Isolation and characterization of the earliest taxon-specific organic molecules (Mississippian, Crinoidea)

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O’Malley et al. (2013) report the extraction and characterization of organic molecules from Mississippian crinoids. Chemical analyses of organic compounds of Paleozoic age can provide valuable information on ancient organisms, but are generally a challenging task requiring great caution in the treatment of samples and the interpretation of data. From the view of an organic geochemist, some statements require comment.

The comparison of the fringelite pigments discovered by Blumer (1951, 1960) with hypericin is obsolete. In a revision of the structures (Wolkenstein et al., 2006), one of the fringelite pigments was identified as hypericin. To avoid confusion, the closely related fossil crinoid pigments, rather, should be termed fossil hypericinoids. Reference should be clearly made that polycyclic quinones including hypericin have been proven in the Jurassic crinoid Litiocrinus and in the Triassic crinoid Carvallicrinus, and that early cementation of the stereom microstructure is an important factor for the preservation of organic material in fossil crinoids (Wolkenstein et al., 2006).

O’Malley et al. give the impression that the crinoids shown in their figure 1 are from the Mississippian of Indiana. However, figure 1 actually shows a crinoid plate from the Mississippian Maynes Creek Formation at Le Grand, Iowa (Gahn and Baumiller, 2004). Although the figure shows an example of color differences among different crinoid species, it should be indicated that these crinoids are from a different locality from those used as samples.

O’Malley et al. state that the crinoids they analyzed originate from two different localities in southern Indiana (Morgan and Monroe Counties). However, it is not indicated from which locality the individual samples originated. A detailed provenance is of particular relevance when comparing the mass spectra of only three crinoid samples (their figures 3A–3C). Moreover, analysis of three different samples is not sufficient to support a taxon specificity of organic molecules and to determine if these molecules indicate phylogenetic relationships as stated. To validate a correlation of the observed data with taxa, more than one sample of the same species should be analyzed.

Another major concern is that crude extracts were analyzed, not isolated compounds as suggested in the title. Chromatographic separation is of crucial importance for the analysis of complex mixtures of unknown compounds, which are typically present in extracts from fossils. Without chromatographic separation, the spectroscopic properties of individual compounds cannot be correlated. For example, it is not possible to evaluate if a compound with a specific molecular mass also shows fluorescence properties. Furthermore, crude organic extracts were obtained by extraction with organic solvents (acetone/methanol 4:1 and methanol), but in the legend of their figure 3, it is indicated that for excitation-emission matrix (EEM) spectroscopy, samples were analyzed in water. Considering the different solvent properties, it is rather unlikely that the mixture of compounds dissolved in water is identical to the mixture of compounds in the crude organic extract.

O’Malley et al. state that the study specifically “seeks to determine whether the coloration distinction among fossil species results from individual crinoids possessing unique organic molecules” (p. 347). However, the ultraviolet-visible light spectra of the fossil samples (their figure 2) show that only very little absorbance occurs within the range of visible light (400–800 nm). The spectra thus indicate that the extracted fossil compounds are not responsible for distinctive coloration differences of the crinoids.

EEM spectroscopy is generally used for characterization of dissolved organic matter in natural waters based on differences in the fluorescence properties of broad groups of compounds (e.g., humic-like fluorescence) (Coble, 1996), not for the determination of specific functional moieties. Comparing the EEM spectra in detail, significant differences can be observed between the fossil crinoids and also with the reference compound, which do not necessarily support the presence of quinone-like compounds in the fossil samples.

Using electrospray ionization time-of-flight (ESI-TOF) mass spectrometry for analysis of mixtures, it should be recognized that ESI is an ionization technique most suitable for the detection of chargeable and polar compounds and that suppression of ions may occur (Pramanik et al., 2002). Compounds occurring in trace amounts may give very intense signals if they ionize very well, whereas other compounds, even if they are abundant, may give no signal at all. Therefore, no relative abundances of compounds can be determined from ESI spectra of mixtures and the phrase “most abundant molecular species” (p. 348) is misleading.

It is very difficult to interpret the ESI-TOF spectra in their figure 3 as shown. O’Malley et al. state that exact mass measurements with internal calibration were performed, but no exact mass data are given in their figure 3 or elsewhere in the article. This is unfortunate, because exact mass data, i.e., with more than two decimal places, would allow for the calculation of molecular formulae of the individual compounds. Based on such data, the identity of the compounds might be verified and possible contaminants could be distinguished from authentic compounds. For example, the succession of peaks with mass-to-charge ratios (m/z) of 281, 355, 429, as well as 519 and 593 with characteristic differences of 74 mass units suggests the presence of polysiloxanes, representing common laboratory contaminants (Schlosser and Volkmer-Engert, 2003). Because of the very high sensitivity of ESI, survey spectra of compound mixtures should also be accompanied by information on background signals from a blank sample.

The statement that a molecular mass “is very close” or “similar” (p. 348) to another mass is not applicable in mass spectrometry because a multitude of structurally completely different molecules may have almost identical m/z values. If no characteristic fragmentation can be observed, a measured molecular mass can only be related to a known compound if the masses are identical within the error range or if an indicative mass difference can be observed. Therefore, it is not possible to relate the signals in the mass spectra to naphthoquinones, antraquinones, or quinone-like compounds. It can also be almost certainly excluded that the m/z 355.06 signal corresponds to a derivative of hexahydrophenanthroperylene (HHPP, with a m/z of 357.16 for [M+H]+, not 358.17 as stated), because apolar polyaromatic hydrocarbons like HHPP cannot be ionized by ESI (Wolkenstein et al., 2002). As mentioned above, the m/z 355.06 signal may correspond to a polysiloxane contaminant ([CmHnSiOx]2, m/z = 355.0699). Without structural information on detected compounds, it is not conclusive to find “similarities and differences in the components of each mixture” (p. 348) to support the hypothesis that the compounds are taxon- or echinodermspecific.

While the report of O’Malley et al. shows that organic compounds are more common in fossils and are also preserved in Paleozoic crinoids, the data are not sufficient to support the claim that the earliest taxon-specific
organic molecules have been isolated and characterized. Further analytic work on the organic content of well-preserved fossils will provide more specific information on the fate and taxonomic significance of molecules in ancient organisms.

REFERENCES CITED