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Strong character incongruence and character choice in phylogeny of sea stars of the Asterinidae

Michael W. Hart,[1,a,b] Sheri L. Johnson,[1,2] Jason A. Addison,[1] and Maria Byrne[3]

[1] Department of Biology, Dalhousie University, Halifax, Nova Scotia B3H 4J1, Canada
[2] Darling Marine Center, University of Maine, Walpole, ME 04573, USA
[3] Department of Anatomy and Histology, F-13, University of Sydney, Sydney, New South Wales 2006, Australia

Abstract. Historically, characters from early animal development have been a potentially rich source of phylogenetic information, but many traits associated with the gametes and larval stages of animals with complex life cycles are widely suspected to have evolved frequent convergent similarities. Such convergences will confound true phylogenetic relationships. We compared phylogenetic inferences based on early life history traits with those from mitochondrial DNA sequences for sea stars in the genera *Asterina, Cryptasterina,* and *Patiriella* (Valvatida: Asterinidae). Analysis of these two character sets produced phylogenies that shared few clades. We quantified the degree of homoplasy in each character set when mapped onto the phylogeny inferred from the alternative characters. The incongruence between early life history and nucleotide characters implies more homoplasy in the life history character set. We suggest that the early life history traits in this case are most likely to be misleading as phylogenetic characters because simple adaptive models predict convergence in early life histories. We show that adding early life history characters may slightly improve a phylogeny based on nucleotide sequences, but adding nucleotide characters may be critically important to improving inferences from phylogenies based on early life history characters.

Additional key words: total evidence, modes of development, life history, mtDNA, Asteroidea

The choice of characters for use in phylogenetic analyses is an important and contentious issue in animal phylogenetics. Since Haeckel (1866), phenotypic characters from the earliest parts of the life cycle (such as the morphological forms of planktonic larvae) have been viewed as a potentially rich source of phylogenetic information. However, even in the early 20th century, there was growing skepticism of the utility of early life history characters for tracing evolutionary relationships (e.g., Conklin 1928; Gould 1977). The subsequent growth of larval ecology as a research discipline led to renewed interest in the phylogenetic history of embryological and larval diversity, with a sharp focus on the study of early life history characters expressed during sexual reproduction or during larval development before metamorphosis to the adult form (McHugh & Rouse 1998). Molecular phylogenetic studies of taxa that include species with diverse early life histories have suggested the evolution of convergent similarities in early life history characters such as egg size, type of parental care, offspring dispersal ability, larval morphology, and fertilization ecology (Hart 2000). Many of these characters reflect functional adaptations of a complex life cycle in which individuals sequentially inhabit planktonic and benthic habitats: the similarities in character states could represent shared ancestral states as a result of constraints on the evolution of early development, shared derived states that evolved prior to speciation events, or convergences that evolved in response to selection (Strathmann 1985; Haszprunar et al. 1995; Wray 1995, 1996; Raff 1996; Smith 1997). The extent of convergence—and the soundness of homology arguments for early life history character states—is often difficult to establish (Strathmann & Eernisse 1994; Haszprunar et al. 1995; Wray 1996; Rouse 1999, 2000). For these reasons, many invertebrate zoologists (see McHugh & Rouse 1998; Hart 2000) have recently chosen molecular characters over characters drawn from reproductive phenotypes or the morphology of embryos and larvae for the evaluation of evolutionary relationships.

[a] Author for correspondence. E-mail: michael.hart@dal.ca
[b] Present address: Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia V5A 1S6, Canada

In cases where several types of phylogenetic characters are available, or different characters can be used to address complementary problems, a potentially powerful approach uses all available molecular and phenotypic characters in a total evidence analysis (Hillis 1987; Kluge 1989; Shaffer et al. 1991; Eernisse et al. 1992; Wheeler et al. 1993; de Queiroz 1996; Moritz & Hillis 1996; Levasseur & Lapointe 2001). Nucleotide characters are especially useful where phenotypic homologies are not clear (Raff et al. 1994; Aguinaldo et al. 1997; McHugh 1997) or few shared derived phenotypic states can be observed (Avise et al. 1994). Molecular and phenotypic data often give results that are congruent or at least not strongly conflicting (Eernisse et al. 1992; Rumbak et al. 1994; Lafay et al. 1995; Littlewood & Smith 1995; Paterson et al. 1995; Reid et al. 1996), and combining these character types may allow the inclusion of fossil or other taxa for which molecular data are unavailable (e.g., Wiens 1998). The total evidence approach has sometimes been cautiously extended to include phenotypic characters from marine invertebrate gametes, embryos, and larvae (Reid 1990; Rouse & Fitzhugh 1994; Smith et al. 1995; Reid et al. 1996; Wray 1996).

In spite of the general suspicion that early life history characters of marine invertebrates might be misleading sources of phylogenetic information, no quantitative analysis has shown the extent of conflict—or consistency—between these characters and phylogenetic information from nucleotides or other molecular characters. Here we give a simple quantitative analysis of congruence between early life history characters and mitochondrial DNA (mtDNA) sequences for asterinid sea stars (including new sequence data for five species). Many asterinid species share apparent derived forms of reproduction, larval morphology, and brood protection that may reflect either homology or convergence (Byrne & Cerra 1996; Hart et al. 1997; Byrne et al. 1999). We use this quantitative analysis to illustrate the extreme conflict between early life history and nucleotide characters, the risks associated with phylogenies based on such characters, and the scant benefits of including certain early life history characters in total evidence analyses.

Methods

Early life history characters of asterinid sea stars

We collated data on the early life histories and breeding systems of asterinids from the literature and from unpublished obsersvations (Byrne 1992, 1996; Byrne & Cerra 1996; Hart et al. 1997; Byrne et al. 1999; Carvalho & Ventura 2002; M. Byrne, unpubl. data; R. Ventura, unpubl. data). We examined ten early life history characters in 17 taxa of Asterinidae (Table 1). These characters vary among species, are related to demographic, ecological, or evolutionary processes, and are frequently of interest to evolutionary biologists studying the development, behavior, and ecology of marine invertebrate embryos and larvae. Some pairs of characters tend to co-vary among asterinid species, but the covariances are not functionally obligate and thus the characters are able to evolve independently (though they might tend to co-evolve). For example, egg size and mode of larval nutrition tend to co-evolve among echinoderms and other marine invertebrates (Strathmann 1985; Emlet et al. 1987), but some echinoderms with large, yolky eggs have feeding larvae (Emlet 1986; Hart 1996), and some asterinids with small eggs depend on maternal resources for early development through metamorphosis (Byrne 1996). Such covariation among characters would tend to increase both the overall consistency among life history characters and their potential conflict with nucleotide characters. The early life history characters were coded as follows:

1. *Adult largest radius:* <30 mm (0) or >40 mm (1). This coding corresponds to a distinct gap in the distribution of adult sizes among asterinid species. This coding is also partially correlated with some other life history characters (e.g., hermaphroditism is restricted to species <30 mm; Strathmann et al. 1984).

