



Molecular phylogeny of the brachyuran crab superfamily Majoidea indicates close congruence with trees based on larval morphology

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ABSTRACT

In this study, we constructed the first molecular phylogeny of the diverse crab superfamily Majoidea (Decapoda: Pleocyemata: Brachyura), using three loci (16S, COI, and 28S) from 37 majoid species. We used this molecular phylogeny to evaluate evidence for phylogenetic hypotheses based on larval and adult morphology. Our study supports several relationships predicted from larval morphology. These include a monophyletic Oregoniidae family branching close to the base of the tree; a close phylogenetic association among the Epialtidae, Pisidae, Tychidae, and Mithracidae families; and some support for the monophyly of the Inachidae and Majidae families. However, not all majoid families were monophyletic in our molecular tree, providing weaker support for phylogenetic hypotheses inferred strictly from adult morphology (i.e., monophyly of individual families). This suggests the adult morphological characters traditionally used to classify majoids into different families may be subject to convergence. Furthermore, trees constructed with data from any single locus were more poorly resolved than trees constructed from the combined dataset, suggesting that utilization of multiple loci are necessary to reconstruct relationships in this group.

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1. Introduction

Decapods are one of the most species-rich groups of Crustacea and are the subject of more papers than all other crustacean groups combined, due to their commercial, economic, and ecological importance (Martin and Davis, 2001, 2006). Phylogenies based on molecular and morphological characters are invaluable tools to trace the evolution of morphological, behavioral, and physiological diversity in decapods (Kitaura et al., 2006; Macdonald et al., 2006; Patek and Oakley, 2003; Porter et al., 2007; Schubart et al., 2000a). Although many studies have examined relationships among the major decapod groups (Ahyong and O'Meally, 2004; Brosing et al., 2007; Guinot, 1978; Jamieson, 1994; Kim and Abele, 1990; Porter et al., 2005; Spears et al., 1992), we know relatively little about phylogenetic relationships within these groups, especially compared with well-studied taxa such as the Vertebrata (Martin and Davis, 2001, 2006).

Within the Decapoda, the brachyuran superfamily Majoidea (Decapoda: Pleocyemata: Brachyura) is a particularly diverse group, estimated to contain over 800 species (Rice, 1988); Rathbun (1925) recorded over 230 species in North America alone. The Majoidea, formerly the family Majidae, were recently reclassified as a superfamily (Hendrickx, 1995; Martin and Davis, 2001; McLaughlin et al., 2005), and we follow this classification throughout.

Monophyly of the superfamily Majoidea is well-supported by adult and larval morphology. All majoids have only two zoeal stages, in contrast to the remaining brachyurans (but see Clark and Ng, 2004) and a terminal molt upon maturity (Rice, 1980, 1983). The majoids are one of the oldest lineages of brachyuran crabs and are thought to branch near the base of the brachyuran tree, based on evidence from spermatozoal ultrastructure (Jamieson, 1994), larval characteristics (Rice, 1980, 1983), and molecular data (Porter et al., 2005; Spears et al., 1992). However, their exact position relative to the remaining Brachyura may depend on accurate placement of two provisionally brachyuran families, the Dromiidae and the Raninidae (Brosing et al., 2007; Spears et al., 1992). Estimates of when the Majoidea diverged from the remaining Brachyura vary; although fossils date the divergence of the majoids close to 70 MYA, in the Upper Cretaceous/Eocene (Spears et al., 1992), studies using model based methods estimated that the majoids diverged from the rest of the Brachyura ~254 MYA, near the Permian–Triassic boundary (Porter et al., 2005).

Although monophyly of the superfamily Majoidea is broadly accepted, internal classifications are less stable, and past taxonomic treatments of this group have frequently transferred genera among different families or subfamilies (Clark and Webber, 1991; Garth, 1958; Griffin and Tranter, 1986; Martin and Davis, 2001; Ng et al., 2008). Familial and subfamilial classifications in the Majoidea are generally based on adult eyestalk and antennal morphology (Garth, 1958; Griffin and Tranter, 1986; Rathbun, 1925). The most current comprehensive taxonomic monographs traditionally

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recognize up to eight majoid families: the Epialtidae, Inachidae, Inachoididae, Majidae, Mithracidae, Pisidae, Tychidae, and Oregoniidae (Griffin and Tranter, 1986; Martin and Davis, 2001; McLaughlin et al., 2005). The majority of these familial associations follow from elevation of traditionally recognized majoid subfamilies (Garth, 1958; Griffin and Tranter, 1986) to familial status (Hendrickx, 1995; Martin and Davis, 2001; McLaughlin et al., 2005). More recently, Ng et al. (2008) has pointed out that many of these families are morphologically poorly defined, and demoted several of the more “problematic” families to subfamily status within more broadly defined families. Although genera were not transferred among families or subfamilies in this classification, Ng et al. (2008) recognized only five majoid families: the Inachidae, Inachoididae, Oregoniidae, Majidae (including the subfamilies Majinae and Mithracinae), and Epialtidae (including the subfamilies Epialtinae, Tychinae, and Pisinae).

Although there are no published molecular phylogenies investigating relationships among or within the different majoid families, previous workers have proposed relationships (Kurata, 1969; Rice, 1980, 1983) and constructed phylogenies using larval morphology (Clark and Webber, 1991; Marques and Pohle, 1998, 2003; Pohle and Marques, 2000). Rice (1980, 1983) proposed that the Oregoniidae retained the ‘ancestral’ larval morphology (i.e., the clade branched near the base of the tree), and proposed two additional groupings of the remaining majoids: the Inachidae, and a second group composed of the Majidae, Pisidae, and Epialtidae (in which the Pisidae and Epialtidae were closely related) (Fig. 1a). Although the Mithracidae and the Tychidae were not considered in these studies, later analyses (Rice, 1988) concluded that the Mithracidae was closely related to the Pisidae and Epialtidae, and past systematic classifications of tychid species (e.g., Rathbun, 1925) support a close Tychidae–Mithracidae relationship. More recent studies using larval morphology (Figs. 1b and c) to construct phylogenies support (1) a monophyletic Oregoniidae clade branching at or near the base of the majoid tree (Clark and Webber, 1991; Marques and Pohle, 1998); (2) a monophyletic Majidae clade branching near the base of the tree (Marques and Pohle, 2003; Pohle and Marques, 2000); (3) an Inachidae–Inachoididae clade (Marques and Pohle, 2003); and (4) close phylogenetic associations among the Epialtidae, Pisidae, and Mithracidae families (Marques and Pohle, 2003; Pohle and Marques, 2000; Rice, 1988). Despite this general concordance in relationships among families, larval morphology provides little support for monophyly of many of the families defined on the basis of adult morphology (Marques and Pohle, 2003). A molecular phylogeny of this group could help resolve such conflict between larval and adult morphological characters by providing independent phylogenetic evidence for or against different hypothesized relationships within and among majoid families.

In this study, we used sequences from one nuclear locus (28S) and two mitochondrial loci (16S and COI) to construct the first molecular phylogeny of the Majoidea superfamily, which we used to evaluate hypotheses about majoid relationships based on larval and adult morphology. We begin with the traditionally recognized classification (Martin and Davis, 2001; McLaughlin et al., 2005), and test the monophyly and relationships among seven of these eight recognized families: the Epialtidae, Inachidae, Majidae, Mithracidae, Pisidae, Tychidae, and Oregoniidae, then also evaluate recent revisions proposed by Ng et al. (2008).

2. Materials and methods

2.1. Taxon sampling and molecular markers

We broadly sampled multiple representatives of the major families in order to reconstruct large-scale relationships within the Majoidea. Our taxon sample primarily consisted of common,

intertidal to shallow subtidal species from the western and eastern coasts of the US and the eastern coast of Japan, although we also obtained specimens from other regions (Table 1). For an outgroup, we selected the parthenopid crab *Heterocrypta occidentalis*, as the Parthenopidae are thought to be closely related to the Majoidea (Rathbun, 1925; Rice, 1983; Spears et al., 1992).

