Available online at www.sciencedirect.com
SCIENCE DIRECT®
Molecular Phylogenetics and Evolution 30 (2004) 563–581
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

Phylogenetic evidence for an ancient rapid radiation of Caribbean sponge-dwelling snapping shrimps (*Synalpheus*)

Cheryl L. Morrison,* Rubén Ríos, and J. Emmett Duffy

The College of William and Mary, School of Marine Science, Gloucester Point, VA 23062-1346, USA

Received 3 December 2002; revised 16 June 2003

Abstract

A common challenge in reconstructing phylogenies involves a high frequency of short internal branches, which makes basal relationships difficult to resolve. Often it is not clear whether this pattern results from insufficient or inappropriate data, versus from a rapid evolutionary radiation. The snapping shrimp genus *Synalpheus*, which contains in excess of 100 species and is a prominent component of coral-reef faunas worldwide, provides an example. Its taxonomy has long been problematic due to the subtlety of diagnostic characters and apparently widespread variability within species. Here we use partial mt COI and 16S rRNA sequences and morphological characters to reconstruct relationships among 31 species in the morphologically well-defined gambarelloides species group, a putative clade of obligate sponge associates that is mostly endemic to the Caribbean and contains the only known eusocial marine animals. Analysis of the combined data produced a single tree with good support for many terminal clades and for relationships with outgroups, but poor support for branches near the base of the gambarelloides group. Most basal branches are extremely short and terminal branches are long, suggesting a relatively ancient, but rapid radiation of the gambarelloides group. This hypothesis is supported by significant departure from a null model of temporally random cladogenesis. Calibration of divergence times among gambarelloides-group species using data from three geminate pairs of *Synalpheus* species separated by the isthmus of Panamá suggests a major radiation between ~5 and 7 Mya, a few My before final closure of the Panamanian seaway during a period of spreading carbonate environments in the Caribbean; a second, smaller radiation occurred ~4 Mya. This molecular evidence for a rapid radiation among Caribbean marine organisms in the late Miocene/early Pliocene is strikingly similar to patterns documented from fossil data for several other Caribbean reef-associated invertebrate taxa. The similar patterns and timing of cladogenesis evidenced by molecular and fossil data for different Caribbean and East Pacific taxa suggests that the radiation involved a wide range of organisms, and strengthens the case that poor basal resolution in the gambarelloides group of *Synalpheus* reflects a real evolutionary phenomenon. The rapid radiation also helps explain the historical difficulty of diagnosing species in *Synalpheus*.

Keywords: Caribbean; Isthmus of Panamá; Mitochondrial DNA; Molecular clock; Phylogenetic resolution; Radiation; Shrimp

1. Introduction

The tempo of evolution is known to vary considerably through time within lineages. In particular, evolutionary opportunity provided by changes in the environment, a paucity of competitors, or acquisition of a key innovation appear to have stimulated rapid bursts of speciation and phenotypic evolution in a wide range of organisms (Givnish and Sytsma, 1997; Schluter, 2000). An expected phylogenetic signal of such rapid radiation is a tree in which internal branches are short, and basal lineages are poorly resolved. In recent years, as the quantity and quality of molecular data have grown, cases of poorly resolved trees have remained common, even when substantial character data from several sources are available (e.g., Fishbein et al., 2001). Often such trees are characterized by very short internal branches, which several authors have interpreted as evidence of rapid radiation. Examples come from

* Corresponding author. Present address: USGS Leetown Science Center, 11700 Leetown Rd., Kearneysville, WV 25430, USA. Fax: 1-304-724-4465.
E-mail address: cheryl_morrison@usgs.gov (C.L. Morrison).

1055-7903/$ - see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/S1055-7903(03)00252-5

saxifragalean plants (Fishbein et al., 2001), microgastrine wasps (Mardulyn and Whitfield, 1999), aphids (Von Dohlen and Moran, 2000), bark beetles (Jordal et al., 2000), and lacertid lizards (Fu, 2000), among others. This interpretation is usually by process of elimination, however, as supporting fossil data are often unavailable.

