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Deep echinoderm phylogeny preserved in organic molecules from Paleozoic fossils

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ABSTRACT

Isolation of organic molecules directly from Paleozoic to Cenozoic fossils has been documented, which raises important, new questions about the conditions of preservation and the range of paleobiological issues that can be addressed with these new data. Herein, molecules are isolated from fossil echinoderms exceeding 445 Ma in age. Previously, morphological data have been insufficient to establish a consensus regarding early echinoderm evolution. Thus, organic molecules extracted from fossil echinoderm specimens (mostly Paleozoic) belonging to the classes Asteroidea, Blastoidea, Crinoidea, Diploporita, Echinoidea, and Edrioasteroidea are used to assess the position of crinoids and blastozoans within competing echinoderm phylogenetic hypotheses. Fluorescence excitation-emission spectroscopy of organic molecules in fossil extracts are used to compare relationships among hypotheses. These new data support the hypothesis that living eleutherozoans diverged early from stemmed echinoderms, crinoids are nested within the clade that includes other blastozoans, and edrioasteroids are a distinct clade.

INTRODUCTION

It is not uncommon today for fossilized DNA to be isolated (see Culotta, 2015; Gibbons, 2015) in ideal conditions, and paleoproteomics is a growing field (Bertazzo et al., 2015; Service, 2015). However, despite the early work of M. Blumer and colleagues on Mesozoic crinoids (e.g., Blumer, 1951, 1960), study of other organic molecules extracted directly from fossil skeletons has received relatively little attention. Wolkenstein et al. (2006, 2008) and Wolkenstein (2015) confirmed and expanded on the work of Blumer et al. (1951, 1960), and Wolkenstein et al. (2010) reported molecules isolated from Mesozoic (ca. 165 Ma) red algae. Further, O'Malley et al. (2005, 2008, 2013) extracted taxon-specific organic molecules from Mississippian crinoids.

The presence of ancient, taxon-specific organic molecules raises many geochemical questions, including how these molecules were altered (from the parent compounds) over millions of years and what diagenetic and geological window is required to preserve such molecules. Further, what paleobiological questions can be addressed with ancient molecules, especially new questions that cannot be addressed using only skeletal morphology? Here, we use preserved organic molecules to compare competing echinoderm phylogeny hypotheses in order to assess the position of crinoids within the Echinodermata.

Using ultraviolet-visible (UV-Vis) spectroscopy, O'Malley et al. (2008) demonstrated that organic molecule signatures were different in different Mississippian crinoid species and that the organic molecule profiles of fossils were distinct from that of the country rock. Further, using both UV-Vis and fluorescence spectroscopy, different fossil crinoids more closely related phylogenetically had more similar organic molecules in extracts (O'Malley et al., 2013). Living echinoderms possess an array of unique organic molecules, such as quinones that are used as predation deterrents (see the GSA Data Repository[1]); therefore, it should not be surprising that the geologically stable forms of these original molecules retain a distinctive signature.

Prerequisites for ancient crinoid organic molecule preservation are, presumably: (1) multielement echinoderm mesodermal skeleton with each element comprised of a single crystal of calcite; (2) burial in fine-grained sediment; and (3) occurrence in strata not subjected to deep burial or metamorphism (O'Malley et al., 2013). These attributes result in a "crystal casket" (Dickson, 2002) and are also prerequisites for preservation of isotope ratios (e.g., Dickson, 2002) and helium thermochronometers (Copeland et al., 2015) in fossil crinoids. The mesodermal skeleton is not unique to crinoids but is a characteristic shared by all echinoderms, indicating that other fossil echinoderms have potential to preserve ancient molecules.

More than 20 class-level echinoderm clades are known (Sprinkle and Guensburg, 1997), but only five remain extant, including Asteroidea, Crinoidea, Echinoidea, Holothuroidea, and Ophiuroidea, each of which originated by the Early to Middle Ordovician (ca. 460 Ma) from now-extinct clades. Whereas genomic information can elucidate relationships among living echinoderms, only morphology has been used previously to understand deeper echinoderm phylogenetic relationships.