2. *Site of fertilization:* external with freely spawned sperm (0) or within the maternal gonad (1). This character is partially associated with parental brood care: all asterinid species with internal fertilization also have internal brood care with live birth, but not all species with external fertilization lack some form of parental care. This character is also partially associated with the type of breeding system: all species with internal fertilization are also hermaphrodites, though some hermaphrodites have external fertilization.

3. *Egg diameter:* <200 μm with little yolk (0) or >400 μm with abundant opaque yolk (1). Large and yolky eggs in asterinids are associated with the loss of larval structures and behaviors used to feed on phytoplankton, but a few species with small eggs depend on maternal resources through brood cannibalism (Byrne 1996).

4. *Larval habitat:* dispersing in the plankton (0) or restricted to the benthos (1). Some species with benthic larval development (in external egg masses or inside the parent) may disperse as juveniles or adults by rafting (Fell 1962).

5. *Parental brood care:* absent (0), external brood guarding (1), or internal brood care with live birth of juveniles (2). Species without brood care include those with planktonic larvae as well as those that produce

Table 1. Mode of larval development, coding of 10 early life history characters, and GenBank accession numbers for mitochondrial DNA sequence data for 17 asterinid sea stars and one outgroup (*Pisaster ochraceus*). Recent synonyms are given in [square brackets]. Life history characters are (1) adult largest radius, (2) site of fertilization, (3) egg diameter, (4) larval habitat, (5) parental brood care, (6) larval brachiolar arm complex, (7) larval nutrition for growth, (8) breeding system, (9) location of gonopores, and (10) hatching stage. See the text for character coding.

Species	Mode of development	Life history characters: 1 2 3 4 5 6 7 8 9 10	GenBank accession
Asterina gibbosa (PENNANT 1777)	egg masses without brooding	0 0 1 1 0 0 1 1 1 1	U50058[1]
A. miniata (BRANDT 1835)	planktotrophic	1 0 0 0 0 0 0 0 0 0	U50056[1]
A. minor HAYASHI 1974	egg masses without brooding	0 0 1 1 0 0 1 1 1 1	AH011641[2]
A. pectinifera (MÜLLER & TROSCHEL 1840)	planktotrophic	1 0 0 0 0 0 0 0 0 0	D16387[3]
A. phylactica EMSON & CRUMP 1978	egg masses with brooding	0 0 1 1 1 0 1 1 1 1	AH011640[2]
A. stellifera (MÖBIUS 1859)	planktotrophic	1 0 0 0 0 0 0 0 0 0	unpublished[4]
Cryptasterina hystera DARTNALL ET AL. 2003 [*Patiriella pseudoexigua*]	viviparous	0 1 1 1 2 0 1 1 0 0	AF509225[2]
C. pacifica (HAYASHI 1977) [*P. pacifica*]	viviparous	0 1 1 1 2 0 1 1 0 0	U50057[1]
C. pentagona (DARTNALL 1971) [*P. pseudoexigua*]	planktonic, lecithotrophic	0 0 1 0 0 0 1 0 0 0	AF509224[2]
Cryptasterina n. sp. DARTNALL ET AL. 2003 [*P. pseudoexigua*]	planktonic, lecithotrophic	0 0 1 0 0 0 1 0 0 0	U50051[1]
Patiriella calcar (LAMARCK 1816)	planktonic, lecithotrophic	1 0 1 0 0 0 1 0 0 0	U50046[1]
P. exigua (LAMARCK 1816)	egg masses without brooding	0 0 1 1 0 0 1 1 1 1	U50053[1]
P. gunnii (H. L. CLARK 1938) [*P. brevispina*]	planktonic, lecithotrophic	1 0 1 0 0 0 1 0 0 0	U50049[1]
P. oriens O'LOUGHLIN ET AL. 2002 [*P. gunnii*]	planktonic, lecithotrophic	1 0 1 0 0 0 1 0 0 0	U50047[1]
P. parvivipara (KEOUGH & DARTNALL 1978)	viviparous	0 1 0 1 2 1 1 1 0 0	U50055[1]
P. regularis (VERRILL 1867)	planktotrophic	1 0 0 0 0 0 0 0 0 0	U50045[1]
P. vivipara DARTNALL 1969	viviparous	0 1 0 1 2 1 1 1 0 0	U50054[1]
Pisaster ochraceus (BRANDT 1835)	planktotrophic	1 0 0 0 0 0 0 0 0 0	X55514[3]

[1] Hart et al. (1997); [2] this study; [3] Asakawa et al. (1995); [4] R. Ventura and H. Lessios; [5] Smith et al. (1990).

benthic egg masses but abandon the egg mass without further protection.

6. *Larval brachiolar arm complex:* well-developed (0) or reduced (1). The brachiolar complex is a set of two or three muscular arms, plus an adhesive disk, used to attach to the substratum prior to metamorphosis in species with planktonic larvae. The brachiolar complex is reduced to nonfunctional bumps in some but not all species with benthic embryos and larvae.

7. *Larval nutrition for growth:* from planktonic food (0) or from maternal resources (1). Maternal resources include metabolism of yolk in large eggs as well as cannibalistic consumption of siblings inside the maternal gonad. This character state could be further divided into yolk consumption versus cannibalism, but cannibalism has not been described in all live-bearing asterinids.

8. *Breeding system:* dioecious (0) or hermaphrodite (1). At least 1 species (*Patiriella exigua*) may be a protandrous hermaphrodite in which functional sperm are present in some small individuals before mature oocytes are found (Lawson-Kerr & Anderson 1978; Byrne 1992). However, not all *P. exigua* are protandrous, and protandry has not been reported in all known hermaphrodites. Some hermaphrodites may be capable of self-fertilization (or some form of parthenogenesis), but others are known to engage in external cross-fertilization. Some species (*P. exigua*) are polymorphic in the laboratory (showing both selfing and outcrossing), but the extent of selfing in nature is not known for any species so we did not attempt to distinguish selfing from outcrossing hermaphrodites.