A potential candidate for such a rapid radiation is the snapping shrimp genus *Synalpheus* (Decapoda: Alpheidae), which comprises one of the most species-rich and abundant genera of crustaceans. Its more than 100 described species (Bruce, 1976; Chace, 1989) and undoubtedly many undescribed species (Duffy, 1996b; Rios and Duffy, in preparation) are prominent members of coral-reef communities throughout the world tropics, where they often dominate the cryptic fauna of reef rubble (Reed et al., 1982; Snelgrove and Lewis, 1989) and form symbiotic associations with various sessile reef invertebrates. In the tropical West Atlantic, the genus is represented primarily by the morphologically distinctive "gambarelloides group" (Coutière, 1909; Dardeau, 1984), a clade of ~36 species of obligate internal associates of living sponges, which is mostly endemic to this region. The gambarelloides-group species of *Synalpheus* span a range in host specificity, body size, and mode of larval development, making the group a promising taxon for comparative approaches to understanding several general problems at the interface of ecology and evolution (Duffy, 1996a,b). Of particular interest, is the group's diversity in social organization, which is unusual for a single genus even among the insects. The gambarelloides-group species span the gamut from asociality, in which heterosexual pairs form but are intolerant of other adult individuals in a host, through communal species in which several to hundreds of pairs cohabit in a host with multiple females breeding, to the only known eusocial marine animals, represented by colonies of a few hundred individuals with only a single female breeding (Duffy, 1996a,b,c, 1998; Duffy and Macdonald, 1999; Duffy et al., 2000, 2002).

Exploiting the comparative potential of the sponge-dwelling shrimps depends on a well-supported phylogenetic tree on which hypotheses of character evolutionary history can be traced. Reconstructing the history of the *Synalpheus* radiation entails several challenges, however. First, *Synalpheus* is a large genus, comprising >100 described (Chace, 1989) and many undescribed species, and the genus has long vexed taxonomists owing both to the subtlety of characters distinguishing species, and the common morphological variability within putative species (Banner and Banner, 1975; Chace, 1972; Christofferson, 1979; Coutière, 1909; Dardeau, 1984). In an attempt to impose some order on the genus, Coutière (1909) erected six informal subgeneric groups, of which the gambarelloides (formerly laevimanus) group is one of three that have been accepted by later workers as "taxonomically useful" (Banner and Banner, 1975; Dardeau, 1984). All but six of the >30 putative species in the gambarelloides group are restricted to the tropical West Atlantic (Dardeau, 1984; Wicksten, 1994). A previous phylogenetic analysis of a subset (13) of its species supported monophyly (Duffy et al., 2000). Thus, the gambarelloides group appears to represent a monophyletic, geographically circumscribed taxon, and an appropriate subunit of the genus for phylogenetic and comparative study. Yet, recent additions of numerous new species to the group, including several key transitional phenotypes in the group's social evolution (Duffy, 1996c,d, 1998; Ríos and Duffy, 1999, in preparation), require a revised and more complete phylogenetic hypothesis before it can be subjected to rigorous comparative analyses. Here, we employ molecular and morphological characters in a phylogenetic analysis of most of the known species within the gambarelloides group. Our analysis reveals a distinctive pattern of short, poorly resolved internal branches, so we employ the formal, null-model approach of Wollenberg et al. (1996) to test the hypothesis of rapid radiation. This test identifies positive or negative departures from temporally random cladogenesis, reflecting rapid radiations in ancient and recent times, respectively. Finally, we use a molecular clock calibrated by three sibling pairs of *Synalpheus* species separated by the isthmus of Panamá to date the radiation of the group, and we explore the circumstances of its radiation in the context of contemporaneous environmental change in the Caribbean region.

2. Materials and methods

2.1. Taxon sampling

For phylogenetic analysis, we sampled 31 putative species within the gambarelloides species group, including most of the described species from the West Atlantic, several undescribed species, and the namesake of the informal gambarelloides species group (Coutière, 1909), *S. gambarelloides* from the Mediterranean (Appendix A). This sample more than doubles the number of species included in a previous phylogenetic analysis (Duffy et al., 2000), allowing a nearly complete phylogenetic hypothesis of the known taxa in the gambarelloides group. Moreover, for several species, we sequenced multiple individuals from geographically distant populations to assess the monophyly of putative species. The sample includes all four taxa previously described as eusocial, i.e., *S. regalis*, *S. filidigitus*, *S. chacei*, and *S.* "*paraneptunus* small" (Duffy, 1996a,d, 1998; Duffy and Macdonald, 1999; Duffy et al., 2000), as well as species that we suspected, based on morphology, to be their closest relatives. The latter include, in

particular, two recently discovered taxa *S.* "*chacei* A" and *S.* "*bousfieldi* blade."