We used three loci that evolve at different rates in an effort to resolve divergences among families and genera at several different time scales. These loci included partial sequences of nuclear 28S ribosomal RNA, mitochondrial 16S ribosomal RNA, and the mitochondrial protein coding gene cytochrome oxidase I (hereafter referred to as 28S, 16S, and COI). Mitochondrial loci, and 16S in particular, are the most commonly sequenced loci used for inter- and intra-specific crustacean phylogenies (Schubart et al., 2000b), and have been used to resolve relationships among taxa within several brachyuran families (Kitaura et al., 2006; Schubart et al., 2006, 2000a,b). COI has also been used in species-level phylogenies (Haye et al., 2004 and references therein). However, mitochondrial markers may be less useful for deeper branches such as those delineating the majoid families. Nuclear loci such as 28S rRNA have been used in multi-locus datasets (in conjunction with mitochondrial markers) to reconstruct major clades in the Arthropoda and Decapoda (Ahyong and O’Meally, 2004; Giribet et al., 2001; Porter et al., 2005) and to construct the evolutionary relationships of panulirid lobsters (Patek and Oakley, 2003). Thus we sequenced 28S in order to assist in resolution of the deeper branches of the majoid tree. In total, we obtained sequence data from 36 majoid species (and the outgroup species, *H. occidentalis*) from seven out of the eight recognized majoid families, primarily by direct sequencing but occasionally from GenBank (see Table 1 for list of collection sources and accession numbers). For COI, we used universal heavy chain/light chain primers (Folmer et al., 1994), and used the heavy chain (HCO) sequences for phylogeny construction. Since evolutionary convergence in nucleotide composition (CNC, i.e., compositional bias) can introduce errors in phylogenetic inference (Foster, 2004; Mahon, 2006), we performed analyses of nucleotide composition to test CNC in a subset of our COI sequences using programs written by J. Neigel in Perl v5.84, and manually examined resulting graphs of AT and CG skew (see Mahon, 2006); none of the taxa exhibited major skew. For 16S, we initially used universal 16AR and 16BR primers (Palumbi et al., 1991), then constructed majoid-specific interior forward (5’-TATT TTGA CCGT GCAA AGGT AG-3’) and reverse (5’-ATTT AAAG GTCC AACA GACC CT-3’) primers that amplified ~430 bp, using Primer3 software v3.0 (Rozen and Skaletsky, 2000). Sequences amplified using universal primers were truncated to capture the interior portion amplified by taxon-specific primers. For 28S, we again used Primer3 software to construct majoid-specific interior primers using a partial 28S gene sequence from the Atlantic spider crab *Maja squinado* in GenBank (accession # DQ079799, Porter et al., 2005) (subsequent analysis by Sotelo et al., 2008 indicates this species is likely *Maja brachydactyla*). These primers (forward: 5’-GCAG TCTC TCAC CGCC TAAG TTAT G-3’; reverse: 5’- GACT CCTT GGTC CGTG TTTC AAGA C-3’) amplified a ~620-bp portion of the 28S gene. For each species, we verified sequences by comparing 3’-5’ reverse sequences against forward sequences and/or aligned 5’-3’ forward sequences from multiple individuals; in all cases, reverse and forward sequences aligned perfectly and/or sequences from multiple individuals were identical or nearly identical (<1% sequence divergence for mitochondrial loci among multiple individuals).

2.2. DNA extraction, sequencing, and alignment

We extracted DNA from 0.5 to 2 mg pieces of crab leg tissue using a Puregene DNA extraction kit (Gentra); final DNA concentrations were 2–70 µg ml⁻¹. We amplified 16S and COI using

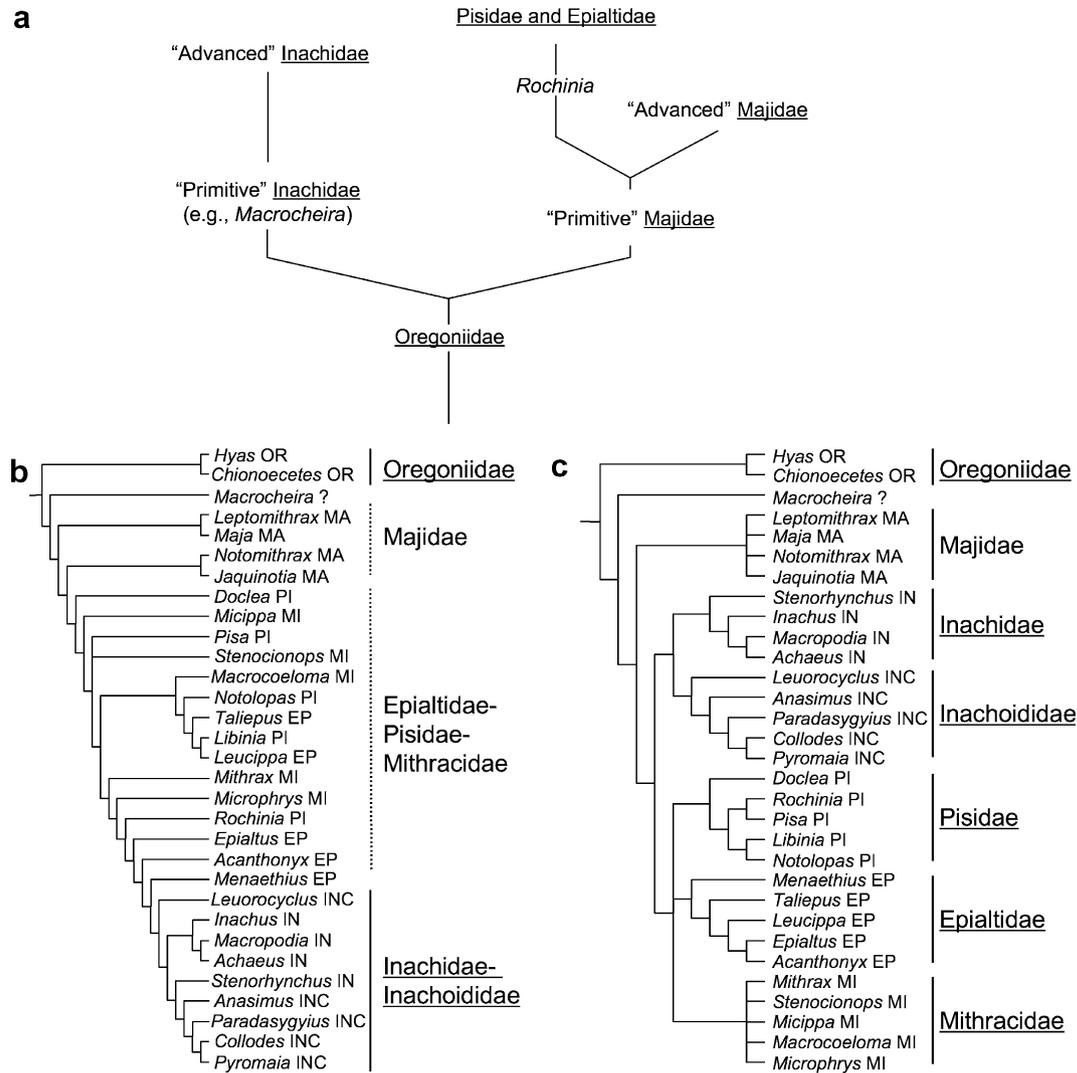


Fig. 1. Previous hypotheses about Majoid relationships based on larval morphology. (a) Suggested relationships of majoid families (modified from Rice, 1980). Underlined text indicates current name of groups. (b, c) Recent trees based on 37 larval morphological characters (modified from Marques and Pohle, 2003); (b) Semi-strict consensus tree of three equally parsimonious trees in an unconstrained analysis. (c) Semi-strict consensus tree of 65 equally parsimonious trees, when families are constrained to be monophyletic. For (b) and (c), EP = Epialtidae, IN = Inachidae, MA = Majidae, MI = Mithracidae, OR = Oregoniidae, PI = Pisidae, TY = Tychidae, ? = incertae sedis; underlined families (and solid lines) indicate monophyletic groupings, and dotted lines indicate non-monophyletic family groupings.