9. *Location of gonopores:* upper (aboral) surface (0) or lower (oral) surface (1). Oral gonopores are associated with external brood care but not with viviparity.

10. *Hatching stage:* blastula or gastrula embryo (0) or brachiolaria (1). This character can be scored even for viviparous brooders, and is correlated with brood care and location of the gonopores.

We inferred phylogenetic relationships by parsimony using PAUP* (Swofford 2002) with all characters considered as unordered and equally weighted. We rooted the trees by the outgroup method using life history characters of the forcipulate sea star *Pisaster ochraceus,* which retains reproductive character states considered to be ancestral for all sea stars (Chia & Walker 1991). We performed a series of 1000 random taxon addition sequences, and estimated nodal support by bootstrapping 1000 times. We used accelerated character transformations (the AccTran option in PAUP*) for all heuristic searches and character optimizations. The limited number of available early life history characters and states will of course not resolve with confidence all relationships among so many taxa, but we were specifically interested in the phylogenetic utility of these particular early life history characters relative to nucleotide characters.

Mitochondrial DNA sequences

We used 12 previously published asterinid mtDNA haplotypes (see Table 1 for GenBank accession numbers) that included complete sequences for a protein-coding gene (cytochrome *c* oxidase I, or COI) and five transfer RNA genes at the 5′ end of COI (for alanine, leucine, asparagine, glutamine, and proline tRNAs; see Hart et al. 1997) and were ~1915 bp in length. Note that some species names have changed based on recent taxonomic and phylogenetic reanalyses (Dartnall et al. 2003; Hart et al. 2003; O'Loughlin et al. 2003; Waters et al. 2004). These sequence data include two closely related species formerly known as *Patiriella pseudoexigua* but recently reassigned to *Cryptasterina* gen. nov. (Dartnall et al. 2003; Hart et al. 2003): *Cryptasterina* n. sp. from Taiwan (U50051), and *C. pacifica* from Japan (U50057), formerly known as *P. pseudoexigua pacifica* (see Komatsu et al. 1990; Clark 1993; Hart et al. 2003). We rooted the trees using a mtDNA haplotype from *Pisaster ochraceus* (Smith et al. 1990) as an outgroup.

Some other available mtDNA sequences from asterinids were not included in this analysis. We had sequenced duplicate individuals of *Patiriella gunnii* (the species formerly known as *P. brevispina*), from eastern and western Australia, but used only the eastern Australia haplotype (U50049) in this analysis because the life histories of the eastern and western populations are not known to differ. Similarly, haplotypes are available for several newly described species of asterinids from southern Australia and New Zealand, including *P. occidens* and *P. oriens* (from western and eastern Australia populations of the species formerly known as *P. gunnii;* O'Loughlin et al. 2002, 2003; Waters et al. 2004). We did not include these haplotypes because life history observations have not been published for these new species (*P. occidens* and *P. oriens* probably have the same early life histories; M. Byrne, unpubl. data).

We obtained sequence data for five other asterinid species whose early life histories are known. For *Asterina minor* and *A. phylactica,* we sequenced 2 portions of the mitochondrial genome including the five tRNAs and the 5′ end of COI, plus a second portion of COI nearer the 3′ end of the gene, totaling 863–871 bp. For two *Cryptasterina* species from northern and central Queensland (Australia) we sequenced just the tRNAs and the 5′ end of COI (totaling 563–565

bp). The species of *Cryptasterina* examined were the dioecious, free-spawning species with planktonic, nonfeeding larvae called *C. pentagona* (AF509224) from near the ''*Patiriella pseudoexigua*' type locality at Airlie Beach, Queensland (see Dartnall, 1971); and a newly described hermaphrodite brooder with viviparous offspring called *C. hystera* (AF509225) from Statue Bay, Queensland (Byrne et al. 2003; Hart et al. 2003). We were given unpublished sequence from the 3′ end of COI for *A. stellifera* (639 bp) by R. Ventura and H. Lessios.

We aligned the tRNA sequences in Clustal W (Higgins & Sharp 1988). We aligned the COI sequences by eye with reference to the translated amino acid sequence (CLUSTAL alignments of complete COI amino acid sequences never included insertions or deletions; Hart et al. 1997). Nucleotide characters that had not been sequenced (e.g., tRNAs in *A. stellifera*) were coded as missing. Gaps in the tRNA alignment were coded as a fifth base (we obtained similar results when gap sites were coded as missing; Hart et al. 1997). We inferred phylogenies for these haplotypes by parsimony using PAUP*. Our earlier analysis showed that parsimony, maximum likelihood, and neighbor-joining methods all produced identical topologies with high bootstrap support for asterinid mtDNA haplotypes (Hart et al. 1997). We treated the nucleotide characters as unordered and weighted all characters equally. We performed 1000 random taxon addition sequences and calculated the 50% majority rule consensus of the most parsimonious trees. We estimated nodal support by bootstrapping 1000 times.

Measuring character congruence

We first evaluated each character set (or data partition) separately to confirm that it contained phylogenetic information. We compared the length of the most parsimonious tree (or strict consensus) to the frequency distribution of lengths of 1000 random phylogenies and the associated *g1* statistic (Hillis & Huelsenbeck 1992) calculated in PAUP*. We then measured congruence between the life history and nucleotide characters first by comparing tree topologies resulting from analysis of each character set separately. We noted all clades that were shared in common by these two tree topologies. We also compared these results to the topology and bootstrap support for a total evidence tree based on the two data partitions combined.

Second, we optimized the changes in each character set on the most parsimonious life history tree and the strict consensus of most parsimonious nucleotide trees in MacClade 3.07 (Maddison & Maddison 1997). We compared tree lengths and consistency indices for each character set mapped onto the most parsimonious trees from either the life history topology or the nucleotide topology. For each character set, we tested the overall degree of congruence by comparing the life history topology to the nucleotide topology using Templeton's test for parsimony in PAUP*. For the nucleotide characters only, we repeated this test using the Shimodaira-Hasegawa test for maximum likelihood (HKY85 + G model) instead of parsimony.

Third, we analyzed the overall congruence between the 2 character sets using the partition homogeneity (or incongruence length difference) test in PAUP* with 1000 replicates. Although this test is a biased indicator of data congruence, it is conservative and unlikely to give a spurious rejection of the hypothesis of data congruence (Cunningham 1997; Barker & Lutzoni 2002; Darlu & Lecointre 2002; Dowton & Austin 2002).

