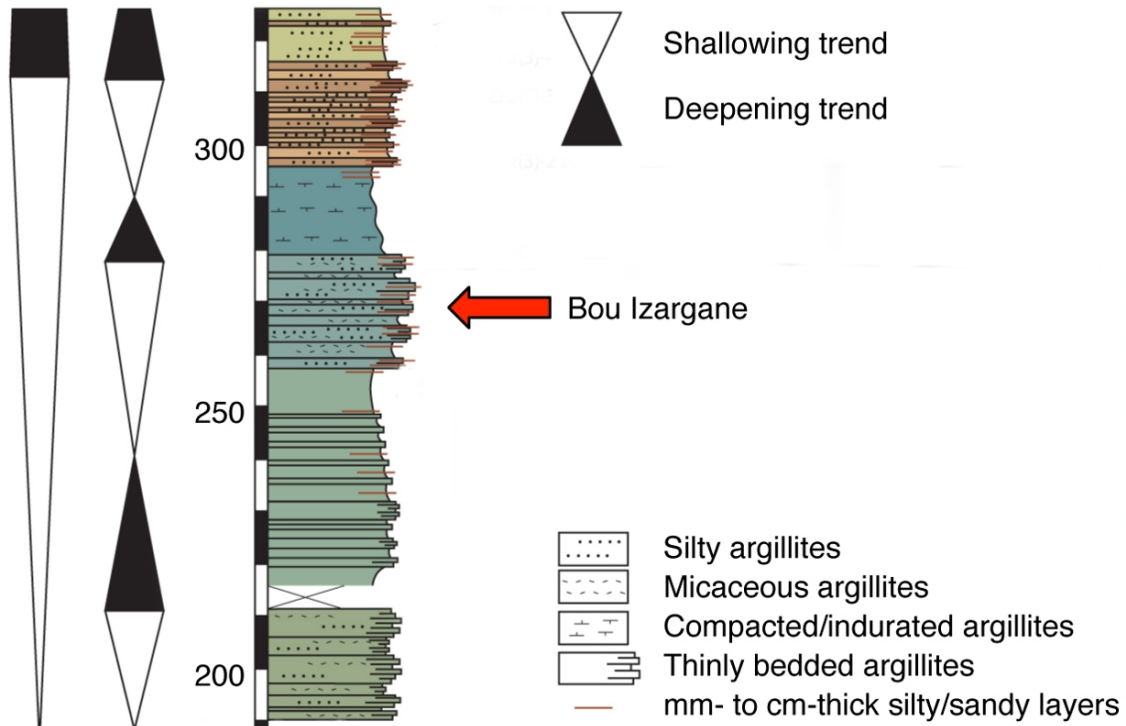
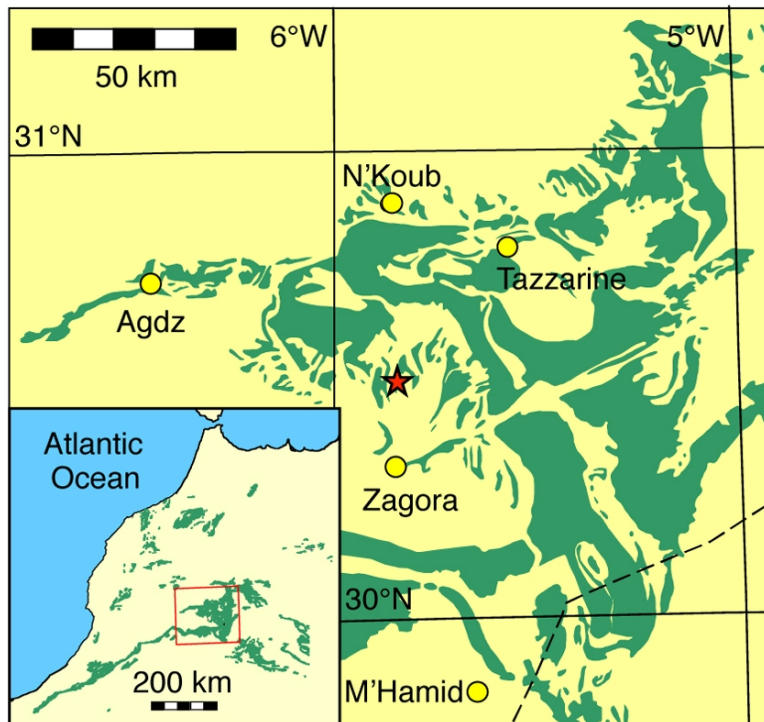
500 **Fig. 8.** Soft-tissue preservation in the proximal appendage of the cornute stylophoran *Hanusia* n.