Finally, we included eight *Synalpheus* species outside the gambarelloides group, comprising four putative geminate species pairs found on opposite coasts of the isthmus of Panamá, with the intention of calibrating a molecular clock for *Synalpheus*. Taxa for which we had data from only one of the two gene regions (mt COI and 16S) were deleted before combining data for simultaneous analysis (Appendix A). No higher level phylogenetic hypothesis exists to suggest a sister taxon to the genus *Synalpheus*, so we used *Alpheus cylindricus* as an outgroup from outside the genus (Appendix A).

Shrimps were collected between 1988 and 2001 from sponges in the San Blas Islands (Caribbean, 9° 34′ N, 78° 58′ W) and the Perlas Islands (Pacific, 8° 39′ N, 79° 03′ W) of Panamá, from Carrie Bow Cay in Belize (16° 48′ N, 88° 05′ W), and from the Florida Keys, USA (24° 48′ N, 80° 46′ W). Live shrimps were obtained from their host sponges and coral rubble, preserved soon after collection in cold 95% ethanol, and stored at −20 °C.

2.2. Morphology

We identified and scored 54 nonautapomorphic morphological characters (Appendix B) for phylogenetic analysis, by direct examination of specimens from 31 gambarelloides-group species and nine other *Synalpheus* and outgroup taxa. Shrimp specimens were stained with methylene blue and examined under dissecting and compound microscopes. Several specimens of each taxon were examined to assess the degree of variation within species. All specimens were scored by the same researcher (R.R.).

2.3. Molecular methods

Genomic DNA was extracted either from eggs or body tissues (major chela or abdomen) of single ethanolpreserved specimens, using the QIAmp tissue kit (Qiagen, Valencia, CA) or G-NOME extraction kit (Bio 101; Vista, CA). For both kits, tissue was first homogenized and incubated overnight at 55 °C in the presence of proteinase K, followed by binding of DNA to a column, washing the DNA, then eluting in sterile water. Genomic DNA was stored at −20 °C.

We obtained partial DNA sequences from the mitochondrial cytochrome oxidase I gene (COI) and the mitochondrial large-subunit ribosomal gene (16S) (see Duffy et al., 2000). To facilitate sequencing, polymerase chain reaction (PCR) primers for both genes were ordered with M13 forward [CACGACGTTGTAAAACG AC] and M13 reverse [GGATAACAATTTCACACA GG] primer tails. A ~510-bp fragment of the 16S gene was amplified using primers 16SAR and 16SBR (Palumbi et al., 1991). A ~620-bp segment of the COI gene was amplified using *Synalpheus*-specific primers COI-GAM4 [CACCCAGAAGTYTATATTCTAAT] and COI-1G [TGTTGGGGGAAGAATGTAAT]. PCR conditions (50 μl reactions) for sequences that are new to this study were as follows: 50 mM Tris–HCl (pH 9.0); 20 mM ammonium sulfate; 0.005% BSA; 2.5 mM $MgCl_2$; 0.2 mM each dNTP; 0.5 μM each primer; and 1.25 U Amplitaq (Perkin–Elmer, Foster City, CA) DNA polymerase. Thermal cycling was carried out on an MJ-Research (Watertown, MA) PTC-200 thermocycler using the following cycle parameters: 94 °C for 60 s; followed by 40 cycles of: 94 °C (30 s), 4552 °C (90 s), 72 °C (150 s); ending with 5 min at 72 °C. PCR products were purified using either the QIAquick (Qiagen) or Wizard (Promega) PCR purification kits. Attachment of M13 tails to PCR primers allowed for direct sequencing of PCR products using fluorescently labeled (IRD-700 and -800, LiCor, Lincoln, NB) M13 primers in sequencing reactions using the SequiTherm EXCEL II DNA sequencing kit (Epicentre, Madison, WI). Sequencing reactions were visualized on a LiCor global IR2 system (LiCor). Both heavy and light strands were sequenced for confirmation.