Results

Phylogenies

Parsimony analysis of the early life history characters suggested an ordered series of clades consisting of species groups with progressively more highly derived life histories (Fig. 1A). All 10 characters were parsimony-informative. We found a single most-parsimonious tree 12 steps long, with a consistency index of 0.92. The lengths of 1000 random trees ranged from 33–55 steps (mean = 46.9; *g1* = -0.50; $P < .01$). The large difference between the most parsimonious and random tree lengths and the highly left-skewed distribution of random tree lengths (Hillis & Huelsenbeck 1992) suggested that the phylogenetic information from the life history characters is highly non-random.

This phylogeny consisted of 6 groups, some of which were resolved into clades with reasonably high bootstrap support (numbers above the nodes in Fig. 1A). The first of these was an unresolved group of species with large bodied, dioecious adults (*P. regularis, A. miniata, A. pectinifera, A. stellifera*) that produce small eggs and dispersing feeding (planktotrophic) larvae. This is widely assumed to be the ancestral mode of larval development for all sea stars (Chia & Walker 1991; Smith 1997), and these species arose from a basal polytomy with the outgroup (*Pisaster ochraceus*). The second group is distinguished by large adult size (*P. calcar, P. gunnii, P. oriens*) and planktonic but nonfeeding (lecithotrophic) larvae that develop from large, yolk-rich eggs. These formed a well-supported clade with other species in which larvae depend on maternal nutrition for development. Third, we found two other species with small adults and le-

cithotrophic development (*Cryptasterina* n. sp., *C. pentagona*) grouped in an unresolved polytomy with two clades that also have small adult radius but have benthic, nondispersing development. Within this latter clade, the fourth group consisted of species (*A. minor, A. phylactica, A. gibbosa, P. exigua*) with benthic development in egg masses. *Asterina phylactica* was further distinguished from other members of this clade by the evolution of external brood care for embryos. Finally, we found a clade of viviparous brooders that included *C. hystera* and *C. pacifica,* with large eggs, and the closely-related sister species *P. vivipara* and *P. parvivipara* in which offspring develop from small, yolk-poor eggs but cannibalize broodmates for growth.

Parsimony analysis of the nucleotide characters suggested a nearly completely different phylogeny. We analyzed 1930 nucleotide sites (including gaps), of which 589 were parsimony-informative. We found 6 most parsimonious trees 2245 steps long, with a consistency index of 0.53. The strict consensus of these trees (the topology in Fig. 1B) was 2254 steps long, with a consistency index of 0.53. The lengths of 1000 random trees ranged from 2579–3042 steps (mean = 2912.3; $g1 = -0.91$; $P < .01$).

The Atlantic species *A. phylactica, A. gibbosa,* and *A. stellifera* formed a sister group to a much larger clade of Pacific or Australian species. The latter group consisted of 2 major clades in the strict consensus tree, though these had low bootstrap support in comparison to other well-supported clades (numbers above the nodes in Fig. 1B). Both of these major clades included species with planktotrophic larvae (e.g., *A. miniata, P. regularis*), species with lecithotrophic planktonic development (e.g., *P. calcar, C. pentagona*), and species with development in benthic egg masses (e.g., *A. minor, P. exigua*). Egg masses are also found among the Atlantic species (*A. gibbosa, A. phylactica*). Viviparous brooding was restricted to 1 of the 2 major clades, but viviparous species (*C. hystera, C. pacifica, P. vivipara, P. parvivipara*) were not all close relatives.

Parsimony analysis of all characters (total evidence) yielded a phylogeny that was completely congruent with the tree derived from the nucleotide data partition. We found 7 most parsimonious trees 2282 steps long, with a consistency index of 0.53. Bootstrap support for clades (numbers below the nodes in Fig. 1B) was similar to bootstrap values for clades in the consensus tree from analysis of nucleotides only. The addition of the early life history characters weakly improved resolution of only 1 node relative to the analysis of the nucleotide sequences alone. We found weak support in the total evidence analysis (54% of bootstrap replicates) for *A. minor* as the sister group to ((*P. calcar* (*P. oriens, P. gunnii*)), (*A. miniata, A pectinifera*)), but this node was collapsed to a polytomy in the consensus nucleotide tree.

In no analysis did we find evidence for monophyly of either *Asterina* or *Patiriella.* Tree topologies in which these taxa were constrained into monophyletic groups were significantly less parsimonious than the most parsimonious trees (results not shown). One potential taxonomic solution suggested by others (Clark 1983; Rowe & Gates 1995) is to retain the genus name *Asterina* for the type species *A. gibbosa* and other Atlantic species (including *A. phylactica* and *A. stellifera*), and refer all remaining species to *Patiriella* or other genera. Our phylogenetic results and those of Waters et al. (2004) support the suggestion of a biogeographic split between genera. These results are consistent with ongoing efforts to rediagnose genera of the Asterinidae into monophyletic clades (O'Loughlin et al. 2002, 2003; Dartnall et al. 2003; Waters et al. 2004).

Character incongruence

The 2 trees in Fig. 1 shared only 1 clade in common: *P. vivipara* + *P. parvivipara.* The highly unresolved nature of the life history tree, based on few characters, limits the potential to identify such shared clades unambiguously. In spite of this limitation, only 4 other clades found in the consensus of most parsimonious DNA trees are consistent with the life history tree: the sister species groups (*A. miniata* + *A. pectinifera*), (*P. calcar* (*P. gunnii* + *P. oriens*)), and (*A. gibbosa* + *A. phylactica*). All other well-supported clades in one tree conflict with well-supported clades in the other.

Early life history characters optimized onto the strict consensus of the 6 most parsimonious DNA trees required more than 3 times the number of character state changes (37) than was needed to optimize the same characters (12) onto the most parsimonious life history tree. The consistency index declined from 0.92 to 0.30. Similarly, optimizing the DNA characters onto the most parsimonious life history tree required more substitutions and insertion-deletions (2841) than needed to optimize these characters on the consensus of the most parsimonious DNA trees (2254), and the consistency index for these characters declined from 0.53–0.42.