Echinoderms display a very high degree of morphological disparity (Fig. 1). This disparity has resulted in varying hypotheses for echinoderm phylogeny (Figs. 2 and 3), and a consensus has never been achieved. In this study, we have extracted organic "quinone-like" molecules from a crinoid and from five other echinoderm classes. We test whether crinoids and blastozoans (blastozoans are stemmed echinoderms, such as blastoids, rhombiferans, diploporites) are sister groups or if each had an independent origin within the Echinodermata.

METHODS

Organic molecules were extracted from specimens belonging to Paleozoic Asteroidea, Blastoidea, Crinoidea, Diploporita, and Edrioasteroidea (Table DR1 in the Data Repository), ranging from Ordovician through Mississippian in age, and from a fossil echinoid of an undetermined age. Specimens were a random collection of fossil echinoderms, being selected based on their availability for destructive sampling (which limits our sample size) and for representation of a wide diversity of echinoderms. Because samples are from many locations, rock types, and diagenetic histories, the expectation is for a random array of results.

Analyses were performed using excitation-emission matrix (EEM) fluorescence spectroscopy, and analytical procedures are discussed in the Data Repository. On some crinoids studied by O'Malley et al. (2013), including *Elegantocrinus hemisphaericus* used here, the same results were obtained with multiple specimens, and when different parts of the same individual were analyzed. Further, O'Malley et al. (2008) demonstrated that extracts from fossils had distinct organic molecules compared to the

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[1]GSA Data Repository item 2016124, discussion of biomolecules in living echinoderms, methods, and Table DR1, is available online at www.geosociety.org/pubs/ft2016.htm, or on request from editing@geosociety.org or Documents Secretary, GSA, P.O. Box 9140, Boulder, CO 80301, USA.