35–40 standard PCR cycles: 94 °C for 30 s (denaturation), 45–50 °C for 30 s (annealing), and 72 °C for 30 s (extension), run on a GeneAmp 9700 thermal cycler (Applied Biosystems). For reactions using majoid-specific 16S and 28S primers, we used higher annealing temperatures suggested by Primer3 software (58 °C for 16S, 65 °C for 28S), and for 28S we used a longer annealing time (1 m) and extension time (1 m 30 s) for each cycle. Amplified PCR products were visualized on 1% agarose gels and formed single bands, including those amplified with 28S primers, and we purified these products using a Shrimp Alkaline Phosphate Exonuclease protocol (USB Corporation). Sequencing reactions were run on an ABI 3730 Capillary Electrophoresis Genetic analyzer using ABI BigDye Terminator v3.1 Cycle Sequencing (Applied Biosystems, Foster City, CA), and we manually checked base spectrographs using the program 4Peaks v1.7 (Griekspoor and Groothuis, 2005).

We calculated the appropriate models of nucleotide substitution for each locus using Modeltest v3.7 (Posada and Crandall, 1998), and used the Akaike Information Criterion (AIC) to select the model of molecular evolution that best fit the data (Posada and Buckley, 2004). To test for saturation, we used PAUP v.

4.0b10 (Swofford, 2002) to plot uncorrected pairwise sequence distances (number of observed changes, or uncorrected 'p') against distances calculated by the ML model for each locus (number of inferred changes), and for COI, we tested different codon positions (positions 1–2 and position 3) for saturation separately.

We used the program MUSCLE 3.6 (Edgar, 2004) to align sequences from each individual locus using default parameters. Although alignment of 16S and COI was fairly straightforward, portions of the 28S were highly divergent among species and difficult to align reliably. We thus used the program GBlocks v0.9 (Castresana, 2000) to locate and exclude ambiguous areas of the alignment for each locus, using relaxed gap selection criteria (allowed gap positions = all). Relaxed gap selection criteria is suggested for short (i.e., single gene) alignments in GBlocks based on simulation studies (Talavera and Castresana, 2007); additional parameters were unmodified from GBlocks defaults. For 16S and COI alignments, GBlocks trimmed only leading and trailing ends of the alignment (21% excluded in 16S, 19% excluded in COI). For 28S, both leading/trailing ends and some hyper-variable regions within the alignment (flanked by conserved sequences) of the alignment

Table 1
Species and individuals used in the study, including collection localities, family affiliations, GenBank accession numbers, and taxa sets

Taxon	Collection locality	Code	Family	Family (Ng et al., 2008)	Accession Nos			Taxa sets		
					16S	COI	28S	Total Evidence	Complete character	Species
<i>Chionoecetes bairdi</i>	GenBank sequence	<i>Chionoecetes bairdi</i> 1	Oregoniidae	Oregoniidae	AY227446	AB21159	—	TE		
<i>Chionoecetes bairdi</i>	GenBank sequence	<i>Chionoecetes bairdi</i> 2	Oregoniidae	Oregoniidae	AB188109	AB21158	—	TE		
<i>Chionoecetes japonicus</i>	GenBank sequence	<i>Chionoecetes japonicus</i> 1	Oregoniidae	Oregoniidae	AB188685	AB211611	—	TE		Species
<i>Chionoecetes japonicus</i>	GenBank sequence	<i>Chionoecetes japonicus</i> 2	Oregoniidae	Oregoniidae	AB188107	AB211160	—	TE		
<i>Chionoecetes opilio</i>	Northwest Atlantic (50° N, 66° W)	<i>Chionoecetes opilio</i> 1	Oregoniidae	Oregoniidae	EU682768	EU682832	EU682875	TE	Complete	Species
<i>Chionoecetes opilio</i>	Northwest Atlantic (50° N, 66° W)	<i>Chionoecetes opilio</i> 2	Oregoniidae	Oregoniidae	EU682769	—	EU682876	TE		
<i>Chionoecetes opilio</i>	Northwest Atlantic (50° N, 66° W)	<i>Chionoecetes opilio</i> 3	Oregoniidae	Oregoniidae	EU682770	EU682833	EU682877	TE	Complete	
<i>Chionoecetes opilio</i>	GenBank sequence	<i>Chionoecetes opilio</i> 4	Oregoniidae	Oregoniidae	AB188684	AB211154	—	TE		
<i>Chionoecetes opilio</i>	GenBank sequence	<i>Chionoecetes opilio</i> 5	Oregoniidae	Oregoniidae	AY227445	AB211153	—	TE		
<i>Hyas araneus</i>	Northwest Atlantic (50° N, 66° W)	<i>Hyas araneus</i> 1	Oregoniidae	Oregoniidae	EU682771	EU682834	EU682878	TE	Complete	Species
<i>Hyas araneus</i>	Northwest Atlantic (50° N, 66° W)	<i>Hyas araneus</i> 2	Oregoniidae	Oregoniidae	EU682772	—	EU682879	TE		
<i>Hyas araneus</i>	Northwest Atlantic (50° N, 66° W)	<i>Hyas araneus</i> 3	Oregoniidae	Oregoniidae	EU682773	—	—	TE		
<i>Hyas coarctatus</i>	Nahant, MA, USA	<i>Hyas coarctatus</i>	Oregoniidae	Oregoniidae	EU682774	EU682835	—	TE		Species
<i>Oregonia gracilis</i>	WA (16S, COI) and CA (28S), USA	<i>Oregonia gracilis</i> 1	Oregoniidae	Oregoniidae	EU682775	EU682836	EU682880	TE	Complete	Species
<i>Oregonia gracilis</i>	Friday Harbor, WA, USA	<i>Oregonia gracilis</i> 2	Oregoniidae	Oregoniidae	EU682776	—	—	TE		
<i>Metoporphaphis calcarata</i>	Gulf Specimen Marine Lab, USA	<i>Metoporphaphis calcarata</i>	Inachidae	Inachidae	EU682777	EU682830	EU682881	TE	Complete	Species
<i>Podochela hemphillii</i>	San Diego, CA, USA	<i>Podochela hemphillii</i>	Inachidae	Inachidae	EU682778	EU682831	EU682882	TE	Complete	Species
<i>Maja brachydactyla</i>	GenBank sequence	<i>Maja brachydactyla</i> 1	Majidae	Majidae	EU000850	EU000811	—	TE		
<i>Maja brachydactyla</i> ^a	GenBank sequence	<i>Maja brachydactyla</i> 2	Majidae	Majidae	DQ079723	—	DQ079799	TE		Species
<i>Maja crispata</i>	GenBank sequence	<i>Maja crispata</i>	Majidae	Majidae	EU000852	EU000836	—	TE		Species
<i>Maja squinado</i>	GenBank sequence	<i>Maja squinado</i>	Majidae	Majidae	EU000851	EU000832	—	TE		Species
<i>Micippa platipes</i>	Japan	<i>Micippa platipes</i>	Mithracidae	Majidae	EU682779	—	EU682884	TE		Species
<i>Micippa thalia</i>	Shimoda, Japan	<i>Micippa thalia</i>	Mithracidae	Majidae	EU682780	EU682844	EU682883	TE	Complete	Species
<i>Microphrys bicornutus</i>	Bocas del Toro, Panama	<i>Microphrys bicornutus</i>	Mithracidae	Majidae	EU682781	EU682843	EU682885	TE	Complete	Species
<i>Mithraculus forceps</i>	Bocas del Toro, Panama	<i>Mithraculus forceps</i>	Mithracidae	Majidae	EU682782	EU682840	EU682886	TE	Complete	Species
<i>Mithraculus sculptus</i>	Bocas del Toro, Panama	<i>Mithraculus sculptus</i> 1	Mithracidae	Majidae	EU682784	EU682841	EU682887	TE	Complete	Species
<i>Mithraculus sculptus</i>	Florida, USA	<i>Mithraculus sculptus</i> 2	Mithracidae	Majidae	EU682783	EU682842	—	TE		
<i>Mithraculus sculptus</i>	Bocas del Toro, Panama	<i>Mithraculus sculptus</i> 3	Mithracidae	Majidae	EU682785	—	EU682888	TE		
<i>Tiarinia cornigera</i>	Kochi, Japan	<i>Tiarinia cornigera</i> 1	Mithracidae	Majidae	EU682786	EU682837	EU682889	TE	Complete	Species
<i>Tiarinia cornigera</i>	Kochi, Japan	<i>Tiarinia cornigera</i> 2	Mithracidae	Majidae	EU682787	—	EU682890	TE		
<i>Tiarinia spinigera</i>	Kochi, Japan	<i>Tiarinia spinigera</i>	Mithracidae	Majidae	EU682788	EU682838	—	TE		Species
<i>Pitho lherminieri</i>	Bocas del Toro, Panama	<i>Pitho lherminieri</i>	Tychidae	Epialtidae	EU682789	EU682839	EU682891	TE	Complete	Species
<i>Acanthonyx petiverii</i>	Gulf Specimen Marine Lab, USA	<i>Acanthonyx petiverii</i> 1	Epialtidae	Epialtidae	EU682803	EU682855	EU682903	TE	Complete	Species
<i>Acanthonyx petiverii</i>	Gulf Specimen Marine Lab, USA	<i>Acanthonyx petiverii</i> 2	Epialtidae	Epialtidae	EU682802	EU682854	EU682902	TE	Complete	Species
<i>Menaethius monoceros</i>	Shimoda, Japan	<i>Menaethius monoceros</i> 1	Epialtidae	Epialtidae	EU682805	EU682857	EU682904	TE	Complete	Species
<i>Menaethius monoceros</i>	Shimoda, Japan	<i>Menaethius monoceros</i> 2	Epialtidae	Epialtidae	EU682804	EU682856	—	TE		
<i>Mimulus foliatus</i>	Bodega Bay, CA, USA	<i>Mimulus foliatus</i> 1	Epialtidae	Epialtidae	EU682808	EU682859	EU682905	TE		
<i>Mimulus foliatus</i>	Bodega Bay, CA, USA	<i>Mimulus foliatus</i> 2	Epialtidae	Epialtidae	EU682806	EU682858	—	TE		
<i>Mimulus foliatus</i>	Bodega Bay, CA, USA	<i>Mimulus foliatus</i> 3	Epialtidae	Epialtidae	EU682809	—	EU682906	TE	Complete	Species
<i>Mimulus foliatus</i>	Bodega Bay, CA, USA	<i>Mimulus foliatus</i> 4	Epialtidae	Epialtidae	EU682807	—	—	TE		
<i>Pugettia dalli</i>	Southern CA, USA	<i>Pugettia dalli</i> 1	Epialtidae	Epialtidae	EU682810	EU682860	EU682907	TE	Complete	Species
<i>Pugettia dalli</i>	Los Angeles, CA, USA	<i>Pugettia dalli</i> 2	Epialtidae	Epialtidae	EU682811	EU682861	—	TE		
<i>Pugettia gracilis</i>	Newport, OR, USA	<i>Pugettia gracilis</i> 1	Epialtidae	Epialtidae	EU682813	EU682863	EU682909	TE	Complete	Species
<i>Pugettia gracilis</i>	Humboldt, CA, USA	<i>Pugettia gracilis</i> 2	Epialtidae	Epialtidae	EU682812	EU682862	EU682908	TE	Complete	
<i>Pugettia gracilis</i>	Newport, OR, USA	<i>Pugettia gracilis</i> 3	Epialtidae	Epialtidae	EU682814	EU682864	—	TE		
<i>Pugettia minor</i>	Mie Prefecture, Japan	<i>Pugettia minor</i> 1	Epialtidae	Epialtidae	EU682815	—	EU682910	TE		Species
<i>Pugettia minor</i>	Mie Prefecture, Japan	<i>Pugettia minor</i> 2	Epialtidae	Epialtidae	EU682816	—	EU682911	TE		
<i>Pugettia producta</i>	Bodega Bay, CA, USA	<i>Pugettia producta</i> 1	Epialtidae	Epialtidae	EU682817	EU682865	EU682912	TE	Complete	Species
<i>Pugettia producta</i>	Bodega Bay, CA, USA	<i>Pugettia producta</i> 2	Epialtidae	Epialtidae	EU682818	—	EU682913	TE		
<i>Pugettia producta</i>	Bodega Bay, CA, USA	<i>Pugettia producta</i> 3	Epialtidae	Epialtidae	EU682819	—	EU682914	TE		
<i>Pugettia producta</i>	Bodega Bay, CA, USA	<i>Pugettia producta</i> 4	Epialtidae	Epialtidae	EU682820	—	EU682915	TE		
<i>Pugettia quadridens</i>	Okayama, Japan	<i>Pugettia quadridens</i> 1	Epialtidae	Epialtidae	EU682824	EU682869	EU682916	TE	Complete	Species