The two trees conflicted strongly when measured by Templeton's test for parsimony. For the early life history characters, the most parsimonious life history tree was significantly better than the consensus of the most parsimonious DNA trees ($z = -2.70$, $P = .007$). For the DNA characters, the contrast was even more strongly significant ($z = -16.54$, $P < .001$). We obtained a similar result using the Shimodaira-Hasegawa

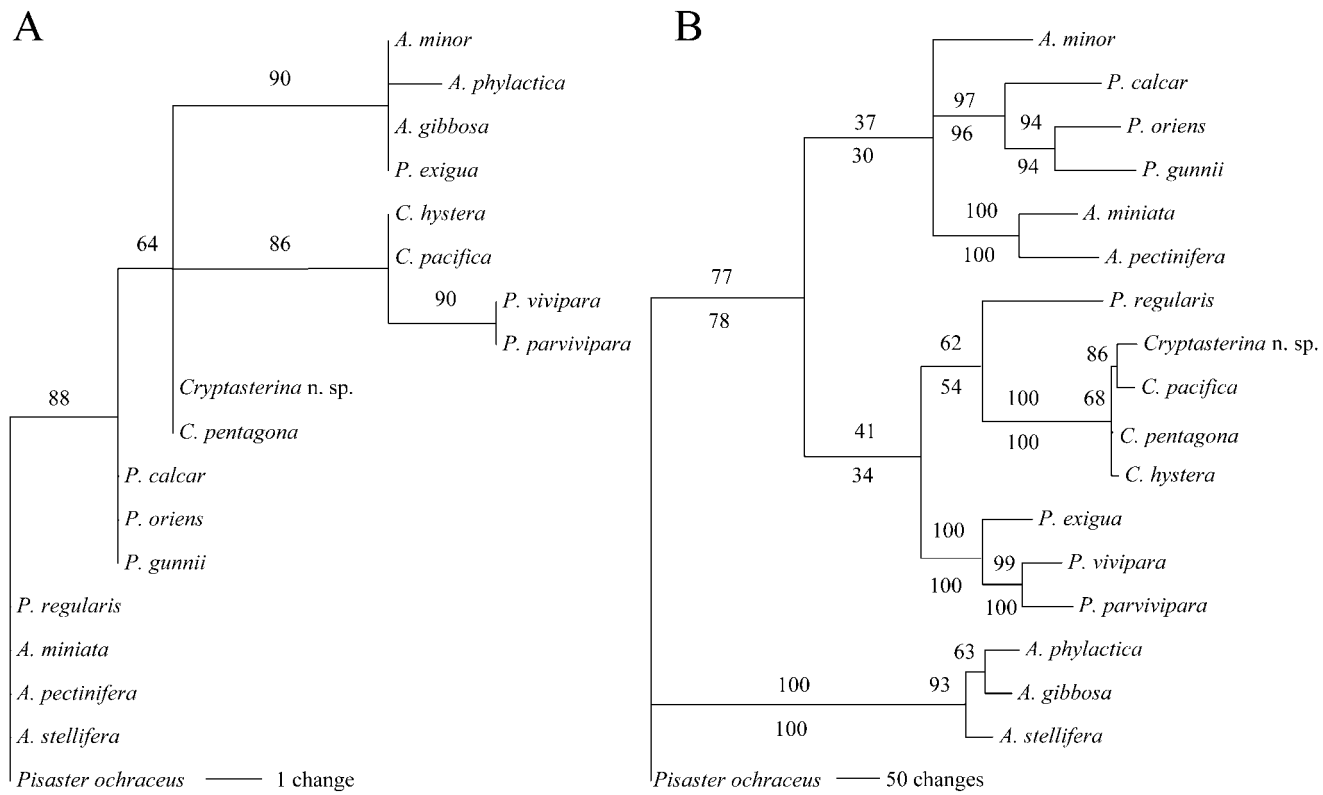

Fig. 1. Phylograms of most parsimonious trees based on analysis of (A) 10 life history characters or (B) 1930 nucleotide characters from asterinid sea stars. *Pisaster ochraceus* was used as the outgroup. Numbers above branches are bootstrap percentages from analysis of single data partitions; numbers below branches in (B) are bootstrap percentages from the total evidence analysis. The topology in (B) is the strict consensus among six most parsimonious trees. Note that bootstrapping analysis of the tree in (A) supported grouping *Asterina minor*, *Cryptasterina hystera*, and other members of these two groups into a single clade with high frequency (83%), but this branch had length 0 in the most parsimonious tree. As a result, this branch is collapsed to a polytomy in (A).

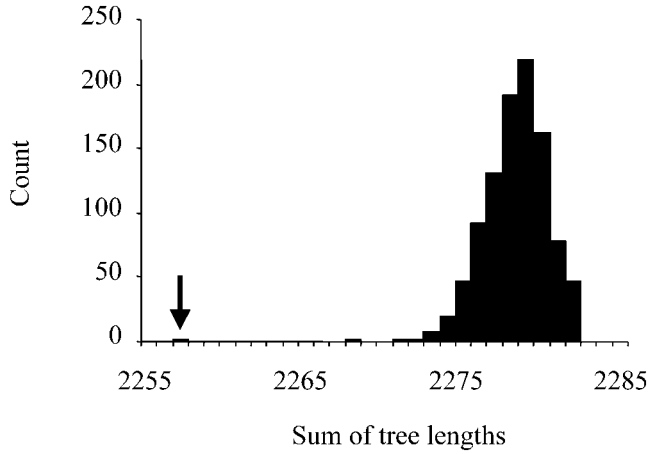

Fig. 2. Results of the incongruence length difference test, showing the frequency distribution of the sum of tree lengths in 999 randomized data partitions consisting of 10 and 1930 characters, respectively (histogram) and the sum of tree lengths from analysis of the real data partitions for early life history and nucleotide characters in asterinid sea stars (arrow).

test for maximum likelihood with the DNA characters alone: the difference between the -ln(likelihood) of the consensus DNA tree (11756.6) and the most parsimonious life history tree (12690.4) was highly significant ($P < .001$). Finally, the incongruence length difference was highly significant ($P < .001$, Fig. 2). Thus, randomized data partitions produced substantially less-parsimonious trees than the real data partitions, which suggests that the real partitions gave significantly conflicting inferences of relationships among these sea stars (and in spite of the extensively analyzed tendency for the ILD test to erroneously confirm congruence between data partitions; Barker & Lutzoni 2002; Dowton & Austin 2002).

