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Decapod Crustacean Phylogenetics

edited by

Joel W. Martin, Keith A. Crandall, and Darryl L. Felder

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Edited by

Joel W. Martin
Natural History Museum of L. A. County
Los Angeles, California, U. S. A.

Keith A. Crandall
Brigham Young University
Provo, Utah, U. S. A.

Darryl L. Felder
University of Louisiana
Lafayette, Louisiana, U. S. A.

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Contents

Molecular Phylogeny of the Family Callianassidae Based on Preliminary Analyses of Two Mitochondrial Genes

DARRYL L. FELDER & RAFAEL ROBLES

Department of Biology, University of Louisiana, Lafayette, Louisiana, U.S.A.

ABSTRACT

Recent revisions in callianassid subfamilies and genera are questionable and appear to be incongruous with relationships evident in morphologically based phylogenetic reconstructions. We generated molecular phylogenetic trees for the closely related families Callianassidae and Ctenochelidae as well as for outgroup representatives of the family Axiidae. Fragments of the 16S and 12S rDNA mitochondrial genes were sequenced for a total of 46 species, representing 18 genera of Callianassidae, two genera of Ctenochelidae, and five genera of Axiidae. Of approximately 1000 potential mitochondrial basepair characters, 903 were used in final alignments. Resolution in our phylogenetic tree was limited at some basal nodes of the topology, as might be expected with the genes chosen for this analysis. Callianassinae formed a well-supported monophyletic group, but Cheraminae was included within it. Support was found for continued recognition of many separate genera in this group and for the naming of additional ones, as opposed to their wholesale reassignment to the clearly separated genus *Callianassa*. Groupings within Callichirinae were not well resolved, though the subfamily appears to be paraphyletic at low support values. Genera of this group were monophyletic except for *Sergio*, which is paraphyletic and of questioned validity. Eucalliacinae appears to be paraphyletic at low to medium support, suggesting that the genus *Calliaxina* may share common lineage with the Ctenochelidae.

1 INTRODUCTION

Recent attempts by Sakai (1999a, b, 2002, 2004, 2005) to comprehensively review and revise systematics of the family Callianassidae and its closest relatives (collectively known as ghost shrimps) have brought together a diffuse taxonomic literature but do not offer objective assessments toward a natural classification. Sakai's major revisions at the level of subfamilies and genera remain questionable (Dworschak 2007), especially in that many appear to be incongruous with relationships evident in phylogenetic reconstructions based upon morphological character analysis (Poore 1994; Tudge et al. 2000). This applies to numerous cases in which previously erected genera of the subfamily Callianassinae were recently synonymized by Sakai (2005), who put them into a very broadly defined genus *Callianassa* Leach, 1814. This action dismissed a restricted definition of the genus previously made by Manning & Felder (1991), while imposing a retrograde taxonomy that potentially masked diversity within the group. Similarly, within the subfamily Callichirinae, Sakai synonymized *Corallianassa* Manning, 1987, with *Glypturus* Stimpson, 1866, on a questionable basis (Dworschak 2007). In the subfamily Eucalliacinae, both *Eucalliax* Manning & Felder, 1991, and *Calliaxina* Ngoc-Ho, 2003, were placed into questionable synonymy with *Calliax* de Saint Laurent, 1973. At a somewhat higher level, membership of the family Ctenochelidae was restricted (Sakai 1999a), and the family Gourretiidae was established to receive *Gourretia* de Saint Laurent, 1973, and *Dawsonius* Manning & Felder, 1991.

The present effort addresses some of the above issues by molecular genetic methods. A previous paper of this volume (Robles et al.) used a combination of 16S mitochondrial and 18S nuclear gene sequences to examine overall phylogenetics of thalassinidean taxa, and a review of previous analyses bearing on all of its member groups was undertaken there. Some of the callianassid taxa that appear in Robles et al. (this volume) are included in the present work, as are yet others treated in earlier brief reports (Felder & Robles 2004; Robles & Felder 2004). With these, we here incorporate additional taxa to potentially enable more robust interpretations at the generic and subfamily levels. Our combined analysis is based strictly upon 16S and 12S mitochondrial gene sequences, rather than on genes more suited to resolution at higher taxonomic levels. The present analysis is considered preliminary in that it is somewhat biased to American materials, along with some available western Pacific and European specimens, the latter including *Callianassa subterranea*, type species of that genus.

2 METHODS

2.1 *Specimens included*

Ghost shrimps were collected in Belize, Brazil, Colombia, Costa Rica, Ghana, Greece, Jamaica, Japan, Mexico, Nicaragua, Panama, Scotland, Spain, USA, and Venezuela, with some of these being obtained as gifts or loans from museums (Table 1). When possible, specimens were initially frozen in seawater or glycerine at −70°C or −20°C. In other cases, or after tissue was extracted for DNA analysis, they were placed directly into 70% ethyl alcohol. Our sample consisted of 74 specimens representing 46 species in 25 genera of the families Ctenochelidae, Callianassidae, and Axiidae, the latter family serving as the outgroup. Outgroup selection was based upon findings of Robles et al. (this volume), which placed Axiidae in a sister clade to that of the aforementioned families within the infraorder Axiidea. Where utilized following a taxon, *s.l.* = *sensu lato* and *s.s.* = *sensu stricto*.