(continued on next page)

Table 1 (continued)

Taxon	Collection locality	Code	Family	Family (Ng et al., 2008)	Accession Nos		Taxa sets		Species
					16S	COI	28S	Total Evidence	
<i>Pugettia quadridens</i> 2	Shizugawa, Japan		Epiplatidae	Epiplatidae	EU682822	EU682867	—	TE	Species
<i>Pugettia quadridens</i> 3	Kochi, Japan		Epiplatidae	Epiplatidae	EU682823	EU682868	—	TE	Species
<i>Pugettia quadridens</i> 4	Shizugawa, Japan		Epiplatidae	Epiplatidae	EU682821	EU682866	—	TE	Species
<i>Pugettia richii</i> 1	Bodega Bay, CA, USA		Epiplatidae	Epiplatidae	EU682826	EU682871	EU682917	TE	Complete
<i>Pugettia richii</i> 2	Bodega Bay, CA, USA		Epiplatidae	Epiplatidae	EU682825	EU682870	—	TE	Complete
<i>Talipeus dentatus</i>	Chile		Epiplatidae	Epiplatidae	EU682827	EU682872	—	TE	Complete
<i>Talipeus nuttallii</i>	Los Angeles, CA, USA		Epiplatidae	Epiplatidae	EU682828	EU682873	EU682919	TE	Complete
<i>Herbstia condyliata</i>	Madiera, Portugal		Pisidae	Epiplatidae	EU682790	EU682845	—	TE	Species
<i>Herbstia parvifrons</i> 1	Catalina Island, CA, USA		Pisidae	Epiplatidae	EU682791	—	—	TE	Species
<i>Herbstia parvifrons</i> 2	Catalina Island, CA, USA		Pisidae	Epiplatidae	EU682792	—	—	TE	Species
<i>Libinia dubia</i>	Fisher Scientific, USA		Pisidae	Epiplatidae	EU682793	EU682847	EU682894	TE	Complete
<i>Libinia dubia</i> 1	Fisher Scientific, USA		Pisidae	Epiplatidae	EU682794	EU682846	EU682893	TE	Complete
<i>Libinia emarginata</i>	Fisher Scientific, USA		Pisidae	Epiplatidae	EU682796	EU682849	EU682896	TE	Complete
<i>Libinia emarginata</i> 1	Fisher Scientific, USA		Pisidae	Epiplatidae	EU682795	EU682848	EU682895	TE	Complete
<i>Libinia mexicana</i>	East Pacific		Pisidae	Epiplatidae	EU682797	—	—	TE	Species
<i>Loxorhynchus crispatus</i> 1	Bodega Bay, CA, USA		Pisidae	Epiplatidae	EU682798	EU682850	EU682898	TE	Complete
<i>Loxorhynchus crispatus</i> 2	Bodega Bay, CA, USA		Pisidae	Epiplatidae	EU682799	EU682851	EU682899	TE	Complete
<i>Scyra acutifrons</i> 1	Bodega Bay, CA, USA		Pisidae	Epiplatidae	EU682801	EU682852	EU682900	TE	Complete
<i>Scyra acutifrons</i> 2	Bodega Bay, CA, USA		Pisidae	Epiplatidae	EU682800	EU682853	EU682901	TE	Complete
<i>Heterocrypta occidentalis</i> ^b	Southern California, USA		Outgroup	Epiplatidae	EU682767	EU682829	EU682874	TE	Complete

Dashes “—” indicate a species was not sequenced for that locus.