These general incongruities are most easily illustrated with specific examples. Figure 3 shows the contrast in inferred state changes for the early life history character egg diameter. This character is ecologically and evolutionarily significant because changes in egg size are associated with differences in larval morphology, clutch size, development time to metamorphosis,

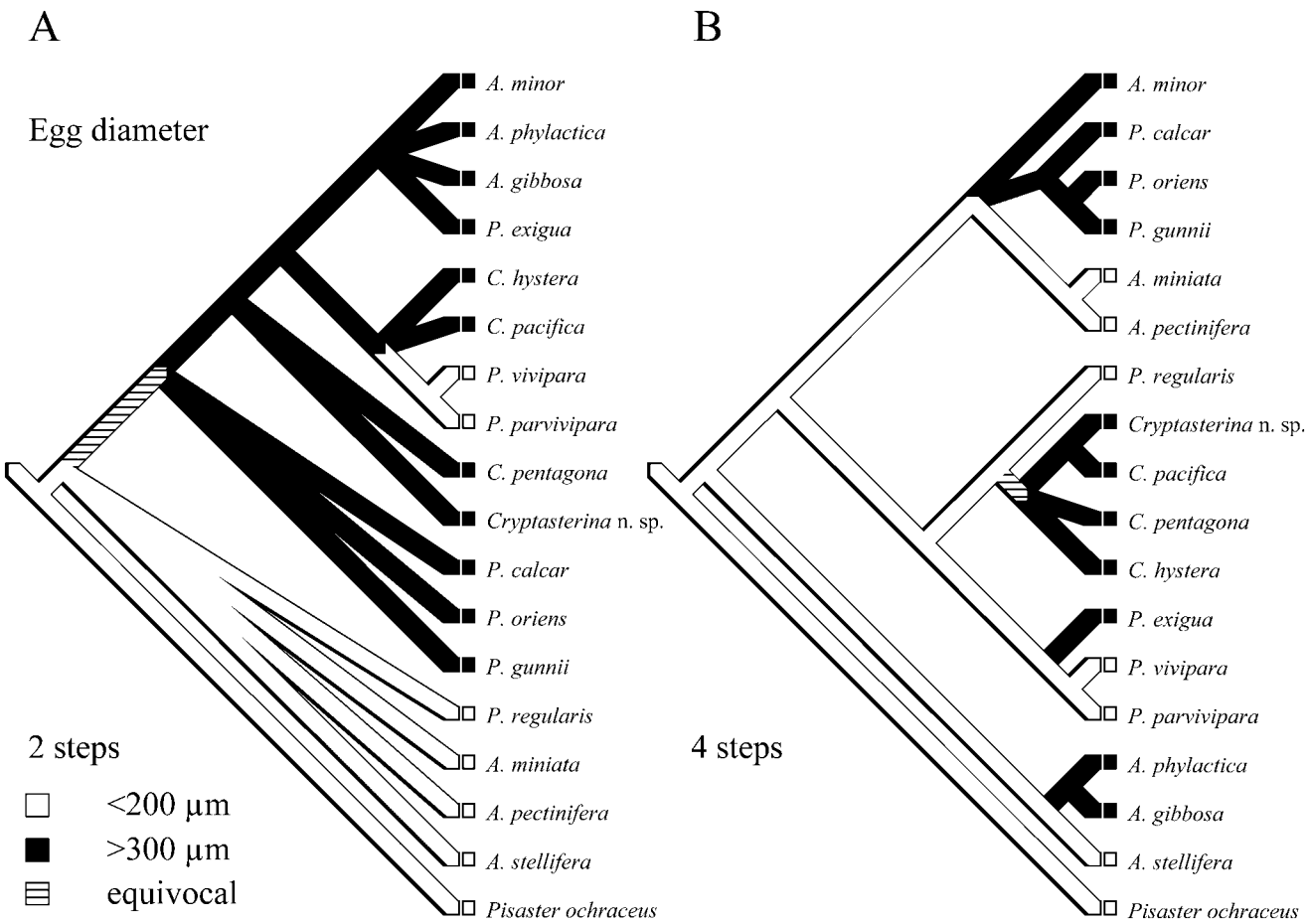

Fig. 3. Examples of character conflict: changes in a life history character (egg diameter) optimized on (A) the most parsimonious life history tree; and (B) the consensus of the most parsimonious nucleotide trees. Shading next to taxon names indicates egg diameter; shading of branches indicates inferred ancestral egg diameter (equivocal ancestral states are indicated by horizontal hatching). Number of inferred evolutionary changes in egg size (steps) for each branching pattern is indicated below each tree. See Fig. 1 for notes on one collapsed branch in (A) with high bootstrap support but 0 length in the most parsimonious tree.

and other early life history features (Strathmann 1985; Emlet et al. 1987). When optimized on the most parsimonious life history tree (Fig. 3A), egg size changed just twice: large eggs evolved once in the common ancestor of species that lack a feeding larval form, followed by a single reversal to small eggs in the brooders *P. vivipara* and *P. parvivipara*, in which offspring grow inside the gonad by sibling cannibalism after metamorphosis. When optimized on the consensus DNA tree (Fig. 3B), large eggs evolved at least 4 times independently in various clades with nonfeeding or nondispersing larval forms.

Figure 4 illustrates the contrasting differences in inferred state changes for a nucleotide character: the third nucleotide in the variable loop (loop III) of the alanine tRNA (nucleotide 11812 in the *A. pectinifera* complete mitochondrial genome sequence; Asakawa et al. 1995). When optimized on the most parsimonious life history tree (Fig. 4A), this nucleotide site evolved at least 5 times. The nature of the inferred substitutions depends on the resolution of the ambiguous ancestral states: the ancestral state that minimizes the number of transversions is C, resulting in a C→T transition and 4 C→A, A→T, or A→C transversions. When optimized on the consensus DNA tree (Fig. 4B), this site evolved 3 times, including a T–C transition and 2 T–A or A–C transversions.

Discussion

One school of phylogenetic theorists has advocated the total evidence approach to inferring evolutionary relationships. In general, the debate over total evidence has largely given way to quantitative evaluation of conflict between character sets (or data partitions) (e.g., Levasseur & Lapointe 2001; Buckley et al.