2.2 *DNA extraction, PCR, and sequencing*

DNA was extracted from muscle tissues excised from the abdomen or pleopods following standard protocols (Robles et al. 2007). Standard PCR amplification and automated sequencing protocols were used to sequence a fragment of approximately 550 bp (basepairs) of the 16S and 450 bp of the 12S rDNA mitochondrial genes. Both strands were sequenced. Primers used for PCR were 16ar (5-CGC CTG TTT ATC AAA AAC AT-3), 16br (5-CCG GTC TGA ACT CAG ATC ACG T-3) (Palumbi et al. 1991), 1472 (5-AGA TAG AAA CCA ACC TGG-3) (Crandall & Fitzpatrick 1996), and 16L2 (5-TGC CTG TTT ATC AAA AAC AT-3) (Schubart et al. 2002). Primers used for the 12S fragment were 12Sai (5'-AAA CTA GCA TTA GAT ACC CCT ATT AT-3') (Palumbi et al. 1991) and 12H2 (5'-ATG CAC TTT CCA GTA CAT CTA C-3') (Colbourne & Hebert 1996).

2.3 *Phylogenetic analyses*

Consensus of complementary sequences was obtained with the Sequencher software program (ver. 4.7, Genecodes, Ann Arbor, MI). Multiple sequence alignment was conducted with the aid of BioEdit v.7.08.0 (Hall 1999) at the following settings: 6-2/6-2 penalty (opening-gap extension, pairwise/multiple alignment respectively). Saturated parts of the alignment were removed with the web-accessible program Gblocks v. 0.91b (Castresana 2000, Talavera & Castresana 2007). Base composition, pattern of substitution for pair-wise comparison, and analysis of variability along both fragments of the 16S and 12S mtDNA were performed as implemented in PAUP 4.0 beta 10 (Swofford 1998). Homogeneity of nucleotide frequency among taxa was also assessed for each gene

Table 1. List of specimens used for molecular analysis. Letter abbreviations following species names refer to collection sites; these are sometimes sequentially numbered to indicate specimens identified as the same species. Catalog numbers refer to the following collections: CNCR = Colección Nacional de Crustáceos, UNAM; ULLZ = University of Louisiana at Lafayette, Zoological Collection. Asterisk (*) indicates sequences also used in Robles et al. (this volume). If a second catalog number is reported for a sample, tissue was donated to the University of Louisiana at Lafayette and a ULLZ catalog number was assigned to it, while the second number belongs to the original voucher that remains in the indicated museum. GenBank accession number (Acc. No.) is listed for each gene.

Family **Taxon Name**	**Collection Site**	**Catalog No.**	**Acc. No. (16S)**	**Acc. No. (12S)**
Outgroup				
Axiidae				
Axiopsis serratifrons (A. Milne-Edwards, 1873) BEL	Caribbean, Belize	ULLZ-5827	EU882909	EU875019
Axiopsis sp. PCR	Pacific, Costa Rica	ULLZ 7750	EU874920*	EU875012
Calaxius sp. GMX	Gulf of Mexico, Mexico	ULLZ 7041	EU874910*	EU875007
Calocaris caribbaeus Kensley, 1996 GMX-1	Gulf of Mexico, Louisiana, USA	ULLZ 7877	EU882902	EU875014
Calocaris caribbaeus Kensley, 1996 GMX-2	Gulf of Mexico, Louisiana, USA	ULLZ 8285	EU874929*	EU875016
Coralaxius nodulosus (Meinert, 1877) GMX	Gulf of Mexico, Mexico	ULLZ 7329	EU874913*	EU875010
Paraxiopsis sp. GMX	Gulf of Mexico, Mexico	ULLZ 7559	EU874917*	EU875011
Ingroup				
Callianassidae				
Callianassinae				
Biffarius biformis (Biffar, 1971) AFL	Atlantic, Florida, USA	ULLZ 6540	EU882910	EU875020
Biffarius fragilis (Biffar, 1970) AFL	Atlantic, Florida, USA	ULLZ 6406	EU882911	EU875021
Biffarius fragilis (Biffar, 1970) CMX-1	Caribbean, Mexico	CNCR 8997	EU882906	EU875017
Biffarius fragilis (Biffar, 1970) CMX-2	Caribbean, Mexico	CNCR 8997	EU882907	EU875018
Biffarius fragilis (Biffar, 1970) JAM	Jamaica	ULLZ 6532	EU882912	EU875022
Callianassa? sp. GMX-1	Gulf of Mexico, Louisiana, USA	ULLZ 8279	EU882903	EU875015
Callianassa? sp. GMX-2	Gulf of Mexico, Louisiana, USA	ULLZ 6058	EU882915	EU875025
Callianassa subterranea (Montagu, 1808) SCO	Atlantic, Scotland	ULLZ 6368	EU882924	EU875034
Gilvossius setimanus (De Kay, 1844) GFL-1	Gulf of Mexico, Florida, USA	ULLZ 4500	EU882934	EU875044
Gilvossius setimanus (De Kay, 1844) GFL-2	Gulf of Mexico, Florida, USA	ULLZ 4500	EU882935	EU875045
Gilvossius setimanus (De Kay, 1844) GFL-3	Gulf of Mexico, Florida, USA	ULLZ 4500	EU882936	EU875046

Table 1. continued.