^a Originally classified as *Maja squinado*, but subsequent taxonomic revisions indicate this is likely *Maja brachydactyla*.

^b Renamed *Latolabrus occidentalis* in Ng et al. (2008).

were excluded (36% of the total sequence). Although removal of ambiguously aligned regions has been shown to significantly improve tree topology in simulations (Talavera and Castresana, 2007), the choice of whether to include these areas (and other portions of the data, such as COI third positions) heavily influences tree topology and should ideally be made in a way that optimizes topological stability (Phillips et al., 2000). We thus examined the stability of the combined sequence alignments under a range of alignment inclusion (untrimmed vs. GBLOCKS-trimmed sequences) and COI third-position weighting (0, 0.5, 1) scenarios using character congruence as a criterion for optimality using the Incongruence Length metric (ILD) (Farris et al., 1995; Mikevich and Farris, 1981), where $ILD = (\text{Length}_{\text{combined}} - \sum \text{Length}_{\text{individual loci}}) / \text{Length}_{\text{combined}}$ (see also Ah Yong and O'Meally, 2004). We measured tree lengths of the combined dataset and datasets of individual loci under different alignment inclusion and COI weighting scenarios using heuristic maximum parsimony analyses in PAUP*, and chose the combination of parameters that minimized the incongruence between the three loci (i.e., had the lowest ILD score).

2.3. Tree construction

To examine the phylogenetic signal of individual loci relative to the combined dataset, we constructed trees using both individual-locus datasets and the combined dataset (16S, COI, and 28S). In this study, we focus on model-based approaches to phylogenetic inference, specifically Bayesian and maximum likelihood methods, because their ability to incorporate information about the model of evolution for a particular locus decreases statistical inconsistency (e.g., by correcting for multiple substitutions) (Bergsten, 2005; Huelsenbeck et al., 2002, 2001; Leache and Reeder, 2002). We used the program MrBayes v3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) to construct trees using single-locus and combined datasets, as MrBayes allows different partitions (i.e., loci) to evolve under different models of evolution. However, since Bayesian posterior probability support values (bpp) can often be inflated for certain clades relative to bootstrap values from maximum-likelihood (ML) and maximum parsimony methods (Erixon et al., 2003; Huelsenbeck et al., 2002; Leache and Reeder, 2002), we also constructed trees using each single-locus dataset using ML. For ML searches, we used the program PhyML (Guindon and Gascuel, 2003; Guindon et al., 2005) to construct 100 ML bootstrap replicates using the full complement of species sequenced for each individual locus. For these searches, we set certain evolutionary parameters calculated by Modeltest (substitution model, number of substitution rate categories) but allowed PhyML to estimate other parameters (rate matrix, base frequencies, gamma shape parameter, proportion invariable sites), and used default PhyML distance-based starting trees. For Bayesian analyses using single-locus datasets, we used information about the general model of evolution for each locus calculated by Modeltest to set general parameters (e.g., shape of rate distributions) but allowed MrBayes to estimate more specific model parameters (proportion invariable sites, base frequencies). For single-locus Bayesian analyses, we ran Markov Chain Monte Carlo (MCMC) searches with four chains for 1×10^6 – 3×10^6 generations, or until runs had converged to a stationary distribution (standard deviation of split frequencies ≤ 0.01). We sampled the chain every 100 generations, and discarded the first 25% of the samples (which generally corresponded to when likelihood values became stationary) as the burn-in. Although utilization of gaps as character data can be phylogenetically informative in parsimony-based analyses, there are few well-tested methods for implementing gap information into model-based approaches to phylogenetic inference (Simmons et al., 2007; Phillips et al., 2000), and we thus treated all gaps as missing data.

Although the majority of species were sequenced for all three loci (Table 1), because of logistical constraints (multiple failed sequencing attempts for some species–locus combinations), not all loci were sequenced for each species, and thus portions of our dataset were missing. Recent simulation studies (Wiens, 2005, 2006) suggest that adding incomplete taxa with >50% complete data may increase the accuracy of the final tree, as long as the overall number of sampled characters is high (>>200 characters). In preliminary analyses of our combined dataset, addition of additional incomplete taxa ($\geq 2/3$ loci sequenced) generally increased posterior probability values for clades, so we included all species for which we had at least 2/3 loci sequenced in the total evidence taxa set ($n = 68$ individuals/37 species). However, we additionally constructed a second taxa set in which we only included species for which we had all three loci sequenced ('complete character set', $n = 33$ individuals/26 species).

We ran Bayesian analyses using both taxa sets (total evidence and complete character set) with the combined dataset (16S, COI, and 28S) using MrBayes. We partitioned the combined dataset by locus, and used information about the general model of evolution (described above) to set parameters for each partition. MCMC searches were run for 1×10^6 – 2×10^6 generations (standard deviation of split frequencies ≤ 0.01 , sampling and burn-in conditions as described previously).

2.4. Bayesian hypothesis testing

To examine phylogenetic hypotheses based on morphological studies, we used Bayes factor analysis (Nylander et al., 2004; Ronquist and Huelsenbeck, 2003) to compare the posterior odds of unconstrained Bayesian tree topologies relative to Bayesian trees where we constrained the monophyly of majoid families or family groupings. We tested support for hypotheses based on both larval morphology (Clark and Webber, 1991; Marques and Pohle, 1998, 2003; Rice, 1983, 1988) and adult morphology, i.e., monophyly of traditional majoid families (Martin and Davis, 2001; McLaughlin et al., 2005). In addition, we also tested support for the most recent majoid taxonomic groupings suggested by Ng et al. (2008) (Table 1). We first trimmed the TE dataset to a single exemplar individual per species ("species" taxa set, see Table 1) to eliminate any biases resulting from unequal numbers of replicates per species, then implemented monophyly constraints in MrBayes using the *prset topologypr = constraint* command. We ran MCMC searches with four chains for 1×10^6 generations (standard deviation of split frequencies ≤ 0.01) and obtained the harmonic mean of tree likelihood values by sampling the post burn-in posterior distribution (*sump* command). We then calculated Bayes factors for each tree (B_{10}) using the difference between the marginal likelihood values of the unconstrained topology (representing H_1) and the monophyly-constrained topology (representing H_0) (following Nylander et al., 2004, Ronquist and Huelsenbeck, 2003). We used these Bayes factors to evaluate whether there was evidence against constrained trees (i.e., different hypotheses based on larval or adult morphology) using the test statistic $2 \log_e(B_{10})$ and the criteria described

by Kass and Raftery (1995). Under this criteria, a value for the test statistic $2 \log_e(B_{10})$ between 0 and 2 indicates no evidence against H_0 ; values from 2 to 6 indicate positive evidence against H_0 ; values from 6 to 10 indicate strong evidence against H_0 ; and values >10 indicate very strong evidence against H_0 (Kass and Raftery, 1995; Nylander et al., 2004).

3. Results

3.1. Alignment and sequence data

The lowest amount of incongruence among different loci (lowest ILD values) was achieved when alignments were trimmed by GBlocks and all positions were weighted equally (COI third position weights = 1; Table 2). Although there was some evidence for mutational saturation in COI third positions, there was little evidence for saturation in remaining loci (data not shown). The final aligned and trimmed dataset consisted of 1447 characters. We obtained 16S sequence data from 72 individuals representing 38 different species; the trimmed dataset consisted a total of 435 sites (216 parsimony-informative sites, CI = 0.402, RI = 0.766), and used best-fit models and parameters calculated by Modeltest (HKY + G, $N_{st} = 2$, rates = gamma). For COI, we obtained sequence data from 54 individuals representing 31 species, resulting in a total of 567 sites after trimming with GBlocks (234 parsimony-informative sites, CI = 0.290, RI = 0.621) and used best-fit Modeltest parameters (GTR + I + G; $N_{st} = 6$, rates = gamma). For 28S, we obtained sequence data from 47 individuals representing 31 species, for a total of 445 bp of data after trimming (119 parsimony-informative sites, CI = 0.631, RI = 0.763); best-fit models and parameters calculated by Modeltest are as follows (GTR + I + G, $N_{st} = 6$, rates = gamma).