501 sp. (AA.BIZ15.OI.80), late Tremadocian (Lower Ordovician), Bou Izargane, Zagora area
502 (Morocco). **A.** Specimen in upper view. **B.** Reconstruction of (A), based on camera lucida
503 drawings. **C.** Closer view of the proximal region of the appendage outlined in (A). **D.**
504 Reconstruction of (C), based on camera lucida drawings. pr. cavity, proximal cavity; pr. rings,
505 proximal rings. Scale bars, 1 mm.

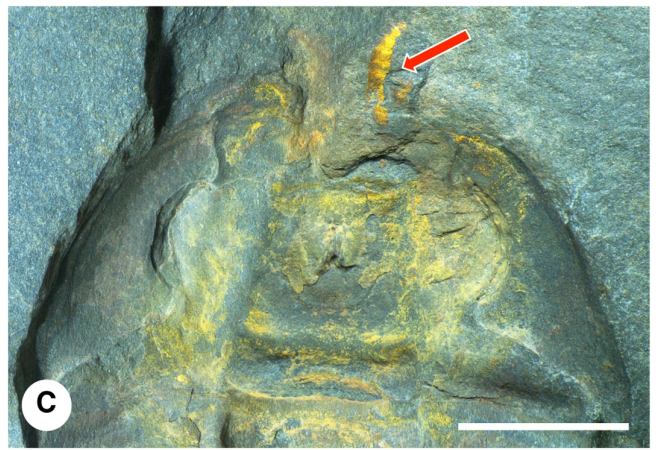
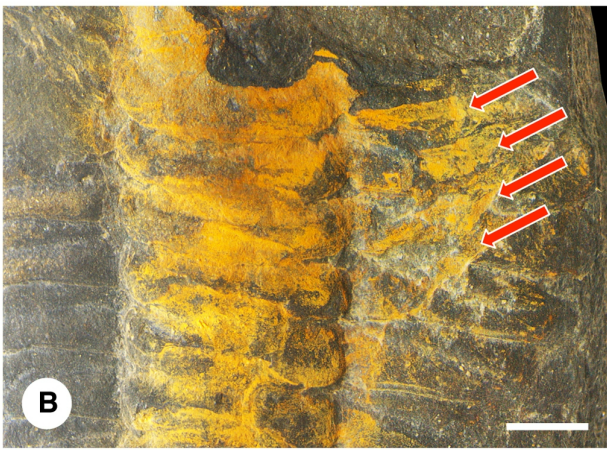