Family Taxon Name	Collection Site	Catalog No.	Acc. No. (16S)	Acc. No. (12S)
Neotrypaea? sp. JAP	Pacific, Hydrocarbon vents, Japan	ULLZ 9414	EU882908	EU875050
Neotrypaea californiensis (Dana, 1854) USA	Pacific, Washington, USA	ULLZ 6405	EU882947	EU875058
Neotrypaea gigas (Dana, 1852) PMX-1	Pacific, Baja California, Mexico	ULLZ 4121	EU882948	EU875059
Neotrypaea gigas (Dana, 1852) PMX-2	Pacific, Baja California, Mexico	ULLZ 4121	EU882949	EU875060
Neotrypaea gigas (Dana, 1852) PMX-3	Pacific, Baja California, Mexico	ULLZ 4121	EU882950	EU875061
Neotrypaea gigas (Dana, 1852) PMX-4	Pacific, Baja California, Mexico	ULLZ 5176	EU882943	EU875054
Neotrypaea gigas (Dana, 1852) PMX-5	Pacific, Baja California, Mexico	ULLZ 5176	EU882944	EU875055
Neotrypaea gigas (Dana, 1852) PMX-6	Pacific, Baja California, Mexico	ULLZ 5176	EU882945	EU875056
Nihonotrypaea harmandi (Bouvier, 1901) JAP	Pacific, Japan	ULLZ 5468	EU882952	EU875063
Nihonotrypaea japonica (Ortmann, 1891) JAP	Pacific, Japan	ULLZ 5470	EU882953	EU875064
Paratrypaea? sp. HWI	Pacific, Hawaii, USA	ULLZ 7080	EU882919	EU875029
Paratrypaea bouvieri (Nobili, 1904) JAP-1	Pacific, Japan	ULLZ 6367	EU882913	EU875023
Paratrypaea bouvieri (Nobili, 1904) JAP-2	Pacific, Japan	ULLZ 6367	EU882914	EU875024
Pestarella tyrrhena (Petagna, 1792) SPN	Mediterranean, Spain	ULLZ 6366	EU882965	EU875078
Pestarella tyrrhena (Petagna, 1792) GRE-1	Mediterranean, Greece	ULLZ 6360	EU882899	EU875005
Pestarella tyrrhena (Petagna, 1792) GRE-2	Mediterranean, Greece	ULLZ 6360	EU882900	EU875006
Callichirinae				
Callichirus major (Say, 1818) BRA-1	Atlantic, São Paulo, Brazil	ULLZ 6055	EU882917	EU875027
Callichirus major (Say, 1818) BRA-2	Atlantic, São Paulo, Brazil	ULLZ 6056	EU882918	EU875028
Callichirus islagrande (Schmitt, 1935) GMX	Gulf of Mexico, Mississippi, USA	ULLZ 6052	EU882916	EU875026
Callichirus seilacheri (Bott, 1955) PNI	Pacific, Nicaragua	ULLZ 6053	EU882921	EU875031
Callichirus seilacheri (Bott, 1955) PMX	Pacific, Baja California, Mexico	ULLZ 6054	EU882920	EU875030
Callichirus sp. PMX	Pacific, Baja California, Mexico	ULLZ 4163	EU882922	EU875032
Corallianassa sp. JAM	Caribbean, Jamaica	ULLZ 6530	EU882923	EU875033
Glypturus acanthochirus Stimpson, 1866 VEN	Caribbean, Isla Margarita, Venezuela	ULLZ 5642	EU882928	EU875038

Table 1. continued.

Family Taxon Name	Collection Site	Catalog No.	Acc. No. (16S)	Acc. No. (12S)
Glypturus acanthochirus Stimpson, 1866 JAM	Caribbean, Montego Bay, Jamaica	ULLZ 6528	EU882929	EU875039
Glypturus acanthochirus Stimpson, 1866 CPA	Caribbean, Panama	ULLZ 6488	EU882930	EU875040
Glypturus sp. GMX-1	Gulf of Mexico, Louisiana, USA	ULLZ 4659	EU882932	EU875042
Glypturus sp. GMX-2	Gulf of Mexico, Louisiana, USA	ULLZ 4659	EU882933	EU875043
Grynaminna tamakii Poore, 2000 JAP-1	Pacific, Japan	ULLZ 5474	EU882937	EU875047
Grynaminna tamakii Poore, 2000 JAP-2	Pacific, Japan	ULLZ 5475	EU882938	EU875048
Grynaminna tamakii Poore, 2000 JAP-3	Pacific, Japan	ULLZ 5476	EU882939	EU875049
Lepidophthalmus jamaicense (Schmitt, 1935) JAM	Caribbean, Jamaica	ULLZ 5189	EU882941	EU875052
Lepidophthalmus louisianensis (Schmitt, 1935) USA	Gulf of Mexico, Louisiana, USA	ULLZ 5617	EU882940	EU875051
Lepidophthalmus turneranus (White, 1861) GHA	Atlantic, Ghana, Africa	ULLZ 4737	EU882942	EU875053
Neocallichirus cacahuate Felder & Manning, 1995 GFL	Atlantic, Florida, USA	ULLZ 3552 USNM 374706	EU882946	EU875057
Neocallichirus grandimana (Gibbes, 1850) AFL	Atlantic, Florida, USA	ULLZ 6491	EU882951	EU875062
Neocallichirus maryae (Schmitt, 1935) AFL	Atlantic, Florida, USA	ULLZ 6492	EU882954	EU875065
Neocallichirus variabilis (Edmondson, 1944) USA-1	Pacific, Hawaii, USA	ULLZ 6043	EU882955	EU875066
Neocallichirus variabilis (Edmondson, 1944) USA-2	Pacific, Hawaii, USA	ULLZ 6039	EU882957	EU875068
Neocallichirus variabilis (Edmondson, 1944) USA-3	Pacific, Hawaii, USA	ULLZ 6045	EU882956	EU875067
Neocallichirus variabilis (Edmondson, 1944) USA-4	Pacific, Hawaii, USA	ULLZ 6047	EU882958	EU875069
Neocallichirus sp. 1 PNI	Pacific, Nicaragua	ULLZ 4838	EU882959	EU875072
Neocallichirus sp. 2 PNI	Pacific, Nicaragua	ULLZ 6536	EU882961	EU875074
Sergio mericeae Manning & Felder, 1995 AFL	Atlantic, Florida, USA	ULLZ 6493	EU882960	EU875073
Sergio trilobata (Biffar, 1970) GFL-1	Gulf of Mexico, Florida, USA	ULLZ 4501	EU882962	EU875075
Sergio trilobata (Biffar, 1970) GFL-2	Gulf of Mexico, Florida, USA	ULLZ 4501	EU882963	EU875076
Sergio trilobata (Biffar, 1970) GFL-3	Gulf of Mexico, Florida, USA	ULLZ 4501	EU882964	EU875077

Table 1. continued.