3.2. Phylogenetic trees

Single-locus trees constructed using ML or Bayesian methods did not generally recover clades deeper than the level of genus (summarized in Table 3). The majority of ML and Bayesian trees constructed from a single-locus resolved a monophyletic Oregoniidae (bootstrap and bpp support = 94–100) and a monophyletic Inachidae (bootstrap and bpp support = 81–100). Bayesian 16S and 28S trees resolved a clade of Mithracidae + Tychidae + Epialtidae + Pisidae (excluding *Micippa*) (bootstrap and bpp support = 51–77). Mitochondrial (16S and COI) single-locus trees supported a clade of eastern Pacific epialtid species (*Pugettia producta*, *P. richii*, *P. dalli*, *P. gracilis*, and *Mimulus foliatus*; bootstrap and bpp support = 69–100). For single-locus trees, Bayesian support values were generally higher than ML bootstrap support, as has been noted in other studies (Erixon et al., 2003; Huelsenbeck et al., 2002; Leache and Reeder, 2002).

Trees constructed from the combined dataset (all three loci) using both taxa sets (total evidence and complete character set) were better resolved than single-locus trees (Figs. 2 and 3) and had higher bootstrap and bpp support values for major clades

Table 2

Tree lengths and ILD values for single-loci and combined analyses under different alignment-inclusion (untrimmed versus GBlocks-trimmed alignments) and COI third-position weighting (1, 0.5, 0)

Alignment inclusion	COI 3rd position weighting	16S	COI	28S	Combined	ILD
GBlocks alignment	1	1128	1553	469	3238	0.027177
GBlocks alignment	0.5	1128	927.5	469	2596.5	0.027730
Untrimmed alignment	1	1234	1721	838	3902	0.027934
Untrimmed alignment	0.5	1234	1048.5	838	3215	0.029393
Untrimmed alignment	0	1234	362	838	2518	0.033360
GBlocks alignment	0	1128	280	469	1949	0.036942

Values for analysis with the lowest ILD value in bold.

Table 3
 Bootstrap (ML = maximum likelihood) and posterior-probability (Bayesian) values for selected clades resolved by single-locus and combined dataset trees using different methods

Method	Dataset	Taxa set	Bootstrap or posterior probability support for clade			
			Oregoniidae	Inachidae	Mith + Pi + Ep + Ty	East Pacific <i>Pugettia</i>
ML	16S	16S	99	99	—	96
ML	COI	COI	94	—	—	70
ML	28S	28S	—	81	—	—
Bayesian	16S	16S	100	100	77	100
Bayesian	COI	COI	—	92	—	69
Bayesian	28S	28S	—	100	51	—
Bayesian	Combined	Total evidence	100	100	99	100
Bayesian	Combined	Complete character	100	100	68	100

Dashes “—” indicate posterior probability or bootstrap support values <50. Values in bold indicate combined analyses. “Inachidae” = *Metoporphaphis* + *Podochela*; “Mith + Pi + Ep + Ty” = Mithracidae + Epialtidae + Pisidae + Tychidae (without *Micippa*), “East Pacific *Pugettia*” = *Pugettia producta*, *P. richii*, *P. dalli*, *P. gracilis* and *Mimulus foliatus*.

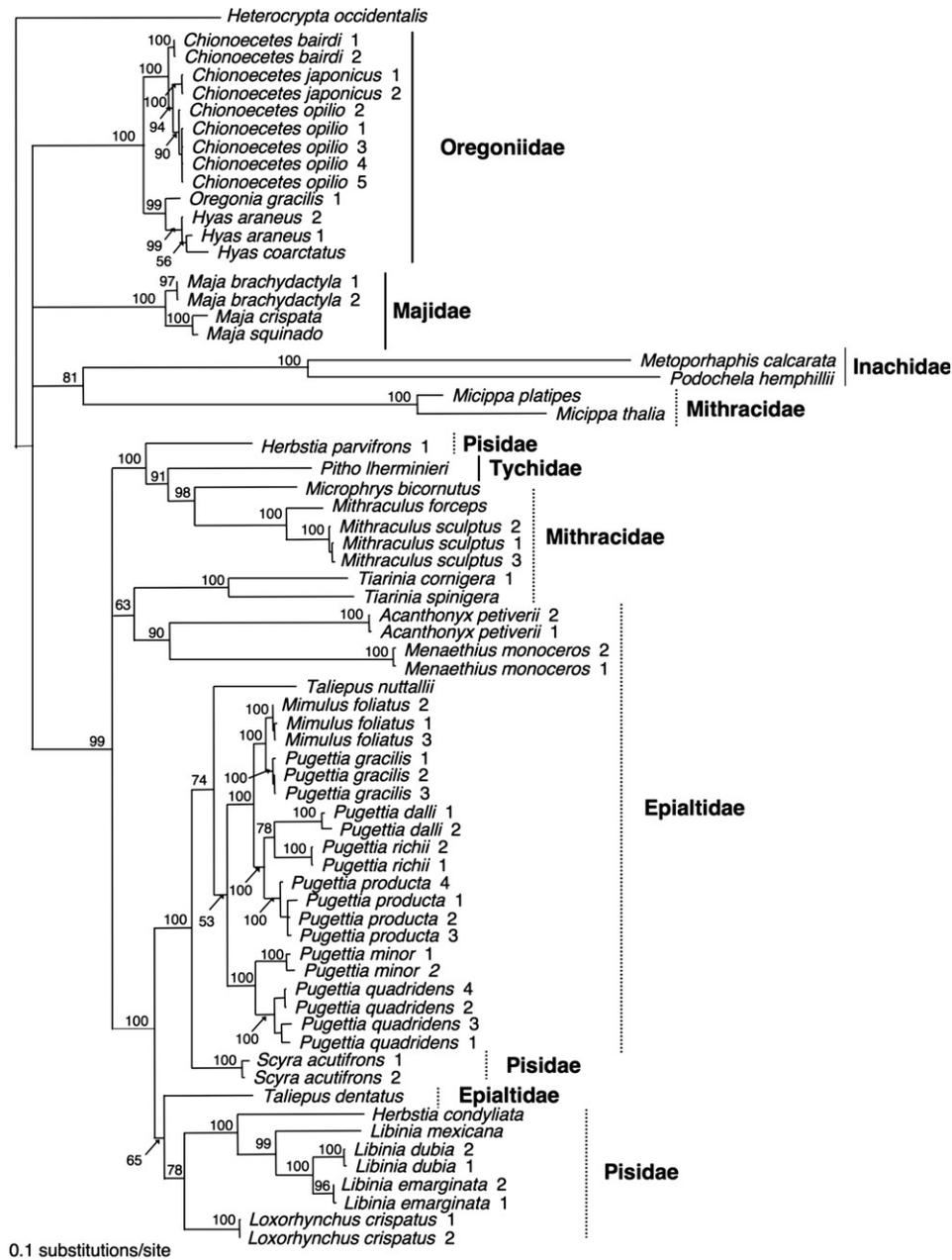


Fig. 2. Phylogenetic tree of the Majoidea, shown as a Bayesian consensus tree based on the combined-locus dataset, using the total evidence taxa set. Numbers above each node indicate Bayesian posterior probability values; posterior probability values below the level of species are omitted. Names in bold indicate family affiliations; dashed lines indicate the family is paraphyletic, solid lines indicate the family is monophyletic. *Heterocrypta occidentalis* is the outgroup.