2.4. Sequence alignment

Forward and reverse sequences for an individual were edited using Sequencher 4.1 (Gene Codes, Ann Arbor, MI). Sequences were deposited in GenBank (Accession Nos. AY344681–AY344768, Appendix A) and the 16S alignment is available from the authors upon request. A multiple alignment of COI sequences was performed using the pairwise alignment program Clustal X ver. 1.4b (Thompson et al., 1994) with default parameters and was straightforward, as no indels were encountered. The 16S sequences were first aligned using Clustal X with several gap-opening and -extension penalties, and visual inspection followed by parsimony analyses were run for each resulting alignment to assess relative quality. The parameters that produced the fewest MP trees were: a gap-opening penalty of 20 and gap-extension penalty of 10. There were several ambiguous regions in this Clustal alignment, however, so we utilized the versatile parameter settings available in MALIGN (Wheeler and Gladstein, 1992, 1994) to make different penalties for leading, internal, and trailing sections of sequence. The Clustal alignment was divided into three segments (A, B, and C) in regions where the alignment was unambiguous. The setting for internal gaps was five for each alignment segment, but the leading and trailing penalties varied, respectively, for each alignment section: section A: 2, 10; section B: 10, 10; and section C: 10, 2. The resulting three alignments were combined and checked by eye.

2.5. Phylogenetic analysis

We determined the most appropriate model of DNA substitution for each gene independently (separating the COI dataset into two partitions for combined first and second codon positions and third positions) and for both genes simultaneously via hierarchical likelihood ratio tests (Posada and Crandall, 2001) using Modeltest 3.06 (Posada and Crandall, 1998). The best-fit models from Modeltest were used in ML analyses, Bayesian analyses, and also as a basis for six-parameter parsimony weighting (6P; Williams and Fitch, 1990; outlined by Stanger-Hall and Cunningham, 1998), in which separate weights are given to each of the six possible substitution classes based on the natural log of the proportion of their inferred frequency. For each of the two molecular datasets, we performed unweighted and 6P weighted maximum parsimony (MP) and maximum likelihood (ML, Felsenstein, 1981) analyses using PAUP* 4.0b8a and/or 4.0b10 (Swofford, 2002). For parsimony analyses, heuristic searches were run with the following settings: starting trees for branch swapping obtained via stepwise addition, 100 random additions of sequences per run, and tree bisection-reconnection (TBR) branch swapping on best trees only. In analyses of the 16S data, results were compared when gaps were treated as missing data and when coded as a fifth base. Maximum likelihood analyses were run in PAUP* with heuristic searches with settings as in parsimony except for as-is addition of sequences, and model parameters as estimated with Modeltest.

Bayesian estimation of phylogeny (Larget and Simon, 1999; Rannala and Yang, 1996) was carried out using MrBayes v2.01 (Huelsenbeck and Ronquist, 2000). The GTR model of substitution was invoked, starting with random trees, and estimating base frequencies, gamma shape parameter, and proportion of invariable sites from the data. Bayesian posterior probabilities were estimated as the proportion of trees that contained each of the observed bipartitions (Larget and Simon, 1999) sampled after a conservative point of convergence of likelihood values had been reached (burn-in = 20,000 generations). Five separate runs were performed using MrBayes, with four chains per run and chain lengths ranging from 300,000 to 1,000,000 generations, sampling trees every 100 or 500 generations. In order to be confident that the Markov chain Monte Carlo (MCMC) simulations had run long enough that parameter values had been sampled in proportion to their posterior probabilities, tree topologies, ML parameters, branch lengths, and clade credibility values were examined from output trees and statistics for appearance of convergence (see Huelsenbeck et al., 2002).

Although it has been suggested (e.g., Wheeler et al., 1993) that phylogenetic relationships generally are most accurately assessed by combining all available data, the decision whether to combine distinct datasets into a single analysis remains controversial (Huelsenbeck et al., 1996). Supporters of conditional combination argue that testing for topological incongruence between data partitions is an important step in data exploration because incongruence can signify that one or more of the data partitions supports the wrong phylogenetic hypothesis (Bull et al., 1993; De Queiroz, 1993; Huelsenbeck et al., 1996; Larson, 1994). The incongruence length difference test (ILD, Farris et al., 1994b) has been shown to be superior to other methods of statistically assessing the degree of such incongruence between data partitions (Cunningham, 1997). However, the behavior of ILD tests in mixed model phylogenetic analysis has recently been questioned (Yoder et al., 2001) and it has been shown to increase perceived congruence when more than one model is incorporated in a phylogenetic analysis, such as in our analyses using 6P parsimony (Dowton and Austin, 2002). Recognizing these reservations, we performed ILD tests between the two molecular datasets, and among the molecular and morphological datasets, as an exploratory step. The same weighting schemes were applied in these analyses as in the original parsimony analyses, and invariant characters were excluded.