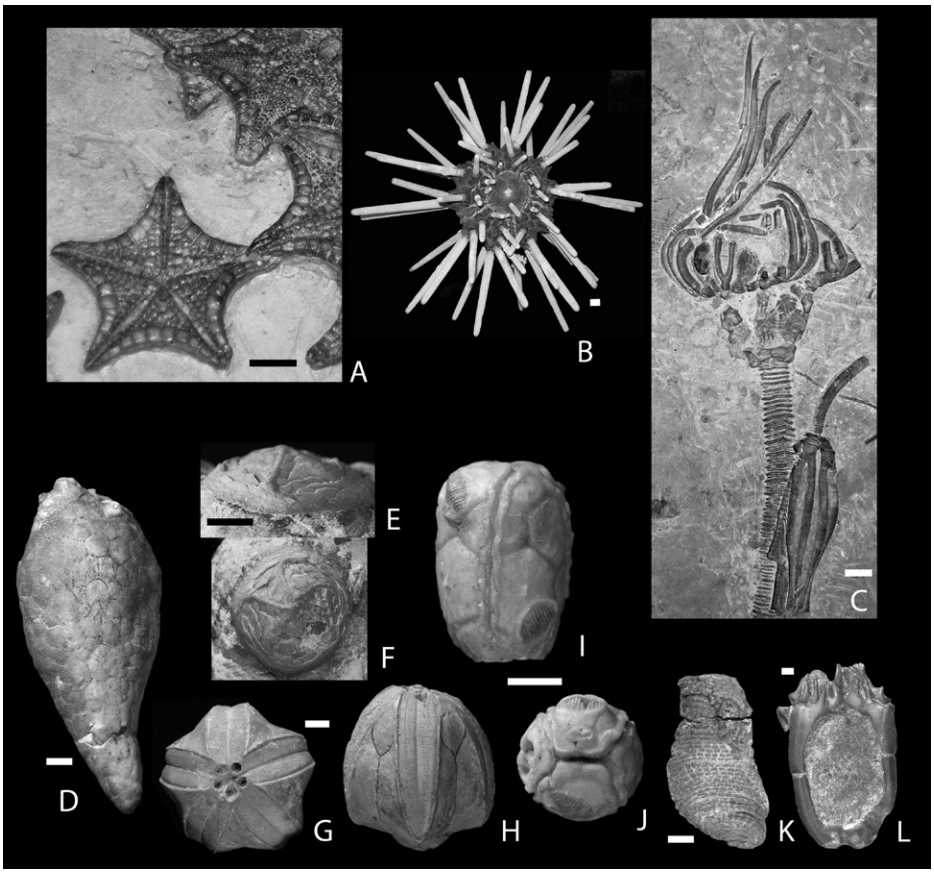

Figure 1. Representative echinoderms. A: *Crateraster mccarteri,* Cretaceous asteroid; scale bar 1 cm. B: Living echinoid; scale bar 1 cm. C: *Actinocrinites gibsoni* and *Scytalocrinus robustus*, Mississippian crinoids; scale bar 1 cm. D: *Holocystites* sp., Silurian diploporoid, scale bar 1 cm. E,F: *Agelacrinites southworthi*, Ordovician edrioasteroid, scale bar 5 mm. G,H: *Pentremites robustus,* Mississippian blastoid; scale bar 1 cm. I,J: *Tetracystis fenestratus*, scale bar 5 mm. K: *Helicoplacus gilberti*, Cambrian helicoplacoid; scale bar 5 mm. L: *Ctenocystis colodon,* Cambrian homalozoan; scale bar 1 mm. Photos in panels C–J and L used with permission of Department of Paleobiology, Smithsonian Institution, Washington, D.C., USA.

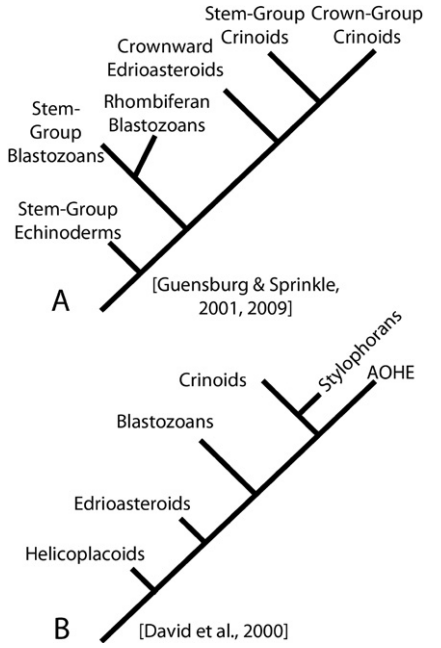

Figure 2. Echinoderm phylogenetic hypotheses with crinoids and Blastozoa separate. A: Crinoids derived from edrioasteroids separately from blastozoans (Guensburg and Sprinkle, 2001, 2009). B: Crinoids more closely related to other living echinoderm clades than to blastozoans (David et al., 2000). AOHE—Asteroidea, Ophiuroidea, Holothuroidea, and Echinoidea.

enclosing rock. This coupled with distinct molecules in different taxa demonstrates that the distribution of organic molecules in that setting were independent of diagenetic effects.

RESULTS

Organic molecules were extracted from specimens of six echinoderm classes, with the oldest from the Ordovician (Katian, ca. 450 Ma) (Table DR1). With the exception of one cyclocystoid, all specimens analyzed yielded organic molecules. Figure 4E is an example of an EEM collected from the camerate crinoid *Elegantocrinus* with a characteristic peak at an excitation of 250 nm and an emission band that ranges from 375 to 400 nm (O'Malley et al., 2013).

One set of hypotheses for echinoderm phylogeny regards crinoids as a monophyletic clade distinct from blastozoans. In this view, crinoids were either derived from edrioasteroids (Fig. 2A) (Guensburg and Sprinkle, 2001, 2009) or more closely related to the other living echinoderm clades (Fig. 2B) (David et al., 2000; Mooi, 2001). Alternatively, crinoids and blastozoans have been regarded as a monophyletic clade (Smith, 1984; Paul and Smith, 1984; Smith and Jell, 1990; Sumrall, 1997) (Figs. 3A and 3B).

As in crinoids, it is reasonable to hypothesize that geologically stable organic molecules of more closely related taxa would be more similar in structure, due to similarities in the parent compounds present when the organisms were alive and assuming that the conditions for diagenesis were similar. In a comparison of stalked echinoderms, one would predict that the two blastozoans analyzed here (blastoid and diploporan) would be more similar to one another than to crinoids. This prediction is borne out by our data (Figs. 4C and 4D). The blastoid and diploporan have excitation and emission (Ex and Em) wavelengths of ~320 and 400 nm, respectively. The crinoid excitation wavelength is markedly lower (~250 nm), but its emission wavelength is similar to that of the blastozoans (Fig. 4E). The extracts from the other echinoderms have very different fluorescence patterns. The asteroid and echinoid have quite similar excitation and emission values (Figs. 4A and 4B), with Ex and