Family **Taxon Name**	**Collection Site**	**Catalog No.**	**Acc. No. (16S)**	**Acc. No. (12S)**
Cheraminae				
Cheramus sp. PCR	Pacific, Costa Rica	ULLZ 7751	EU882901	EU875013
Cheramus marginata (Rathbun, 1901) GMX	Gulf of Mexico, Louisiana, USA	ULLZ 7313	EU874912*	EU875009
Eucalliacinae				
Calliaxina sakaii (de Saint Laurent, 1979) JAP-1	Pacific, Japan	ULLZ 8894	EU882904	EU875070
Calliaxina sakaii (de Saint Laurent, 1979) JAP-2	Pacific, Japan	ULLZ 8894	EU882905	EU875071
Eucalliax sp. COL	Caribbean, Rosario Islands, Colombia	ULLZ 6543	EU882926	EU875036
Eucalliax sp. JAM	Caribbean, Montego Bay, Jamaica	ULLZ 6531	EU882927	EU875037
Ctenochelidae				
Dawsonius latispina (Dawson, 1967) GMX	Gulf of Mexico, Mexico	ULLZ 7306	EU874911*	EU875008
Gourretia sp. GMX	Gulf of Mexico, Louisiana, USA	ULLZ 4673	EU882925	EU875035
Gourretia biffari Blanco & Arana, 1994 CPA	Caribbean, Panama	ULLZ 5757	EU882931	EU875041

with a χ^2 test as implemented in PAUP. Previous to the analysis of the combined data, we performed an incongruence length difference (ILD) test or partition homogeneity test (Bull et al. 1993), as implemented in PAUP, to determine whether the 16S and 12S genes could be considered samples of the same underlying phylogeny.

Phylogenetic analyses were conducted using MRBAYES for Bayesian analysis (BAY) and PAUP 4.0 beta 10 (Swofford 1998) for both maximum parsimony (MP) and neighbor joining (NJ) analyses; maximum likelihood (ML) analysis was conducted with RAxML v.7.0.4 (Stamatakis 2006) using the online version at the Cyberinfrastructure for Phylogenetic Research (CIPRES) website (Stamatakis et al. 2008). Prior to conducting the BAY and NJ analyses, the model of evolution that best fit the data was determined with the software MODELTEST (Posada & Crandall 1998). Maximum likelihood analysis was conducted with the default parameters for RAxML for the GTR model of evolution. Bayesian analysis was conducted by sampling one tree every 1,000 generations for 2,000,000 generations, starting with a random tree, thus obtaining 2,001 trees. A preliminary analysis showed that stasis was reached at approximately 75,000 generations. Thus, we discarded 101 trees corresponding to the first 100,000 generations and obtained a 50% majority rule consensus tree from the remaining 1,900 saved trees. NJ analysis was carried out with a distance correction set with the parameters obtained from MODELTEST (Posada & Crandall 1998). MP analysis was performed as a heuristic search with gaps treated as a fifth character, multistate characters interpreted as uncertain, and all characters considered as unordered. The search was conducted with a random sequence addition and 1,000 replicates, including tree bisection and reconnection (TBR) as a branch-swapping option; branch swapping was performed on the best trees only. To determine confidence values for the resulting trees, we ran 2,000 bootstrap pseudo-replicates for NJ and MP analysis, based on the same parameters as above. For ML analysis, we selected the option to automatically determine the number of bootstraps to be run in RAxML. Thus, 200 bootstrap pseudo-replicates were run. On the molecular trees, confidence values >50% were reported for ML, MP, and NJ analyses (bootstraps), while for the BAY analysis values were reported for posterior probabilities of the respective nodes among all the saved trees. Sequences as well as alignments have been submitted to GenBank as a Popset.

3 RESULTS

3.1 *Description of datasets and model selection*

We obtained sequences for 37 species of Callianassidae belonging to 18 genera. Our final alignment included 903 bp, 520 for the 16S and 383 bp for the 12S sequence data (excluding primer regions, saturated and ambiguous fragments of both genes). From these, 386 characters were found to be constant, 62 were variable but parsimony-uninformative, and 455 were parsimony-informative. The ILD test showed no significant incongruence (P = 0.110). Thus we used the combined 16S and 12S dataset for our phylogenetic analysis. The nucleotide composition of this dataset can be considered homogeneous (χ^2 = 180.21, df = 219, p = 0.97), with a larger percentage of A-T (33.34%–34.54%, respectively). The best fitting model of substitution, selected with the Akaike information criterion (AIC, Akaike 1974) as implemented in MODELTEST (Posada & Crandall 1998), was the transversional model with invariable sites and a gamma distribution (TVM+Γ+δ) (Rodríguez et al. 1990) and with the following parameters: assumed nucleotide frequencies: A = 0.3716, C = 0.1258, G = 0.1317, T = 0.3710; substitution rates A-C = 1.1541, A-G = 8.3551, A-T = 1.5835, C-G = 0.5502, C-T = 8.3551, G-T = 1.0000; proportion of invariable sites Γ = 0.3104; variable sites followed a gamma distribution with shape parameter δ = 0.5690. These values were used for both NJ and BAY analyses.

