

LETTER TO THE EDITOR

Re: Spectral Analysis of Echinoderm Small Subunit Ribosomal RNA Gene Sequence Data

To the Editor:

Elucidating relationships between the echinoderm classes has proven to be a difficult problem. Low bootstrap support and competing topologies indicated by Littlewood *et al.* (1997) suggested conflict in the dataset. We employed spectral analysis in an effort to search for alternative topologies that might be supported by the small subunit ribosomal RNA gene (SSU rDNA) sequence dataset and to examine patterns under different transformations/models of evolution. To help elucidate the relationships of the asteroids and ophiuroids, we added four new ophiuroid DNA sequences to the database. There was a large amount of conflicting information in the untreated sequences. Correcting the data for unequal nucleotide composition, unequal rate distribution, proportion of invariable sites, and transition transversion (ti/tv) ratio significantly decreased the amount of conflicting signal. There was support for the topology (C(A(O(E,H))))). There was absolutely no support for placing holothurians as primitive echinoderms and very weak support for ophiuroids and asteroids as sister taxa.

DNA extraction from four ophiuroids (*Amphiura filiformis*, *A. chiajei*, *Acrocrida brachiata*, and *Ophiocomina nigra*), PCR amplifications of the SSU rDNA, and DNA sequencing were performed as described (McCormack *et al.* 1995; McCormack, 1998) and sequences were deposited in GenBank under Accession Nos. AJ011141 to AJ011144. Additional echinoderm SSU rDNA sequences were retrieved from the RDP anonymous FTP server and from Dr. Tim Littlewood (NHM, London). DNA sequences were realigned using ClustalW, edited by eye in the Genetic Data Environment (Smith, 1993) with reference to published secondary structure models (Gutell *et al.*, 1994) and differed from previously published alignments. The final alignment is available from the authors. The spectral analysis method of Hendy *et al.* (1994) was employed to quantify the relative amounts of support and conflict for different hypotheses in the data (see Lento *et al.*, 1995, for a review of the method), using the SPEC-TRUM program of Charleston and Page (1995). Sequences from 19 echinoderm taxa were used in this analysis. We compared the spectra and trees produced from the untreated sequence data, from DNA sequence after the hadamard transform step was performed,

from transformed distance matrices with adjustments made for unequal nucleotide composition using the LogDet model (Lockhart, 1995), and from unequal rate distribution across sites, ti/tv ratio, and proportion of invariable sites using the K3P model (Kimura, 1980) with the parameters optimised using maximum likelihood criteria. The program was instructed to compute the amount of support for all splits in the dataset and to keep all splits with lengths greater than 0.001. A tree was inferred for each model using the Manhattan tree method (Hendy, 1991).

True relationships within the phylum were masked by a high degree of conflict within the untransformed data. Echinoid and asteroid classes were not recovered as monophyletic groups (Fig. 1 (top), splits 6 and 4, respectively). This conflict was reduced when the data were transformed using the hadamard conjugation. However, echinoids and asteroids remained mixed on the tree and overall relationships did not change. In both datasets there was some support for an asteroid/ophiuroid clade (split 5) and an echinoid/holothurian clade (split 6), although these relationships did not appear on the tree because of the large number of conflicting signals. Producing a distance spectrum from the LogDet model dramatically changed the support for and against some edge partitions. The monophyly of the echinoids and asteroids was established (Fig. 1 (bottom)). There was some support for placing asteroids with crinoids (split 7) with no conflict for this relationship. While there was relatively more support for placing echinoids with holothurians (split 5), there was also some conflict for this relationship. With less evidence and a greater amount of conflict, the bipartition that grouped ophiuroids with holothurians did not appear on the tree (split 8). By employing distances obtained using the K3P model, the amount of support for an echinoid/holothurian clade was increased (not shown).