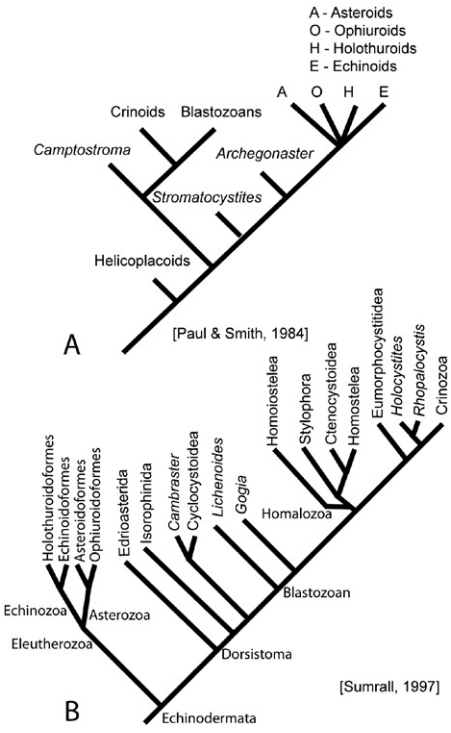

Figure 3. Echinoderm phylogenetic hypotheses with crinoids and Blastozoa in a single clade. A: Paul and Smith (1984). B: Sumrall (1997); note terminal taxon, Crinozoa, includes crinoids, paracrinoids, glyptocystitids, blastoids, and other Blastozoa.