3.2 *Tree topologies, relations to Ctenochelidae, and basally positioned groups*

All four phylogenetic methods produced similar tree topologies (Fig. 1). We illustrated one of two equally parsimonious trees of length 3013, CI = 0.326, and RI = 0.713, noting that both MP trees produced the same topology. Within the family Callianassidae, representatives of the four subfamilies included in our analysis were not uniformly monophyletic. The subfamily Eucalliacinae was not only paraphyletic (partitioned between Clades A and B, Fig. 1) but also more basally positioned than traditional classification would predict. Members of the genus *Calliaxina* were unexpectedly placed as a sister clade to members of Ctenochelidae, albeit only at low to moderate support levels. Regardless of their topological placement in our tree, three species representing two genera of Ctenochelidae formed a well-supported monophyletic group.

3.3 *The Callichirinae*

Clade C (Fig. 1) included all sampled genera presently assignable to the subfamily Callichirinae, except for *Lepidophthalmus*. *Lepidophthalmus* was instead positioned in clade D immediately basal to the Callianassinae, but at low support in ML and BAY analyses and without support in the MP and NJ analyses (75/–/–/59). Thus, *Lepidophthalmus* is here regarded as a monophyletic clade of unresolved subfamily assignment in our molecular analysis. Grouping of the Callichirinae was not well-resolved, but present topology suggests it is paraphyletic, though some clades are presently positioned at low support values. While clade C topologically grouped all members of the Callichirinae other than *Lepidophthalmus*, this node was not supported. Furthermore, genera assigned to the Callichirinae were not well-resolved in terms of intergeneric relationships, but with one exception were separated from one another with strong support. Only a single representative of *Corallianassa* was included, but multiple specimens were grouped for each of the genera *Callichirus* Stimpson, 1866, *Glypturus*, *Grynaminna* Poore, 2000, and *Neocallichirus* Sakai, 1988. Those for *Grynaminna* were all *a priori* assignable to *G. tamakii*, but all of the other three included multiple species, even when species names could not be assigned. Clearly grouped as a genus, species of *Callichirus* included at least one new species to be named from the eastern Pacific. Likewise, *Glypturus* included a long-recognized but unnamed species from the Gulf of Mexico, and *Neocallichirus* included two unnamed species from the Pacific coast of Nicaragua. An exception to monophyly was seen in branch positioning for two of the species presently assigned to *Sergio* Manning & Lemaitre, 1994, as *S. mericeae* and *S. trilobata* were positioned paraphyletically. It was also evident that *S. mericeae*, the species closest to *S. guassutinga* (Rodrigues, 1971) (type species of the genus), was placed unambiguously within what is otherwise a monophyletic grouping of species assignable to *Neocallichirus*. This raises a question as to the validity of the genus and, regardless of that issue, argues for generic reassignment of *S. trilobata*.

3.4 *The Cheraminae and Callianassinae*

Clade D (Fig. 1) included representatives of seven genera usually assigned to the subfamily Callianassinae and one assigned to the Cheraminae, in addition to *Lepidophthalmus*, which, as noted above, was questionably positioned as a basal branch with low support. Callianassinae formed a well-supported monophyletic group, but Cheraminae was included within it, also with strong support. While the two species representing the Cheraminae were clearly assignable to the genus *Cheramus* Bate, 1888, only one was assignable to a known species, given the need for further comparative studies and formal descriptions of several new congeners. Support was found for continued recognition of many separate genera in the Callianassinae, including *Pestarella* Ngoc-Ho, 2003, *Gilvossius* Manning & Felder, 1992, *Biffarius* Manning & Felder, 1991, *Neotrypaea*