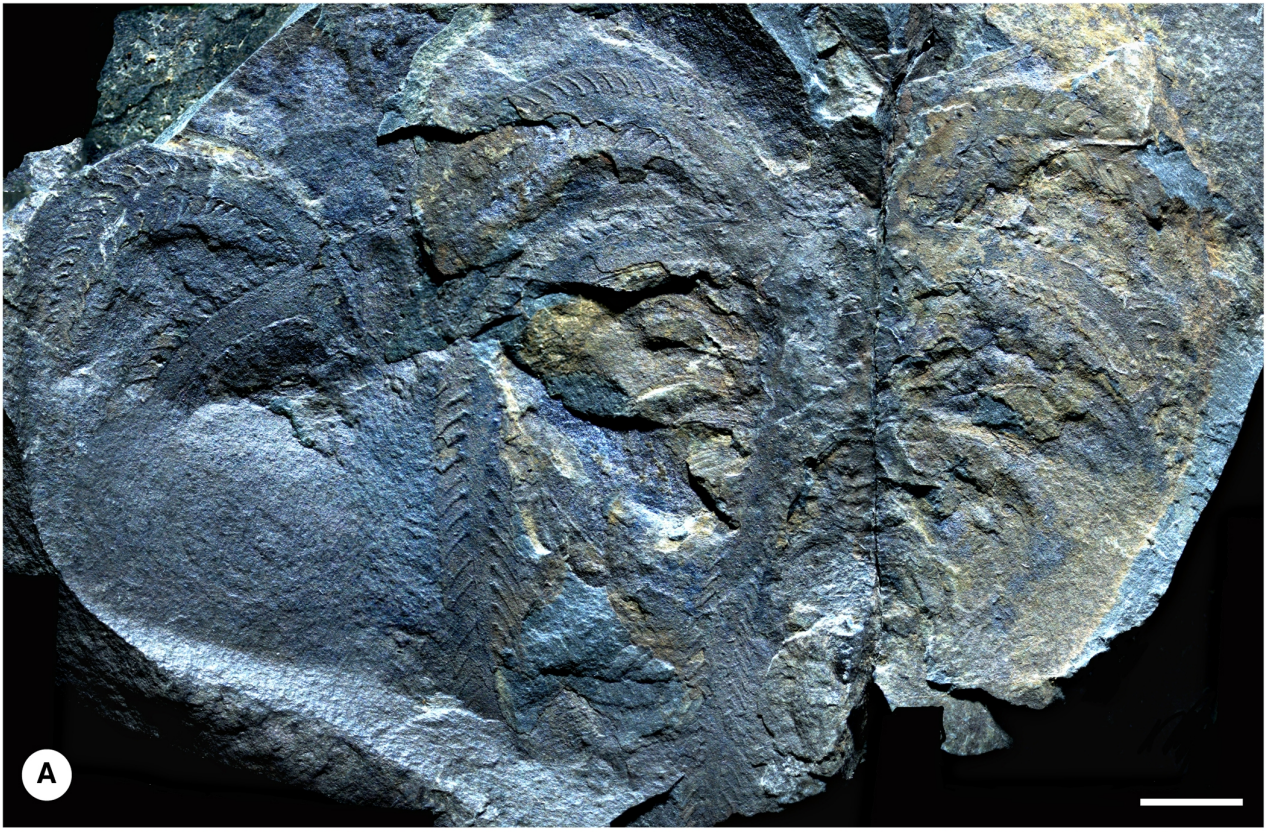
506

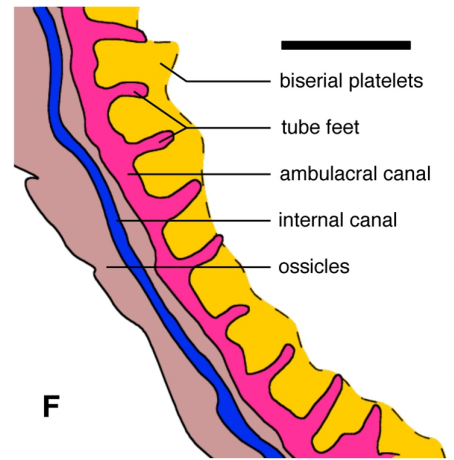
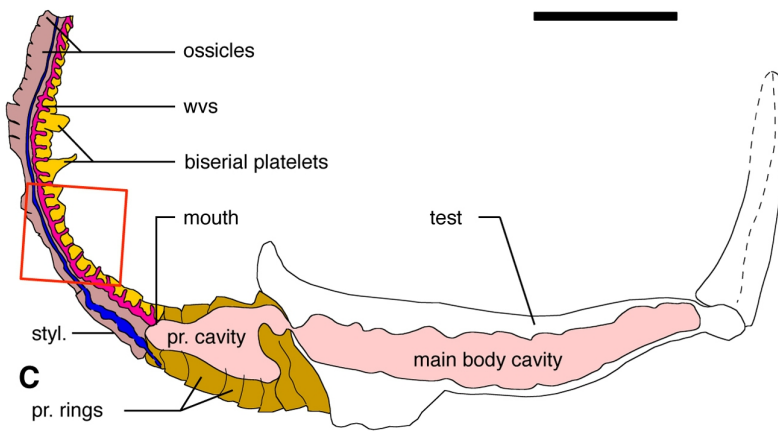
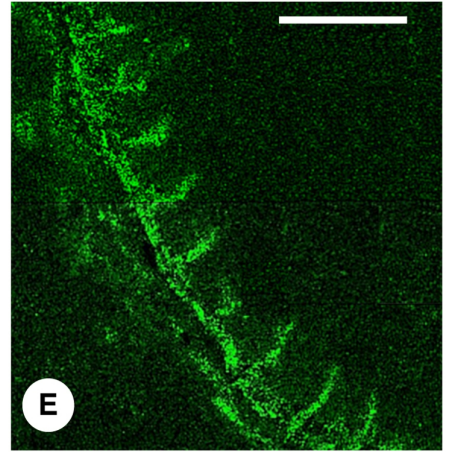
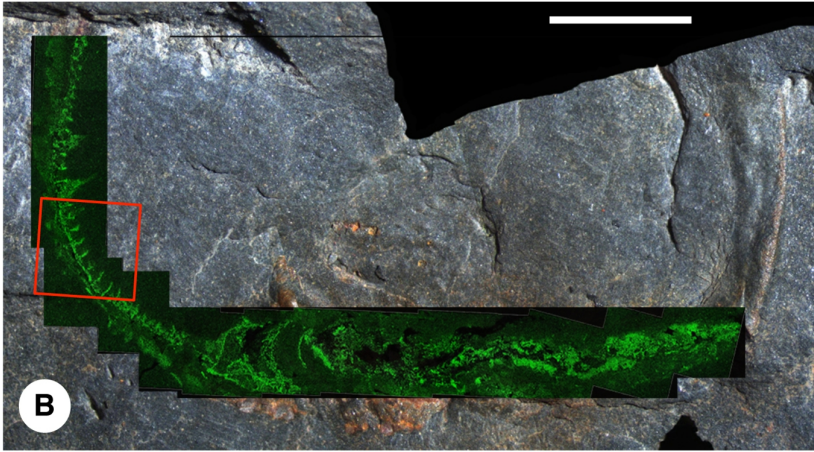
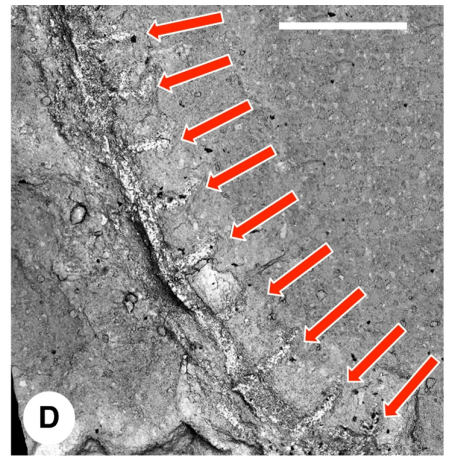