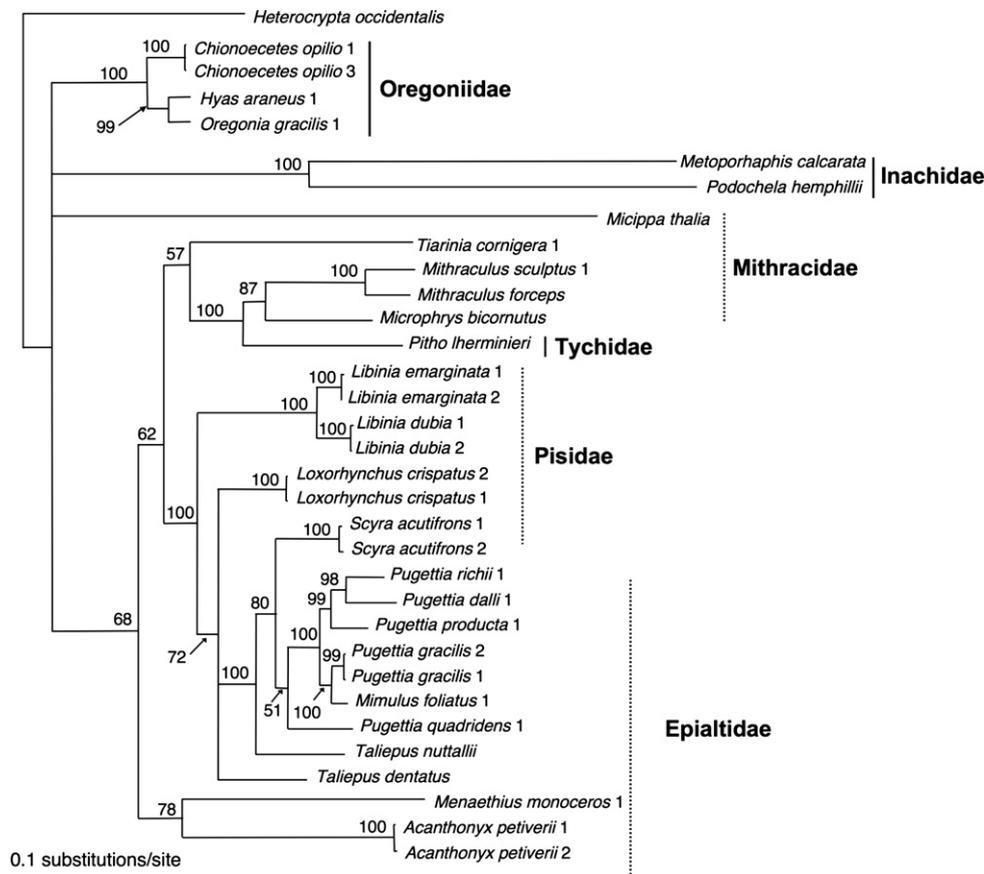


Fig. 3. Phylogenetic tree of the Majoidea, shown as a Bayesian consensus tree based on the combined-locus dataset, using taxa for which all three loci were sequenced (complete character taxa set). Family affiliations, Bayesian posterior probabilities, and outgroup as in Fig. 2.

(Table 3). Most of the major phylogenetic relationships recovered from unconstrained topologies supported hypotheses based on larval morphology. The total evidence tree (Fig. 2) and complete character set tree (Fig. 3) resolved the majoids into several major clades forming a polytomy at the base of the tree. Both Bayesian trees constructed with the combined dataset supported a monophyletic Oregoniidae (bpp support = 100), and a monophyletic Inachidae (bpp support = 100). The total evidence tree resolved a monophyletic Majidae clade (the genus *Maja*, bpp = 100). Species from the Mithracidae, Tychidae, Epialtidae, and Pisidae families (with the exception of mithracid species in the genus *Micippa*) formed a clade in trees constructed with both the complete character set (bpp = 68) and the total evidence taxa set (bpp = 99) trees (Figs. 2 and 3). *Micippa* formed a clade with the inachids *Metoporphaphis* + *Podocheila* in the total evidence tree (bpp = 81, Fig. 2), but *Micippa* did not group with the inachids in the complete character set tree (Fig. 3). The Pisidae, Epialtidae, and Mithracidae families were individually paraphyletic in both the total evidence and complete character set trees (Figs. 2 and 3).

3.3. Bayesian hypothesis testing

Bayes factor testing and/or posterior probability support for certain clades indicated much stronger evidence against hypotheses (i.e., constrained topologies) based on adult morphology rather than larval morphology (summarized in Table 4). The only monophyletic families recovered in unconstrained analyses—Oregoniidae, Inachidae, and Majidae—were families that were also predicted to be monophyletic using larval morphology (i.e., representing hypotheses supported by larval and adult morphol-

ogy; Table 4). Evidence against alternate topologies predicted from larval morphology was generally weaker ($2 \log_e(B_{10}) \leq 12.7$) than topologies based on adult morphology ($2 \log_e(B_{10}) \gg 10$, range 47–163) (Table 4). For example, there was no evidence against an alternate topology constraining the Oregoniidae family to branch basal to the remaining majoids ($2 \log_e(B_{10}) = 1.44$), as predicted by larval morphology. Although there was strong support against a strict Mithracidae-Epialtidae-Pisidae-Tychidae clade ($2 \log_e(B_{10}) = 12.7$), these groups formed a well-supported monophyletic group in unconstrained trees when the mithracid genus *Micippa* was excluded, (Table 4, Figs. 2 and 3). Conversely, Bayes factor testing indicated much stronger evidence against all topologies predicted only from adult morphology, such as strict monophyly of the Epialtidae, Pisidae, and Mithracidae families ($2 \log_e(B_{10}) \geq 57.72$; summarized in Table 4). There was also very strong support ($2 \log_e(B_{10}) \geq 47$) against monophyly of the most recent familial classifications erected by Ng et al. (2008), with the exception of the Oregoniidae and the Inachidae families supported in unconstrained analyses.

4. Discussion

This study, representing the first molecular investigation of systematic relationships within the Majoidea superfamily and encompassing 37 majoid species across 20 different genera, illustrates several important results. First, single-locus trees were generally poorly resolved, and only combined-locus trees provided enough resolution to consistently infer relationships deeper than the level of genus (Table 3, Figs. 2 and 3). Second, despite the utility of the combined dataset in recovering deeper clades, increased taxon

Table 4
Bayes factor testing of phylogenetic hypotheses based on morphological studies, ranked by the degree of evidence against the hypothesis

Morphology-based hypotheses (H_0)	Morphological character set	Bayesian posterior probability support for hypothesis	$2 \log_e(B_{10})$	Evidence against H_0
Monophyletic Oregoniidae	Larval and adult	100	n/a	None (H_0 supported)
Monophyletic Majidae	Larval and adult	100	n/a	None (H_0 supported)
Monophyletic Inachidae	Larval and adult	100	n/a	None (H_0 supported)
Oregoniidae branches basal	Larval	—	1.44	Minimal (H_0 supported)
Mithracidae–Tychidae	Larval	—	12.70	Very strong
Pisidae–Epialtidae				
Family Epialtidae (Ng et al., 2008)	Adult	—	47.00	Very strong
Monophyletic Mithracidae	Adult	—	57.72	Very strong
Monophyletic Epialtidae	Adult	—	74.80	Very strong
Monophyletic Pisidae	Adult	—	76.76	Very strong
All families monophyletic	Adult	—	151.96	Very strong
Family Majidae (Ng et al., 2008)	Adult	—	152.32	Very strong
All families monophyletic (Ng et al., 2008)	Adult	—	163.82	Very strong

Higher values of $2 \log_e(B_{10})$ indicate stronger support against the morphology-based hypothesis (H_0). Dashes “—” indicate hypothesis was not supported in unconstrained trees; “n/a” indicates that Bayes factor testing was not done (i.e., the hypothesis was supported in unconstrained trees). Evidence against individual hypotheses assessed using the criteria of Kass and Raftery (1995).

sampling and/or utilization of additional characters is necessary to adequately resolve branching relationships at the base of the majoid tree. Nevertheless, molecular topologies were generally more congruent with phylogenetic hypotheses based on larval morphology and provided little support for phylogenetic hypotheses implied from adult morphology, i.e., monophyletic families. Several lines of evidence, which we discuss in more detail below, suggest this lack of familial monophyly is because adult morphology within the majoids may be subject to widespread convergent evolution as a result of the radiation of different lineages into similar ecological habitats and niches.