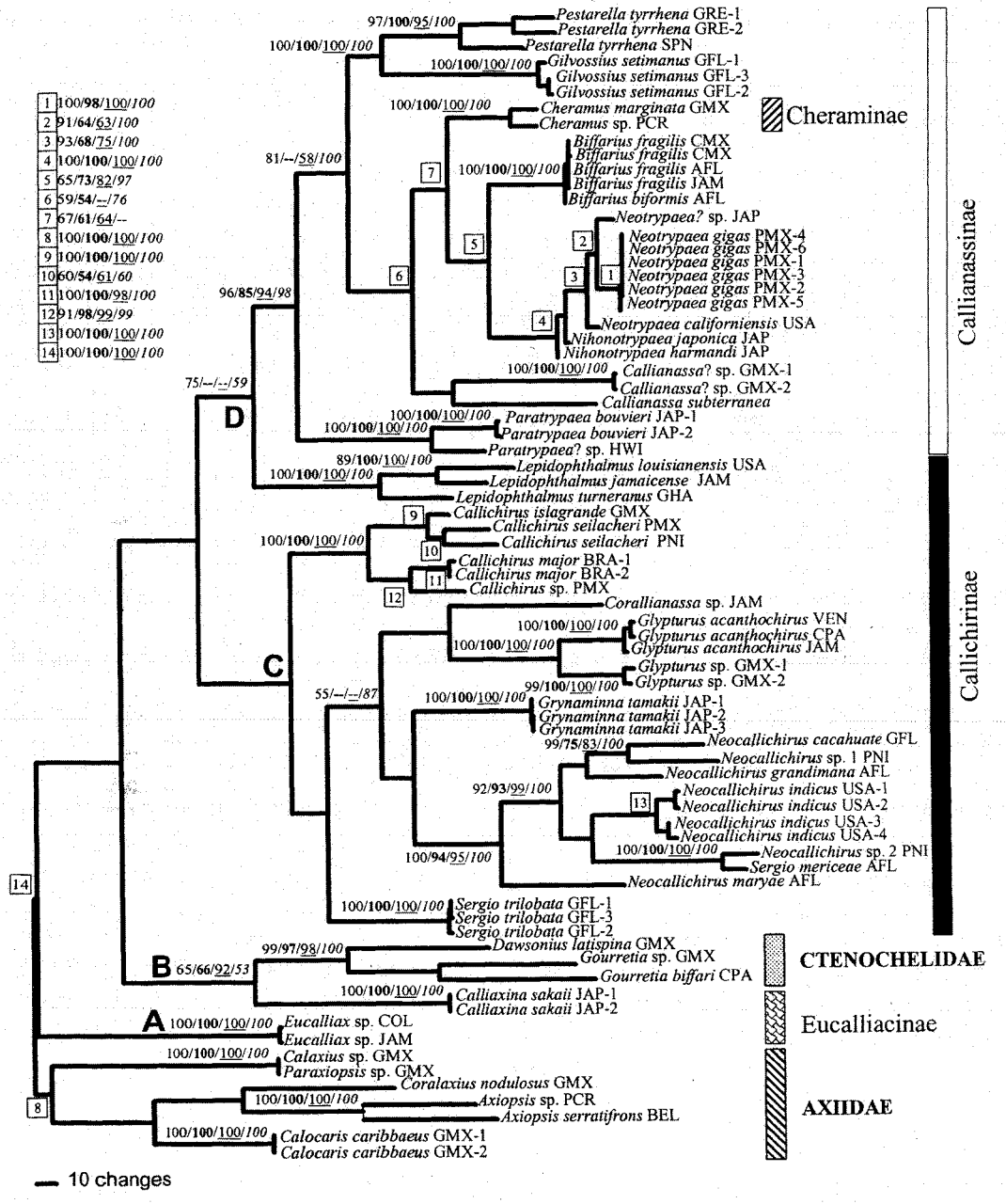

Figure 1. Evolutionary relationships of 18 genera of Callianassidae, two genera of Ctenochelidae, and five outgroup genera of Axiidae, inferred from an MP analysis of 16S and 12S rDNA data. Letters A–D adjacent to major nodes define clades that are referred to in Results. Support values shown from left to right are for ML, **MP**, NJ, and *BAY* respectively; – represents value equal to or lower than 50%.

Manning & Felder, 1991, and *Callianassa* s. s., rather than for their wholesale reassignment to the genus *Callianassa*. It is important to note that sequence data we have here identified with *C. subterranea*, type species of the genus, do not represent the same specimen and species for which sequence data are presently archived as GenBank Acc. No. DQ079706, originally reported in Porter et al. (2005). We propose that this previously published sequence possibly represents a source specimen assignable to *Pestarella*, as was also noted by Robles et al. (this volume). Extractions of *C. subterranea* for our present analyses were instead made from a more recently collected specimen taken in Scotland, for which we have carefully confirmed identification by morphological examination (Table 1). Thus identified, this type of the genus *Callianassa* defines a distinctly separate branch among other major clades of the subfamily, regardless of their present generic assignments. Support values are inadequate to confidently place an undescribed species listed as *Callianassa*? sp. from the Gulf of Mexico (GMX-1 and GMX-2) into this genus, despite its positioning in an immediate sister clade (albeit at long branch lengths). However, there is clear evidence to support the recent removal of *Paratyrpaea bouvieri* from *Callianassa* by Komai & Tachikawa (2008), while also suggesting that an apparently undescribed species from Hawaii is its likely congener.

Our samples of *Pestarella tyrrhena* and *Gilvossius setimanus* sort into a sister clade relationship at high support values. While samples represent multiple populations of both species, those of *P. tyrrhena* suggest, at very least, evidence of conspicuous population genetic structure. While all three specimens generally fit the present diagnosis for *P. tyrrhena*, the specimen from Spain appears to have a slightly broader telson and other features somewhat like those of *P. convexa* de Saint Laurent & LeLoeuff, 1979, from western Africa. Samples of the species we included to represent *Biffarius* reflect, in contrast, little measured genetic divergence between two species that separate readily on the basis of morphology. Finally, the representatives of *Neotrypaea* grouped together with those of *Nihonotrypaea* in a strongly supported monophyletic clade, encompassing somewhat less supported subclades that do not clearly resolve the status of the genus *Nihonotrypaea*.

The only present conclusion we draw for the two included species of *Nihonotrypaea* is that both are placed basally in the topology of this lineage, one without support. It is noteworthy that an undescribed species "*Neotrypaea*?", tentatively assigned by us to this genus on the basis of morphology, did indeed group among the other two, *N. californiensis* and *N. gigas*.

4 DISCUSSION

4.1 *Relationships of the family Callianassidae*

As the subfamily Eucalliacinae is placed within the Callianassidae by most recent authors (Manning & Felder 1991; Tudge et al. 2000; Ngoc-Ho 2003; Sakai 2005), present molecular phylogenetic placements for both of its clades are problematic, especially as one appears allied to the family Ctenochelidae. We can interpret that either the family Ctenochelidae is, undeserving of present rank, embedded within an otherwise monophyletic Callianassidae, or that the family Callianassidae is paraphyletic in present composition. The latter interpretation would infer that the subfamily Eucalliacinae is an unnatural grouping that encompasses at least one genus, *Calliaxina*, of ctenochelid affinities, and another, *Eucalliax*, which perhaps represents a yet-to-be-recognized family. We note that no such affinities were evident for a species of *Calliaxina* previously included in a morphological analysis under its earlier generic assignment, *Calliax punica*, by Tudge et al. (2000). However, relative development and positioning of the appendix masculina and appendix interna on the male pleopod 2 in members of Eucalliacinae is more like that seen in ctenochelids than in most callianassids (Felder & Manning 1994).