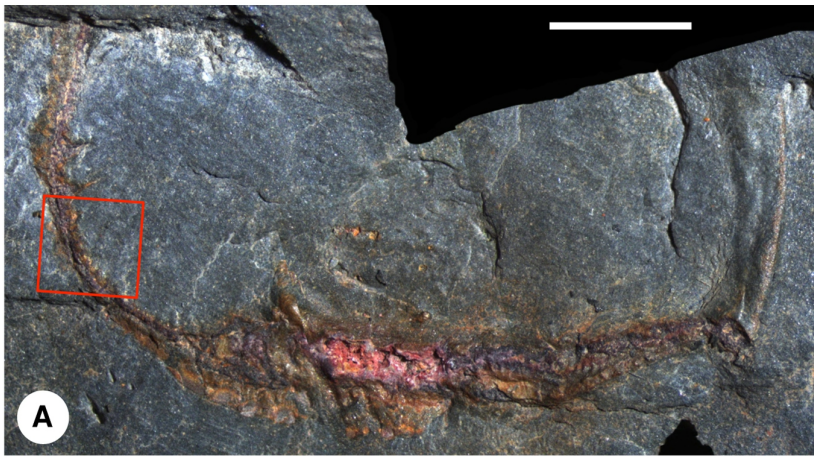
507 **Fig. 9.** Reconstruction of the cornute stylophoran *Thoralicystis* n. sp., late Tremadocian (Lower
508 Ordovician), Zagora area (Morocco), with the tube feet protruding beyond the cover plates. The