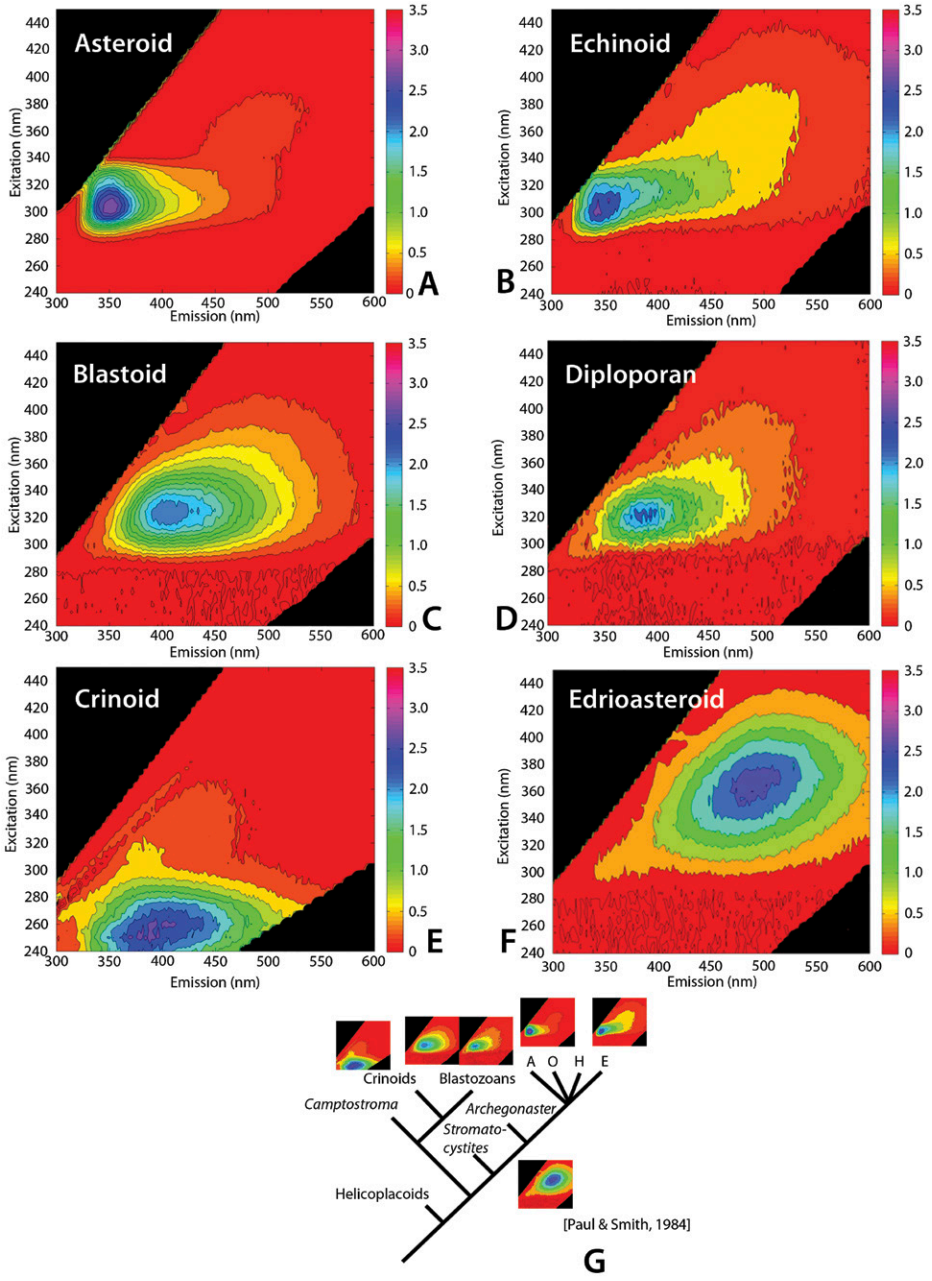

Figure 4. Excitation-emission matrix (EEM) results for Paleozoic echinoderms and supported phylogeny. A: *Stenaster* sp. EEM, asteroid. B: Echinoid EEM, echinoid. C: *Pentremites* sp. EEM, blastoid. D: *Holocystites* sp. EEM, diploporoid. E: *Elegantocrinus hemisphaericus* EEM, crinoid. F: *Isorophus cincinnatiensis* EEM, edrioasteroid. G: EEMs placed on phylogenetic hypothesis with crinoids and blastozoans in a single clade (Paul and Smith, 1984; Smith and Jell, 1990). The color scale on the right of each panel is relative intensity. AOHE—Asteroidea, Ophiuroidea, Holothuroidea, and Echinoidea.

Em wavelengths (300 and 325 nm, respectively) blue-shifted relative to the blastoid, diploporan, and crinoid. Edrioasteroid extracts fluoresce very differently, with red-shifted Ex-Em pairs (~370 and 500 nm) (Fig. 4F). These results suggest the existence of organic compounds with fluorophores capable of absorbing and emitting light at longer wavelengths.

Crinoid EEM results differ from those of the blastoid and diploporan by shifts in the excitation wavelengths only, whereas the crinoid differs from the asteroid, echinoid, and edrioasteroid by shifts in both the excitation and emission wavelengths (Fig. 4). These EEM data support the hypothesis of Smith (1984), Paul and Smith (1984), Smith and Jell (1990), Sumrall (1997), and others who grouped all stemmed echinoderms separately from eleutherozoans (Fig. 4G). It also supports the conclusions of Kammer et al. (2013) that blastozoans and crinoids are part of one large clade based on the structure of the oral region. The fluorescence patterns for the Paleozoic echinoderms do not support two independent originations of stemmed echinoderms (crinoids and blastozoans) (David et al., 2000; Guensburg and Sprinkle, 2001, 2009).

DISCUSSION

Morphological disparity among echinoderm classes is sufficiently high that standard character-based phylogenetic methods have not clearly resolved the question of evolutionary relationships. Analyses of oral surface characters (Sumrall and Waters, 2012; Kammer et al., 2013) indicated that crinoids and blastozoans are closely related, supporting the phylogenetic hypothesis of Smith (1984), Paul and Smith (1984), Smith and Jell (1990), and Sumrall (1997). Results from organic molecules, presented here, also support this phylogenetic hypothesis. Accordingly, the stalked echinoderms should be regarded as one clade, as originally proposed by Leuckart (1848).

Results presented here demonstrate considerable promise for the use of organic molecules derived from fossils as a tool to resolve numerous fundamental paleobiological questions.

CONCLUSIONS

Organic molecules are extracted from fossil specimens representing six echinoderm classes through the Paleozoic to assess the relationship of crinoids and blastozoans relative to other echinoderms. The results support the hypothesis that living eleutherozoans (at least asteroids and echinoids) diverged early from stalked echinoderms, crinoids are nested within the clade that includes blastozoans (Fig. 4G), and edrioasteroids are distinct from these two clades.

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