Given the low to medium support values that group *Calliaxina* with two other ctenochelid genera, we are not yet committed to family or subfamily level revisions reflecting this in taxonomy. Rather, we await inclusion of additional taxa in our analysis. Ideally, inclusion of *Calliax s.s.* and *Paraglypturus* Türkay & Sakai, 1995, would more comprehensively represent Eucalliacinae in this

analysis, along with perhaps *Ctenocheles* Kishinouye, 1926, *Callianopsis* de Saint Laurent, 1973, *Anacalliax* de Saint Laurent, 1973, and *Paracalliax* de Saint Laurent, 1973, to represent likely members of the Ctenochelidae (*sensu* Manning & Felder 1991; Poore 1994; rather than that of Sakai 2005).

Sakai (2005) treated both *Calliaxina* and *Eucalliax* as junior synonyms of the genus *Calliax* de Saint Laurent, 1973. Clearly, molecular phylogenetic placement of at least *Calliaxina* corrects an error of that synonymy, but we do not yet have a molecular basis upon which to judge the other synonymy. In other revisions, Sakai (2004, 2005) removed both *Gourretia* and *Dawsonius* (see Sakai 2005: 245) from Ctenochelidae, placing them into separate subfamilies of a new family, Gourretiidae. Lacking representation of *Ctenocheles*, which Sakai left as the only genus assigned to Ctenochelidae, we cannot yet speak to the merits of this separation. However, the highly supported present grouping of *Dawsonius* and *Gourretia* raises doubt as to their warranting separation at the level of subfamily. These genera were also supported as a monophyletic group in a combined 18S and 16S molecular genetic analysis of higher-level thalassinidean relationships (Robles et al. this volume), where in the absence of eucalliacine representatives, Ctenochelidae was positioned immediately outside the Callianassidae. Similarly, where represented by a single species of *Ctenocheles* and a smaller group of callianassid taxa (Tsang et al. 2008), analysis of the same two genes placed the Ctenochelidae immediately outside the Callianassidae.

4.2 *Relationships within the subfamily Callichirinae*

No support was found for continued treatment of the genus *Lepidophthalmus* as a member of the subfamily Callichirinae, despite its previous placement among members of that group and wide separation from the Callianassinae in the morphological analysis of Tudge et al. (2000). We found weak support for its sharing a basal relationship with the subfamily Callianassinae but no evidence to contradict this topological placement on the basis of combined 16S and 18S sequence analyses (Robles et al. this volume; Tsang et al. 2008). In combined analysis of 16S, 18S, and 28S rDNA sequences (Tsang et al. 2008), there is in fact support for its separation from *Sergio* and *Callichirus*, the only other callianassid genera included, both of which are members of Callichirinae, though support for definition of that family, as traditionally defined, was lacking in our analysis. *Lepidophthalmus* was clearly monophyletic in our analysis, as in the morphological analysis of Tudge et al. (2000). In terms of habitat, physiology, and larval development, the genus is unique among the callianassids (Nates et al. 1997; Felder 2001, 2003), being highly adapted to muddy euryhaline coastlines and estuaries.

By contrast, members of the genus *Callichirus* are adapted to generally quartzite sandy sediments of high energy beaches and differ markedly from *Lepidophthalmus* and known members of the Callianassinae in varied aspects of larval morphology and life history (Strasser & Felder 1999, 2000; Felder 2001). The representatives in our analysis reflect a few of many remaining taxonomic problems at the species level and also a sister-clade relationship between members with eyes that end in long terminal spines (*C. islagrande* and *C. seilacheri*) and members with eyes that end in short terminal spines or blunt angles (*C. major* and relatives). Eastern Pacific populations of *C. seilacheri* obviously are separated into two populations, one of which may be identifiable with *C. garthi* Retamal, 1975. The latter species was placed into synonymy with *C. seilacheri* by Sakai (1999b) but without apparent study of its type or topotypic materials. Similarly, though our present tree represents only topotypic materials of *C. islagrande*, a sister lineage of *C. islagrande* is known to occur in the western Gulf of Mexico and may also warrant separate taxonomic treatment (Bilodeau et al. 2005). While only Brazilian populations (provisionally assigned to *C. major*) and yet another unnamed eastern Pacific species are included in the alternative major clade of this genus, it should also be noted that this group encompasses several divergent western Atlantic populations that potentially warrant further taxonomic revisions, and not all are represented in the present work (Staton & Felder 1995; Strasser & Felder 1999).

The highly supported grouping for two species of *Glypturus* included the widespread Caribbean species, *G. acanthochirus*, along with a Gulf of Mexico species that lacks a valid species name (without fixation of a holotype, see Dworschak 2007). While Sakai (2005) placed *Corallianassa* into synonymy with *Glypturus*, these genera were well separated in the morphological analyses of Tudge et al. (2000). There was also no support in our own analyses for placing of these genera into close relationship. However, our present analysis is based upon only one species of *Corallianassa* and two closely related species of *Glypturus*. Inclusion of additional members of these groups is needed to definitively resolve their generic status.