Support for nodes on parsimony trees was estimated using bootstrap resampling (Felsenstein, 1985) with 1000 replicates, each with 100 random additions of sequences and the full heuristic search algorithm. The decay index, or Bremer support index (Bremer, 1988, 1994; Donoghue et al., 1992) was also calculated using TreeRot (Sorenson, 1999) for nodes occurring in strict consensus trees from unweighted parsimony. ML bootstrap analyses were run with the "fast" stepwise addition algorithm and 100 replicates.

Because initial analyses suggested a pattern of rapid radiation in part of the tree (see Section 3), we tested whether the observed tree from the combined molecular data departed from a stochastic temporal pattern of cladogenesis using the method of Wollenberg et al. (1996). This required estimation of the relative temporal placements of the nodes (or speciation events) among *Synalpheus* gambarelloides-group taxa, which requires a "contemporaneous-tips" tree in which all extant taxa coexist at the same time (present) on the temporal scale of the tree, i.e., are right-justified. To obtain this tree, we calculated branch lengths for the gambarelloides species via Fitch–Margoliash least-squares estimation, using the program KITSCH in Phylip 3.572c (Felsenstein, 1993), which assumes a molecular clock and produces a "least-squares-with-contemporaneous-tips," or KITSCH, tree. We performed two separate analyses, one using *A. cylindricus*, and one using *S. brevicarpus* (a *Synalpheus* species shown in our analyses to fall outside of the gambarelloides group) as the outgroup. In order to assess relative temporal placement of all internal nodes on

the tree, the KITSCH tree branch lengths were standardized to a temporal scale ranging from zero (the earliest node in the tree) to one (the present). A frequency histogram of the scaled branching times for all nodes in the tree ($n = 31$ nodes) was plotted and then converted to a cumulative frequency distribution (CFD; Sokal and Rohlf, 1995). We compared this cumulative frequency histogram to that expected with the same number of taxa under a Markovian model of temporally random cladogenesis using the approach of Wollenberg et al. (1996). Since we had 32 taxa, we interpolated the cumulative frequency distributions for this number of taxa from those given for 30 and 35 taxa in Wollenberg et al. (1996). The probability that the temporal spacing of cladogenetic events in the empirical data differed from that of a phylogeny generated by a Markovian branching and extinction process was tested using the Kolmogorov-Smirnov goodness of fit test (K–S D-statistic, see Wollenberg et al., 1996).

3. Results

3.1. Data matrix description

The COI multiple alignment for 60 individuals contained 541 bp, of which 288 were constant and 224 were parsimony-informative (Table 1). Translation of the COI sequences to amino acids revealed no stop codons, and variance at third positions was considerably greater (99.44%) than at first and second positions (30 and 16%, respectively), suggesting that pseudogenes (Williams and Knowlton, 2001) were not a problem in our dataset. In both mtDNA genes, base frequencies were skewed towards A and T, as has been seen in other invertbrate mtDNA genes (Simon et al., 1994). The best-fit model chosen by Modeltest for the COI dataset was the Tamura and Nei model (TrN; 1993), with correction for among-site rate variation estimation (Γ) and proportion of invariable sites (I). Parameters were: bases = ($A = 0.3694$, $C = 0.2319$, $G = 0.0773$, $T = 0.3214$); R matrix = (1.0, 13.2082, 1.0, 1.0, 17.3252); $\Gamma = 0.5968$; $I = 0.4701$. The 16S multiple alignment included 526 bp of sequence for 50 individuals, with 52 gapped sites, 219 constant sites, and 246 parsimony-informative sites. Gaps were not included as a fifth character in parsimony analyses of the 16S dataset since inclusion of gaps greatly increased the number of MP trees (Table 1). Based on results from Modeltest, the most appropriate model for the 16S data partition was the general time-reversible model (GTR; Lanave et al., 1984; Rodriguez et al., 1990), with Γ and I corrections. Parameters were: bases = ($A = 0.2908$, $C = 0.0868$, $G = 0.2326$, $T = 0.3898$); R matrix = (0.2231, 8.2905, 1.6339, 0.7830, 6.2332); $\Gamma = 0.686$; $I = 0.2728$. The GTR model was also chosen by Modeltest for the combined molecular data: bases = ($A = 0.3382$, $C = 1595$, $G = 0.1495$, $T = 0.3528$); R matrix = (0.4814, 5.4673, 1.0112, 0.3548, 7.0993); $\Gamma = 0.8112$; $I = 0.4007$.