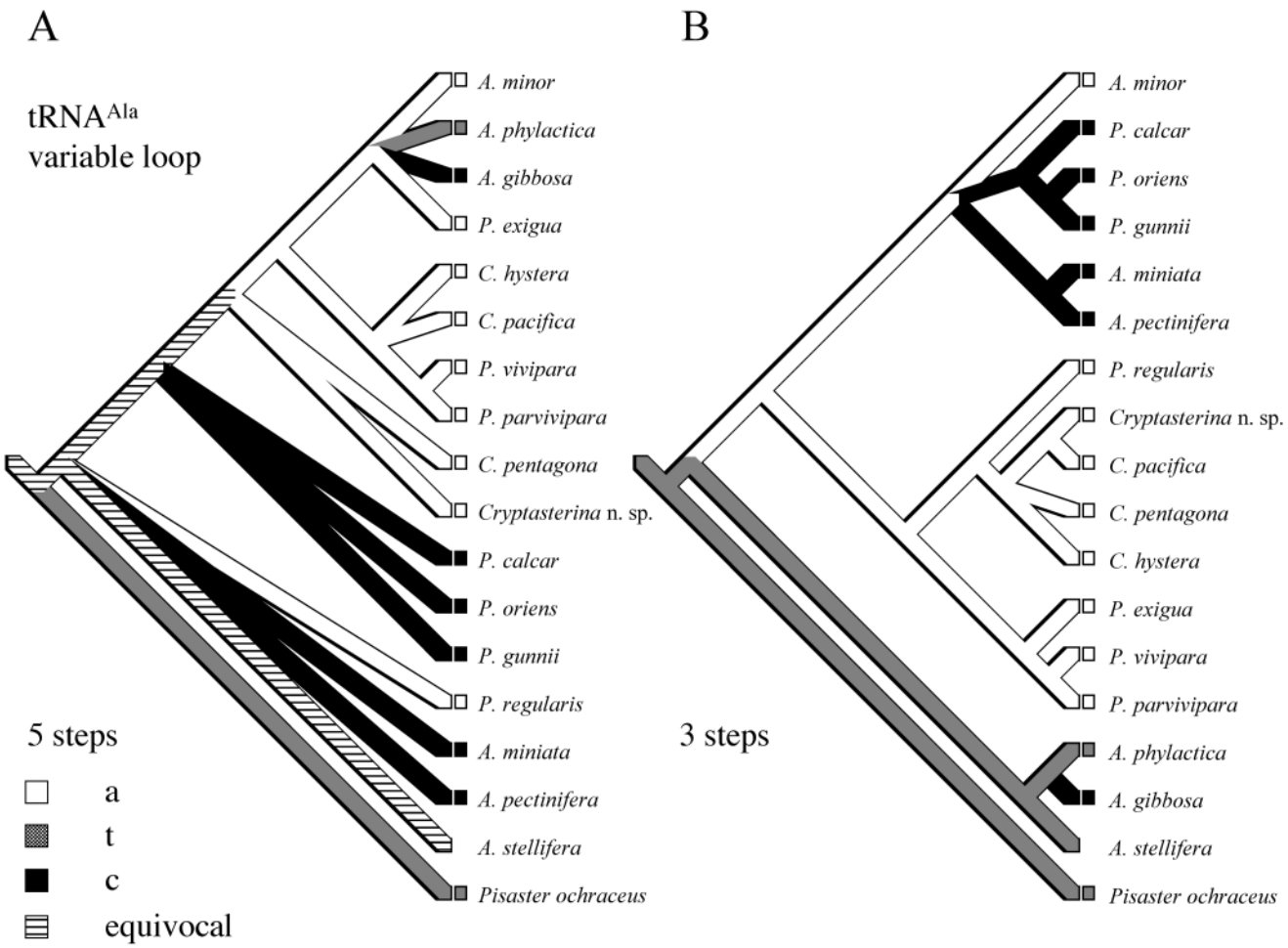

Fig. 4. Examples of character conflict: changes in a nucleotide character (from the variable loop of the alanine transfer RNA gene) optimized on (A) the most parsimonious life history tree; and (B) the consensus of the most parsimonious nucleotide trees. Shading next to taxon names indicates nucleotide identity; shading of branches indicates inferred ancestral nucleotide identity under the assumption that all nucleotide changes are equally likely (equivocal ancestral states are indicated by horizontal hatching). Number of inferred nucleotide substitutions (steps) for each branching pattern is indicated below each tree.

2002). We set out to evaluate the conflict between early life history and nucleotide characters for inferring phylogenies of asterinid sea stars because these life history characters have been cautiously used in other studies for phylogenetic information and because these characters are especially diverse among asterinid species. We do not specifically advocate for or against the prior utility of these early life history characters for phylogenetic analysis. Rather, we aimed for a more objective evaluation of these characters relative to some nucleotide characters from the mitochondrial genome that are generally acknowledged to provide reliable phylogenetic information for resolving relationships among congeners and other closely related species.

The quantitative analysis of character conflict between these two data partitions is a significant improvement over previous qualitative demonstrations of character conflict (e.g., Hart et al. 1997). Many of the earlier qualitative demonstrations were published before the quantitative tests were readily available together in PAUP* and other software packages. Qualitative analyses, such as the mapping of single early life history characters onto molecular phylogenies, will underestimate overall incongruence between data partitions because, even for highly diverse characters, some clades in one tree will have synapomorphic character states from the other data partition (e.g., Figs. 3B, 4A). However, these shared character states vary among clades for different characters. As a result, overall consistency between the data partitions is much less than is implied by the parsimony mapping of single characters.

Several of the quantitative results are striking. First, the mapping of each character set onto the alternative phylogeny shows highly reduced consistency among character states: in the case of the early life history characters (which have a high consistency index on the life history tree), the consistency of these characters declined to 0.30, almost in the range of consistency indices expected for randomly assigned character states. Second, the ILD test result places the sum of tree lengths for the real data partitions (2257 steps) ~10 standard deviations away from the mean of tree lengths for randomized data partitions (2278.3±2.1 steps). Third, all quantitative comparisons of the two most parsimonious tree topologies (Fig. 1) suggest that each tree is a significantly poorer fit to data from the other partition. Each result alone suggests potentially strong conflict between these two data partitions, but the various quantitative analyses together suggest overwhelming conflict that is not entirely evident in simple graphical analyses (e.g., Figs. 3, 4)

Together, these results could be used to argue against the inclusion of both character sets together in a combined data analysis because the character conflict could obscure the clear phylogenetic results inferred from the nucleotides alone. However, the conflict between astrinid early life history and nucleotide characters did not produce an equivocal result in our total evidence analysis: this phylogeny was not different from the phylogeny based on nucleotide characters (Fig. 1B), and the resolution of some clades was slightly improved in comparison to the nucleotide results. This particular outcome from total evidence probably depends greatly on the very large difference in size of the two character sets: 10 parsimony-informative early life history characters versus 589 parsimony-informative nucleotide sites. The conflicting phylogenetic signal from a few early life history characters is overwhelmed by phylogenetic information from numerous nucleotide sites. Adding a few life history characters in this case helps to resolve relationships among *A. minor, P. calcar,* and their relatives because 5 of these species share in common several life history features associated with dispersing larval development in the plankton, but share only 8 unambiguous nucleotide synapomorphies. As a result, bootstrap support for such weak nodes can be slightly increased by the inclusion of a few critical early life history characters. Whether such results are general for other combined analyses of early life history and nucleotide characters will depend in large part on the relative size of the 2 data partitions and the sharing of early life history synapomorphies among clades. Our results suggest that these combined analyses will at best reflect inferences from nucleotide sequences alone.