509 appendage is about 20 mm long.

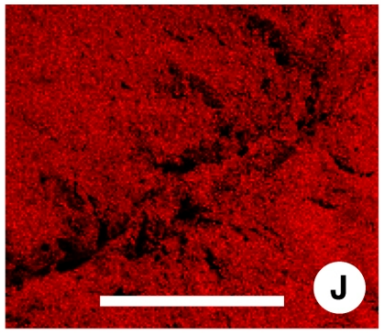
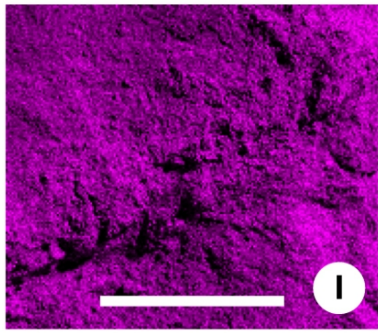
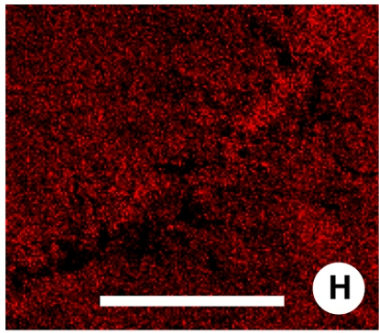
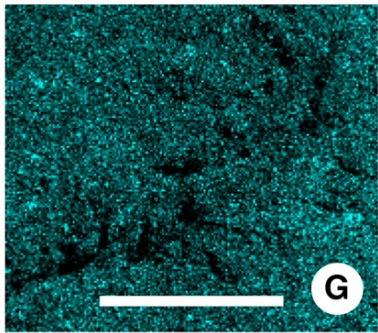
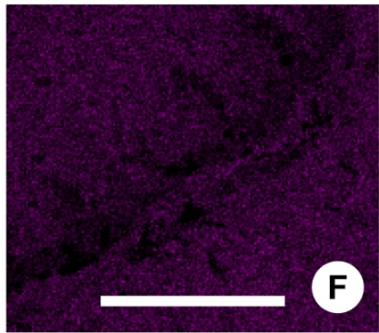