Numbers of transitions and transversions were plotted against uncorrected proportional distances (p-distances) for all pairwise comparisons of *Synalpheus* gambarelloides taxa in order to assess the potential impact of saturation in certain classes of sites. For COI data, transitions and transversions were plotted by codon position, or at combined first and second codon positions, and at third positions. Generally, numbers of transitions outnumbered transversions in all comparisons by approximately 2:1, and numbers of substitutions did not appear to reach a plateau at p-distances between gambarelloides taxa ($\leqslant$22%; data not shown). Therefore, it did not appear as if saturation was interfering in our ability to assess relationships among *Synalpheus* gambarelloides taxa. Transition biases exist in both datasets, yet for opposite types of transitions. Among COI sequences, C $\leftrightarrow$ T transitions outnumber A $\leftrightarrow$ G transitions 3.7:1, and the opposite trend is seen in the16S data where A $\leftrightarrow$ G transitions outnumber C $\leftrightarrow$ T transitions 2.43:1. Our use of ML and 6P parsimony in phylogenetic analyses took these biases into account.

Corrected pairwise sequence divergence estimates (K2P, Kimura, 1980) were generally lower for 16S than for COI. Between conspecific individuals from the same

Table 1
Statistics for phylogeny estimation using parsimony

Analysis	Figure	# Characters/ PI characters	# Taxa	# Trees	# Steps	CI	RI	Support for gambarelloides clade
COI, unweighted		541/224	60	1	1959	0.2149	0.5883	—
COI, weighted (6P)	1A	541/212	60	3	2654	0.1952	0.6529	—
16S, unweighted		526/240	50	20	1368	0.3165	0.5430	—
16S, weighted (6P)	IB	526/246	50	6	1967	0.2822	0.5585	—
16S, unweighted w/gaps		526/251	50	41	1501	0.3185	0.5415	—
Morphology	1C	54/54	40	163	344	0.2849	0.5692	71
16S/COI unweighted		1067/463	50	8	3335	0.2549	0.5100	—
16S/COI weighted (6P)	2A	1067/456	50	3	4655	0.2281	0.5580	—
Combined data, unweighted		1134/508	40	1	3555	0.2616	0.4228	73
Combined data, weighted (6P)	3	1134/502	40	1	4837	0.2349	0.4720	65

location, K2P distances averaged 0.34 and 1.60% for 16S and COI, respectively. Between conspecific individuals from different locations (e.g., Belize and Panamá), these values averaged 1.66 and 4.15% for 16S and COI, respectively. Between members of morphologically defined species complexes, K2P distances ranged from 7.86 to 16.05%, or 10.43% on average for 16S, and 4.96 to 22.41%, or 14.93% on average for COI. 16S and COI K2P divergence estimates, respectively, for transisthmian sister taxa were: 7.89 and 8.66% for *S. brevicarpus*; 6.21 and 9.65% for *S. minus/digueti*; 8.23 and 12.34% for *S. fritzmuelleri*; and 14.93 and 22.52% for *S. bannerorum/dominicensis*. K2P distances between gambarelloides and nongambarelloides *Synalpheus* taxa ranged between 14 and 29% (mean = 19.74%) for 16S, and between 17 and 29% (mean = 22.02%) for COI. K2P distances between *A. cylindricus* (the outgroup) and gambarelloides species averaged 39.5% for COI and 25.26% for 16S. K2P distances between *A. cylindricus* and nongambarelloides species averaged 37% for COI and 23.2% 16S.