Our comparison of early life history and mtDNA characters in asterinids shows that these character sets each contain significant non-random phylogenetic information but give strongly conflicting inferences about phylogenetic relationships. Three earlier studies provided some specific qualitative documentation of phylogenetic conflict between phenotypic characters from larval stages and molecular characters in echinoderms. Littlewood & Smith (1995) developed a phylogenetic hypothesis for families and orders of sea urchin (Class Echinoidea) based largely on 18S and 28S rRNA sequences. This phylogeny was later used by Smith et al. (1995) and Wray (1996) to compare phylogenetic hypotheses based either on DNA sequences or on life history characters (many of them morphological characteristics of larval stages) largely compiled by Wray (1992). The results of both analyses were clear: at a relatively high taxonomic level, shared derived similarities in life history characters were rarely congruent with shared derived nucleotide similarities. This general conclusion was also reached by Wray (1992) in an analogous comparison of phylogenetic hypotheses for sea urchin families and orders based on adult or larval morphological characters: larval characters were in strong conflict with adult characters (which were, in turn, largely congruent with nucleotide characters; Smith et al. 1995).

This extreme character conflict is evident in many marine invertebrates with complex life cycles and is probably not an artifact of the high taxonomic level used for previous analyses in sea urchins. Molecular phylogenetic hypotheses for gastropods (*Conus:* Duda & Palumbi 1999; turritellids: Lieberman et al. 1993), clams (*Lasaea:* ÓFoighil & Smith 1995), soft corals (*Alcyonium:* McFadden et al. 2001), sea cucumbers (*Cucumaria:* Arndt et al. 1996), and tunicates (*Molgula:* Hadfield et al. 1995) all suggest strong character conflict between early life history characters and DNA sequences (Hart 2000).

Examples of strongly supported congruence between early life history characters and molecular characters in marine invertebrates are rare. Reid (1990) developed a morphological hypothesis of relationships among littorinid snails, and identified a clade of 10 *Littorina* species in which lecithotrophic larval development had evolved as a synapomorphy. The mtDNA analysis of Reid et al. (1996) left some relationships between lecithotrophic and planktotrophic species unresolved but was at least consistent with a single origin of lecithotrophy. Similarly, ÓFoighil & Taylor (2000) used rDNA sequences to analyze relationships among oyster species and discovered a well-supported clade

of 11 species in which brood care had evolved as a synapomorphy.

Which data partition is misleading in the case of asterinid sea stars? Smith (1997) concluded that early life history characters like those studied here in asterinids are much more prone to convergence and reversal than are either adult morphological characters or nucleotide characters. Smith argued that plausible models of life history evolution, based on the functional and ecological consequences of changes in larval nutrition, egg size, breeding system, and other characters, predict convergent adaptive changes in early life history characters as suites of correlated traits in echinoderms and other marine invertebrates (Strathmann 1985; Wray 1992, 1996; Strathmann & Eernisse 1994; McHugh & Rouse 1998).

In spite of the potential for misleading phylogenetic information from early life history characters in marine invertebrates with complex life cycles, such characters are sometimes used in cladistic analyses without prior confirmation of the congruence between the life history characters and other sources of phylogenetic information (e.g., Rouse & Fitzhugh 1994; Rouse & Gambi 1998; Rouse 2000). Such analyses are not necessarily problematic: larval morphology, breeding system, and other characters scored from the embryonic and larval stages of complex life cycles are sometimes congruent with other characters. However, our quantitative analysis of this character conflict in asterinids can be viewed as a kind of cautionary tale with the following moral: use of early life history characters in total evidence analyses will probably need prior confirmation of congruence (or at least lack of strong conflict) between the life history characters and some other source of phylogenetic information such as nucleotides. The difference between our total evidence analysis and the analysis of single data partitions alone suggests that adding early life history characters to a molecular phylogeny might be slightly beneficial, but adding molecular characters to an early life history phylogeny could be crucial to the discovery of well-supported phylogenetic relationships.

Is this commonly observed character conflict simply an artifact of incomplete knowledge of taxonomic diversity? The identification of higher species diversity could lead to increased congruence between early life history and nucleotide characters if there are many unrecognized clades that consist of undescribed sibling species with similar life histories (Knowlton 1993, 2000). Among asterinids, the sibling species *Patiriella oriens* and *P. occidens* (formerly known as *P. gunnii;* Hart et al. 1997; O'Loughlin et al. 2003) probably have similar nonfeeding, planktonic larval development. If confirmed, many such results would reduce the extent of character conflict between nucleotide and life history characters (Waters et al. 2004). However, we have also found cryptic species pairs within the *Cryptasterina* species complex that have highly divergent life histories (*Cryptasterina* n. sp. versus *C. pacifica; C. pentagona* versus *C. hystera*). In addition, *P. regularis* and *P. mortenseni* are newly distinguished from each other as cryptic species but do not appear to be closely related based on mtDNA sequence data (O'Loughlin et al. 2002). If *P. mortenseni* has planktotrophic larval development like *P. regularis,* then their distant phylogenetic relationship might increase the measured conflict between nucleotide and life history characters. Our results and other recent studies (O'Loughlin et al. 2002; Byrne et al. 2003) illustrate the need for an expanded survey of taxonomic and reproductive diversity in asterinids. However, at least for asterinids, it is not clear whether unrecognized species diversity should generally be expected to decrease the observed conflict between nucleotide and early life history characters, or to improve the otherwise weak utility of early life history characters for phylogenetic analysis.

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