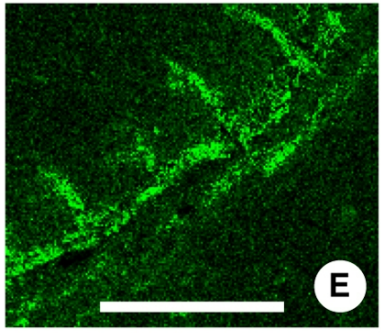
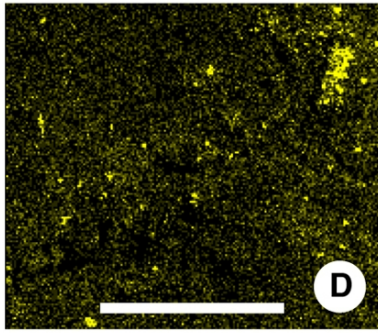
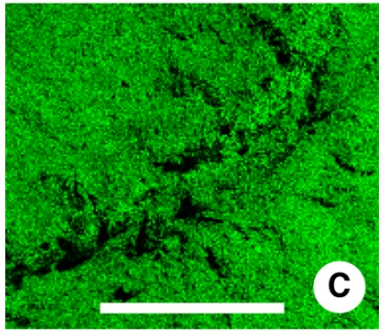
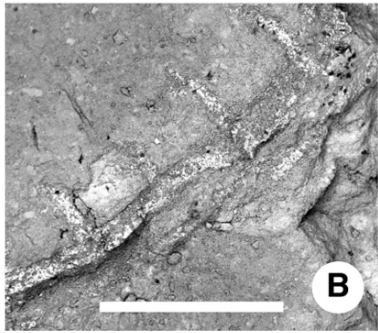
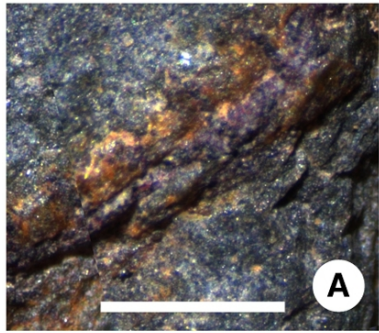


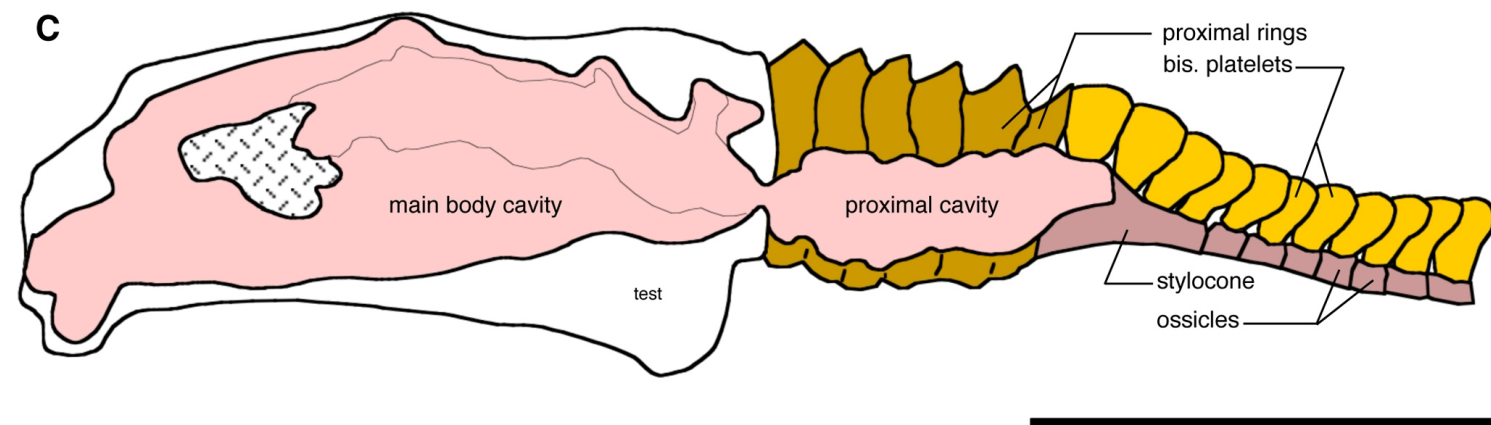