We have for now retained use of the genus *Grynaminna* for the species *G. tamakii*, instead of placing the genus into the synonymy of *Podocallichirus* Sakai, 1999, as called for by Sakai (2005) on rather subjective bases. As the genus *Podocallichirus* was derived by Sakai from subdivision of the genus *Callichirus*, it is of interest that *Grynaminna* was, with limited support, placed in a separate lineage from *Callichirus*. However, support is again low, and typical representatives of the genus *Podocallichirus* were not available for inclusion in our analysis.

As in the morphological analysis of Tudge et al. (2000), members of the genus *Neocallichirus* constituted a monophyletic group in our analysis, with the exception that *Sergio mericeae* was included among its subclades. The only other species of *Sergio* in our analysis, *S. trilobata*, was positioned independently, showing this genus to be paraphyletic, as was also evident in a combined analysis of 16S and 18S sequence data (Robles et al. this volume). This separation of *S. trilobata* from supposed congeners (including the type of the genus) was likewise the case in the previous morphological analysis of Tudge et al., where multiple species assigned to this genus were distributed among several clades. We continue to regard *S. mericeae* as a very close sibling species of *S. guassutinga*, type species of the genus, rather than placing it in synonymy with the latter species as advocated by Sakai (1999b). However, they are admittedly close, and thus we regard the clade including *S. mericeae* in our analysis to conservatively represent membership of the genus *Sergio*. If we hereafter treat these most typical members of *Sergio* to be *Neocallichirus*, as did Sakai (1999b), present congeners like *S. trilobata* must be assigned to one or more new genera. Thus, while we find no reason to disagree with Sakai (1999b) in placement of *Sergio s.s.* in synonymy with *Neocallichirus*, we cannot agree that such reassignment is justified for all members of *Sergio s.l.*

4.3 *Relationships within the subfamily Callianassinae*

In the course of deriving what has been termed a "controversial and retrograde classification" (Dworschak 2007), Sakai (1999b, 2005) merged a previously erected 12 genera of callianassids into synonymy with one large genus, *Callianassa*. Conceived as such, *Callianassa* in our analysis could be rationalized as monophyletic, but only provided one merged (from our analysis alone) eight monophyletic clades into it, thus giving high support at the same basal node for the genus that in our analysis defines a full subfamily. Were this to be adopted, a host of well-supported monophyletic genera evident in our phylogeny and that of Tudge et al. (2000) would be merged, serving to obfuscate evolutionary relationships and informative synapomorphies rather than to reflect them in classification and taxonomy. Virtually all nodes defining the represented generic membership of the Callianassinae prior to revisions by Sakai (1999b, 2005) are highly supported in our analysis. In addition, a basally positioned branch apparently defines *Paratrypaea*, recently separated from *Callianassa* on the basis of morphology (Komai & Tachikawa 2008).

While our continued recognition of these and perhaps other callianssine genera is in distinct disagreement with the recent works of Sakai, we submit that insight to reasonable generic groupings is best gained from overall study of tree topologies, branch lengths, and support values—based upon both morphological and molecular data when possible. Even so, outcomes of molecular and morphological analyses do not always agree in full and should not be expected to do so, given varied character sets and inconsistent taxonomic coverage among alternative studies. While the species

set represented in our analysis produced strong evidence of monophyly for callianassine genera and supports the need for naming of generic-level monophyletic clades like that for *Paratrypaea*, inclusion of more species is certain to even further complicate this picture. For example, studies including other species of *Biffarius* analyzed with a different combination of genes do not definitively show monophyly among the represented species (Tsang et al. 2008; Robles et al. this volume). These could resolve differently in expanded analyses with additional genes or more likely become segregated into additional monophyletic clades supported by synapomorphies. We agree with Tudge et al. (2000:142) in that generic names are needed for these additional small groups of species, but those erected to date "should stand for the time being."

We do not support relegation of *Cheramus* to the synonymy of *Callianassa* as proposed by Sakai (1999b), but we cannot disagree with his conclusion that it belongs among the Callianassinae, rather than in its own subfamily. We thus advocate abandoning of the Cheraminae. Our analysis included only two species of the genus (one apparently unnamed), but they formed a well-supported monophyletic group that was unambiguously positioned in topology, quite differently from the findings of Tudge et al. (2000).

A well-defined understanding of *Callianassa s.s.* was deemed essential to our analysis, so we made a concerted effort to ensure accurate representation of *C. subterranea*, the type species of the genus, in our analysis. Thus, the topological positioning for *C. subterranea* in the present work differs significantly from that for the currently available GenBank sequence of "*C. subterranea*" as depicted in Robles et al. (this volume), ostensibly for reasons already stated above in Results. The clade to which the specimen of *C. subterranea* is assigned in our analysis is not strongly supported and reflects a long-branch pairing with undescribed materials from hydrocarbon vent habitats of the Gulf of Mexico, provisionally assigned by us to this genus (*Callianassa*? sp. GMX-1, 2). While incomplete, our morphological studies suggest these materials may warrant treatment under a separate genus.

4.4 *Pending analyses*

Currently in progress, a molecular genetic analysis of all available species of *Lepidophthalmus* and its closest putative relatives should soon provide a somewhat more robust look at relationships of that genus. Likewise, a separate analysis targeted to the relationships of *Neotrypaea*, *Trypaea*, and *Nihonotrypaea* will address the unresolved status of the latter genus. In addition, collaborative work is currently under way to build the broadest overall taxonomic representation we can for a combined morphological and molecular analysis of not only the family Callianassidae but also other families in its infraoder, the Axiidea (*sensu* Robles et al. this volume).

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