There was evidence for very few alternative class relationships apart from the (C(A(O(E,H)))) topology in the SSU rDNA dataset. There was absolutely no support for Smiley's suggestion (1988) placing holothurians as the most deeply branching of the echinoderms. Likewise, there was no support for grouping echinoids with the ophiuroids as Hyman (1955) suggested. Both in the untreated spectrum and in the spectrum produced from transforming the DNA sequences using the hadamard conjugation, there was a small amount of support for placing ophiuroids as sister taxa to the

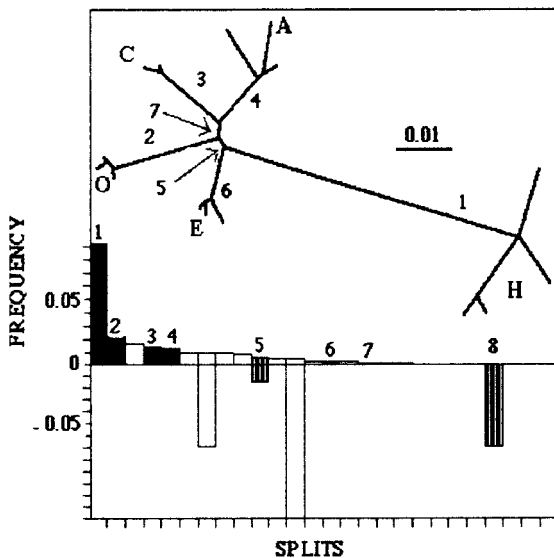
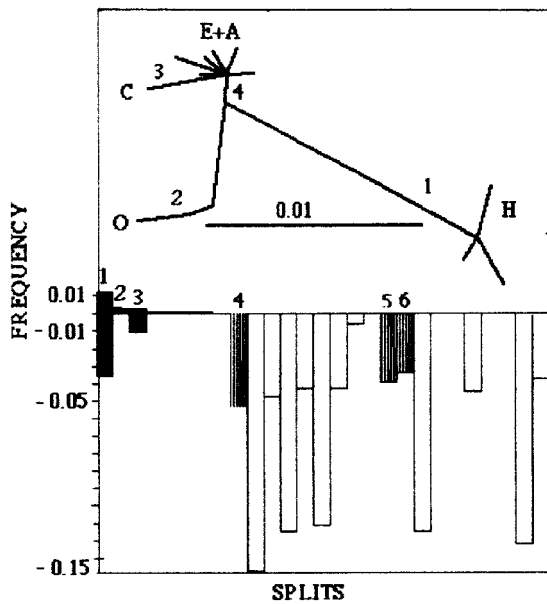


FIG. 1. (Top) Ranked sequence spectrum from untransformed sequence data and (bottom) ranked distance spectrum from genetic distances calculated using the LogDet transformation, with conflicts normalized. The bars above the X axis show the amount of support for each split, while the bars below the X axis show the sum of all conflicts against the corresponding split above the X axis. H, Holothuroidea; O, Ophiuroidea; E, Echinoidea; and C, Crinoidea. Solid bars (and numbers) represent splits of interest. The optimal tree returned from SPECTRUM is shown as insets with the numbers on the tree corresponding to the splits in the spectrum.

asteroids, a relationship that appeared in Littlewood *et al.*'s (1997) analysis and was previously suggested by Fell (1962). However, there was a large amount of conflicting signal within the asteroid and echinoid classes and this relationship did not appear on the tree. When corrections were made for unequal nucleotide composition, ti/tv ratio, and unequal rate distribution across sites, any evidence that was present for placing asteroids and ophiuroids as sister taxa vanished and instead there was stronger support for splitting the taxa in to [crinoids and asteroids] and [echinoids and holothurians]. Ophiuroids are positioned intermediate to these two groups but associated with the latter due to some support for a split placing them with holothurians. Despite the conflict present in the SSU rDNA dataset (largely within the echinoid and asteroid classes) there appears to be only one true set of class relationships supported, i.e., (C(A(O(E,H))))). Much of the conflict present was removed by incorporating various model parameters/corrections to reveal much clearer relationships. On the basis of this we feel that the topology (C(A(O(E,H)))) originally proposed by Smith (1984) and more recently confirmed by Littlewood *et al.* (1997) is the true topology.

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