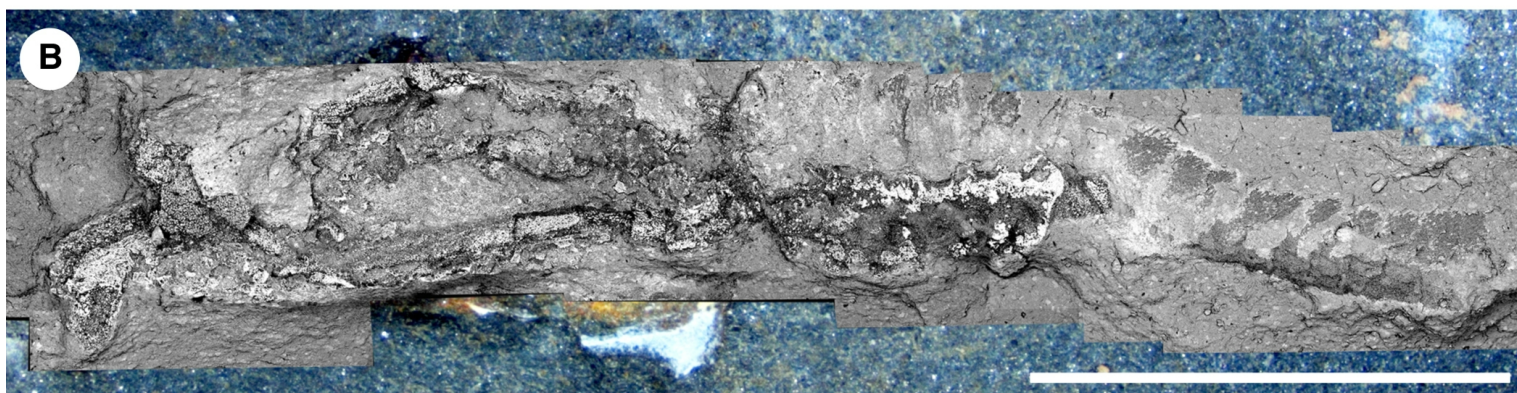


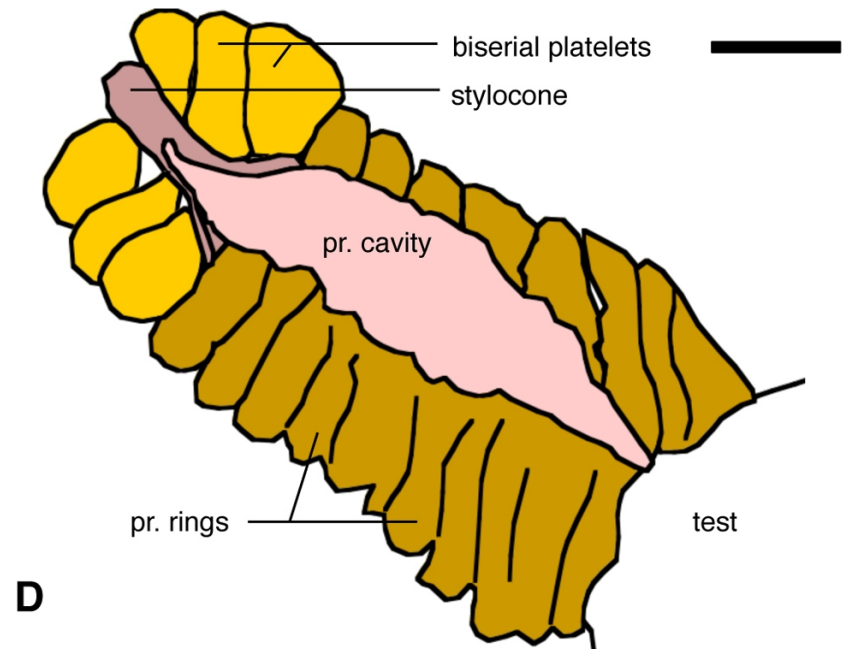
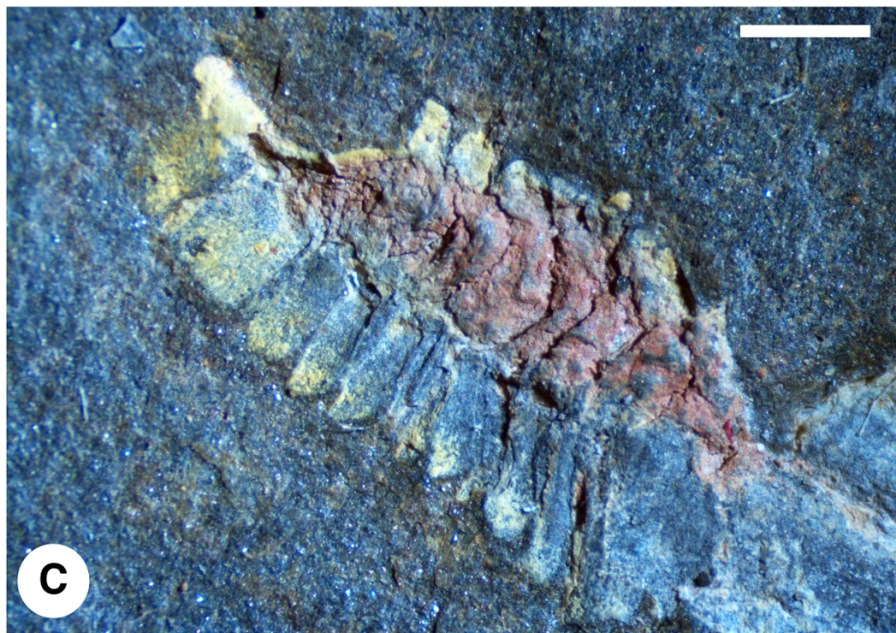
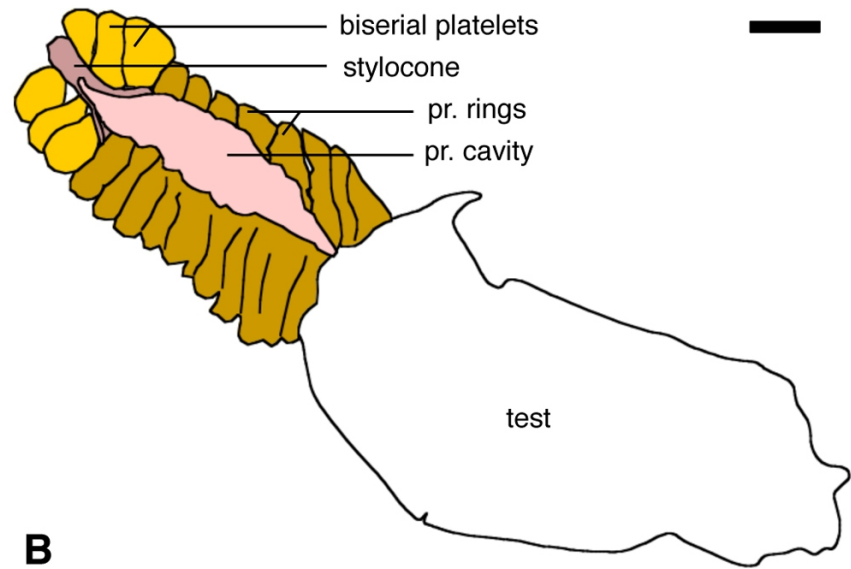
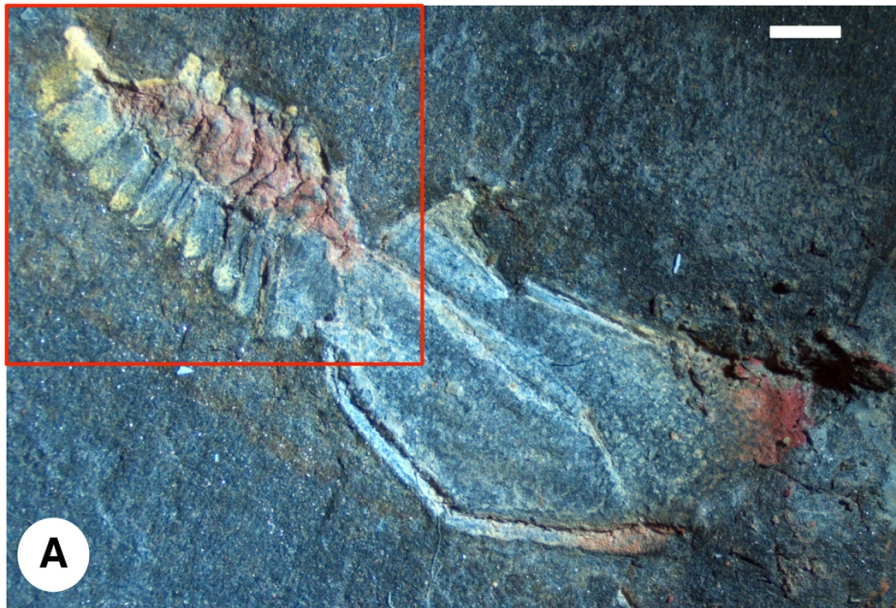


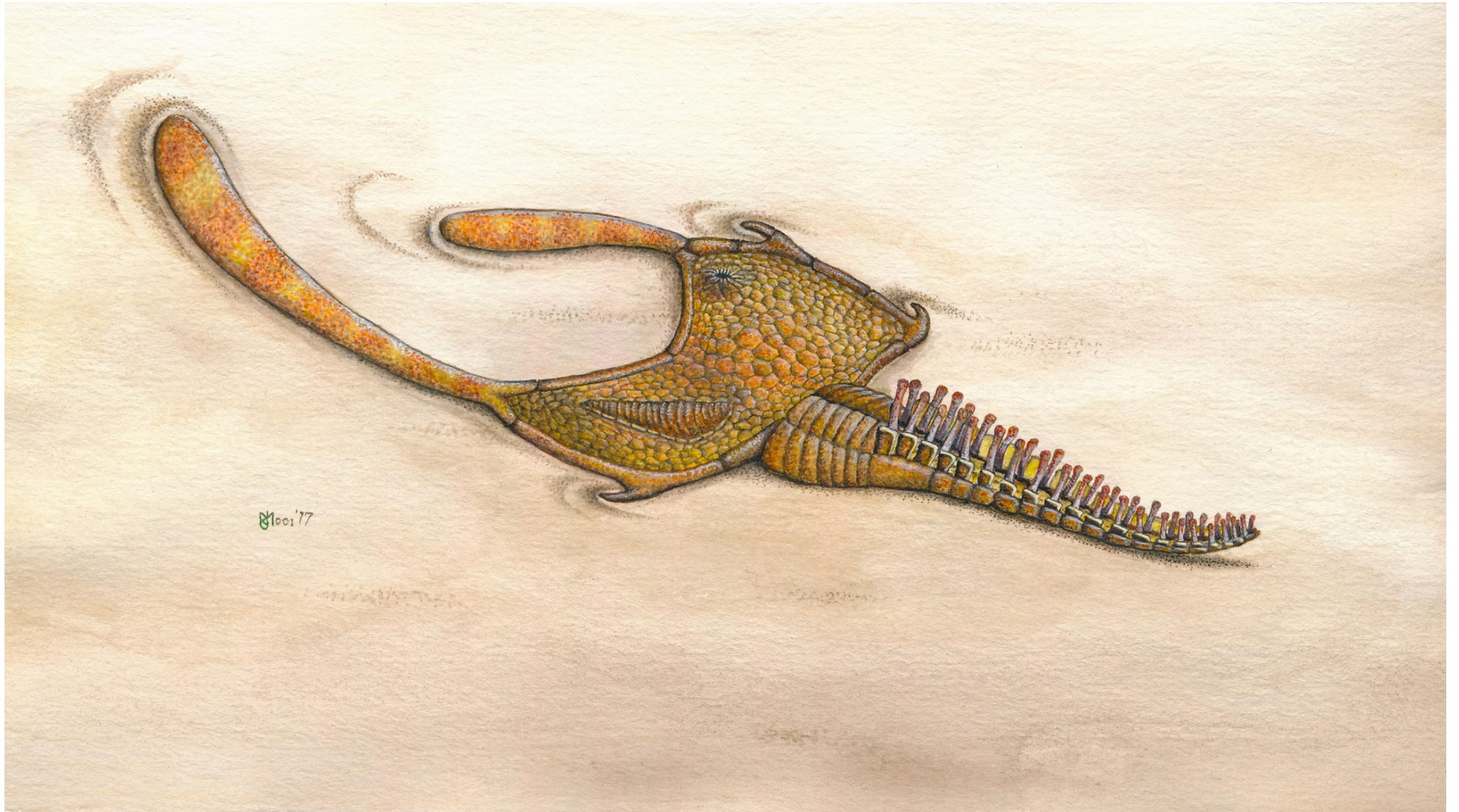












Moos '77