Our study underscores the importance of both multiple-locus studies and adequate taxon sampling in decapod systematics. In general, only combined-locus datasets provided enough information to resolve most clades deeper than the level of genus with >50% Bayesian posterior probability support. However, despite the utility of the combined dataset in recovering clades unresolved by single-locus analyses, there was poor support for internal nodes near the base of the tree. Widespread divergence in portions of 28S and difficulties with alignment of these regions, especially within the Inachidae, may have contributed to this instability. For example, grouping of the mithracid genus *Micippa* and an inachid clade (*Metoporphaphis* + *Podochela*) could have been influenced by long branch attraction, possibly affecting to unstable placement of both clades in the total evidence and complete character set trees (Figs 2 and 3). Long branches can be problematic even in model-based analyses (Bergsten, 2005; Sanderson and Shaffer, 2002; Wiens, 2005, 2006), and the exact position of the Inachidae will be difficult to determine without utilization of additional characters and additional taxon sampling in the Inachidae and Inachoididae.

Closely related species living in the same regions often formed strongly supported monophyletic clades (e.g., the east Pacific *Pugettia* clade), and in at least two cases, species that branched outside of their genus were from different geographic regions than their congeners on the tree. For example, *Herbstia parvifrons* (from the US west coast) and *H. condylaita* (from Madeira) did not form a monophyletic clade in combined-data or single-locus analyses, and sampling additional populations or congeners (*H. camptacantha*, *H. tumida*, *H. pyriformis*: Hendrickx (1995)) from under-represented geographic regions in our study might help in more accurate placement of these species. Similarly, the two species of *Taliepus* in our study (*T. dentatus* from Chile and *T. nuttallii* from the US west coast) never formed a monophyletic clade regardless of the method of tree construction, despite strikingly similar adult morphologies and ecologies (Garth, 1958; Rathbun, 1925). Sampling additional

populations of these species may help determine if their disparate locations on the tree reflect radiations of different lineages into ecologically similar, but geographically distinct environments: namely temperate kelp forests of southern and northern hemisphere, respectively.

Many workers have noted convergent evolution of adult morphology in majoids (Gore et al., 1982; Marques and Pohle, 2003; Rice, 1988), and independent sources of data such as larval morphological characters could also aid in corroborating the placement of taxa that consistently branch outside of their family. For example, in our molecular trees the mithracid genus *Micippa* was only distantly related to the rest of the Mithracidae, branching near the base of the majoid tree (Figs. 2 and 3). In this case, larval morphology studies (Gore et al., 1982; Marques et al., 2003) also find that *Micippa* zoeae are morphologically distinct from the rest of the Mithracidae. These two independent lines of evidence suggest the systematic position of *Micippa* may need to be revised.

In a few cases our study supported earlier morphology-based studies of adults that questioned the placement or existence, of certain genera. For example, while our study only included one species from the Tychidae family (*Pitho lherminieri*), it consistently grouped with a clade of mithracid genera (*Mithraculus* + *Microphrys*), and early taxonomic studies also placed *Pitho* in the same subfamily as *Mithraculus* and *Microphrys* (i.e., the Mithracidae; Rathbun, 1925). Additional taxon sampling of the Tychidae is needed to resolve whether the family is monophyletic and if it is phylogenetically distinct from the Mithracidae. In another case, the most taxonomically well-sampled group in our tree—the *Pugettia* genus—was consistently paraphyletic, in part because the monotypic epialtid species *Mimulus foliatus* (from the east Pacific) was always nested within a clade of other east Pacific *Pugettia* species (*P. producta*, *P. richii*, *P. dalli*, and *P. gracilis*). This placement concurs with the assessment of one early majoid systematist (Rathbun, 1894) who stated “there seems to be no good reason for placing this [*Mimulus*] in a genus distinct from *Pugettia*.” *Mimulus* is also morphologically and ecologically similar to other *Pugettia* species (Hultgren and Stachowicz, 2008), and these data suggest *Mimulus* should be reclassified as a member of the *Pugettia* genus.

Molecular trees constructed in our study support many phylogenetic hypotheses of relationships among majoid families based on larval morphology (Table 4). Combined dataset trees provided support for a clade containing the Mithracidae, Tychidae, Pisidae, and Epialtidae (with the exception of the genus *Micippa*), consistent with some larval morphology-based topologies (Marques and Pohle, 2003). Data from all three single-locus trees and both

combined dataset trees support monophyletic Oregoniidae, Inachidae, and Majidae families, in concurrence with data based on larval morphology (Marques and Pohle, 1998; Rice, 1980, 1983). Bayes factor testing also indicated some support for the Oregoniidae as the most basally branching group of majoids. However, utilization of additional characters, especially more slowly evolving nuclear loci, is necessary to adequately resolve deep internal nodes at the base of the Majoidea to determine which of three groupings—the Inachidae, Oregoniidae, or Majidae—represent the most basally-branching majoid family.

As suggested above, support for relationships predicted from adult morphology (e.g., monophyly of individual families) was variable. Although we found evidence for the monophyly of three families (Oregoniidae, Majidae, and Inachidae), these families have also been found to be monophyletic in studies based on larval morphology. However, we have adequate taxon sampling only for the Oregoniidae family (three out of four oregoniid genera sampled); we sampled only 2 inachid genera (<10% of recognized inachid genera) and only one genus within the Majidae (*Maja*) in this study. Thus, we cannot comment definitively on the monophyly of the Inachidae and Majidae families. Conversely, there was little support for monophyly of additional families represented by multiple genera in our study (Mithracidae, Epialtidae, and Pisidae). In particular, in both our study and previous larval morphology studies, species from the Pisidae were consistently paraphyletic, and constraining the Pisidae family to be monophyletic resulted in strong support *against* monophyly (in this study) or significantly longer tree lengths (Marques and Pohle, 2003). Paraphyly of the Pisidae family may be due in part to the close, and consequently difficult to distinguish, phylogenetic relationships among the epialtid and pisid species sampled in our study. Many of these species are denizens of giant kelp forests (*Loxorhynchus crispatus*, *Scyra acutifrons*, *Taliepus nuttallii*, *Pugettia* spp., and *Mimulus foliatus*) and may have undergone rapid kelp-associated diversification, as has been proposed for several other kelp-associated taxa (Jacobs et al., 2004). Diversification of this lineage into distinct kelp forest microhabitats may have selected for the widely divergent adult morphologies that forms the basis for the classification of these species into distinct families, despite evidence from this study for recent shared phylogenetic history.

As our study is necessarily based on an incomplete sampling of the superfamily Majoidea, the topologies presented here should be regarded as hypotheses to be tested in future studies with additional taxa and/or additional sources of molecular and morphological data. In particular, directed taxon sampling is necessary to more definitively resolve the grouping and monophyly of groupings branching near the base of the tree (Inachidae, Majidae, and Oregoniidae) and relationships within the Mithracidae–Pisidae–Epialtidae clade. Inclusion of characters based on larval morphology (Marques and Pohle, 2003; Pohle and Marques, 2000) and/or additional nuclear loci could help resolve taxa that are particularly difficult to place due to long-branch attraction and strengthen support for relationships between and among majoid families. Well-resolved phylogenies of the Majoidea can serve as important tools for evolutionary studies investigating what factors may drive quantitative variation in and among species in “decoration” behavior, the well-described habit by which many majoids decorate their carapace to avoid detection (Berke et al., 2006; Hultgren and Stachowicz, 2008; Wicksten, 1993). Strong links between decoration behavior and morphology (hooked setae; Wicksten, 1993), broad diversification into a wide variety of marine habitats (Hines, 1982; Rathbun, 1925; Williams, 1984), and the demonstrated adaptive anti-predatory consequences of decoration (Hultgren and Stachowicz, 2008; Stachowicz and Hay, 1999; Thanh et al., 2003) make the superfamily Majoidea an ideal group for comparative studies examining the evolution of anti-predatory behaviors in decapod crustaceans.

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