3.2. Phylogenetic analyses

3.2.1. Cytochrome oxidase I

Weighted parsimony analysis of 60 individuals, with *Alpheus cylindricus* designated as the outgroup, resulted in three equally parsimonious trees (Fig. 1A). All geographic populations putatively considered conspecific are strongly supported as such, with the possible exception of *S.* "*pandionis* giant," which may be paraphyletic. This suggests that using single exemplar populations to represent species in the overall analysis is justified. Monophyly of the gambarelloides species group was not supported by the COI dataset, as *S. bannerorum*, *S. dominicensis*, and *S. fritzmuelleri* are nested within the clade containing the gambarelloides taxa. Most deep nodes in the phylogeny were poorly

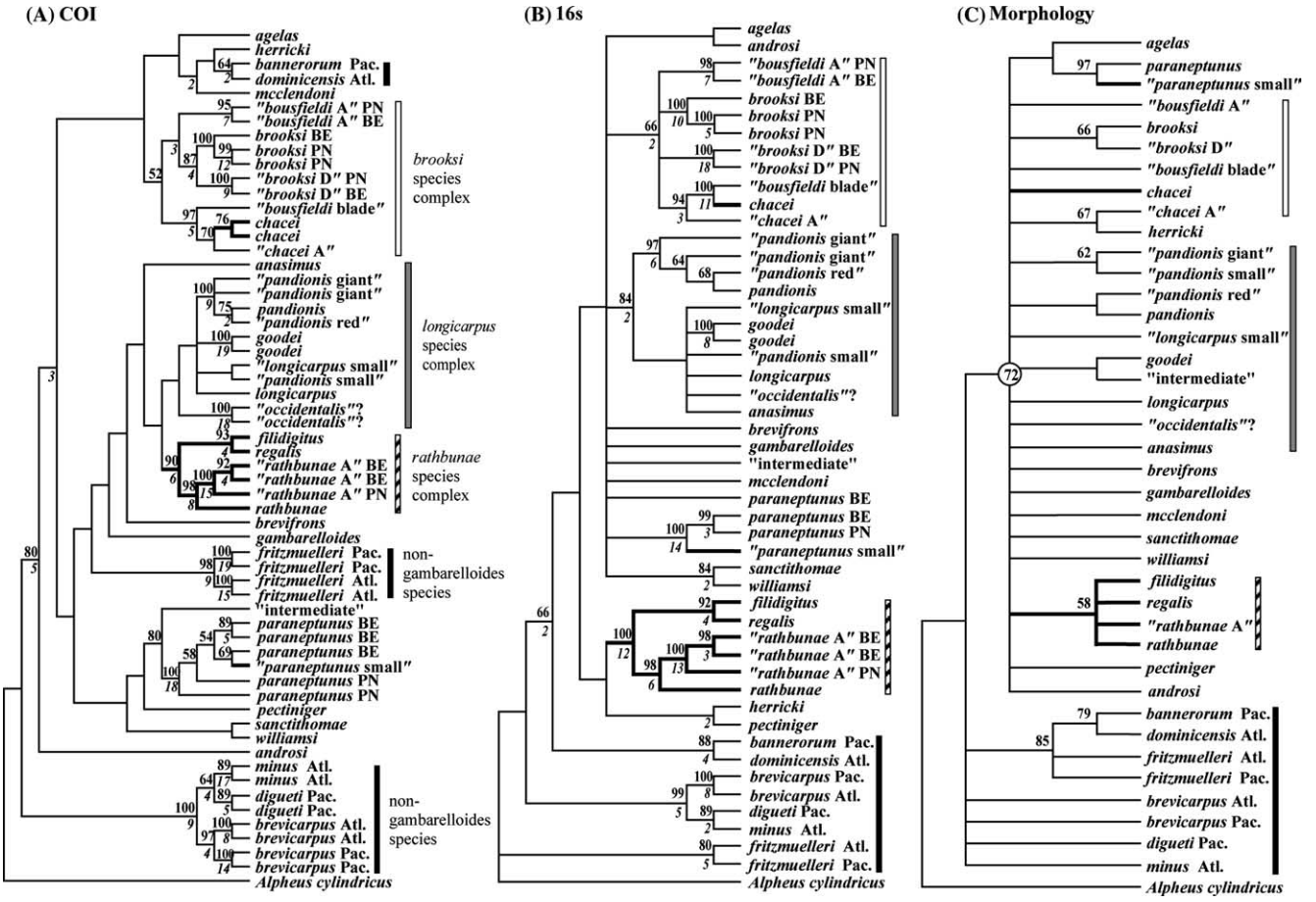


Fig. 1. Phylogenetic hypotheses for West Atlantic *Synalpheus* species in the gambarelloides group based on: (A) weighted-parsimony analysis of partial mtCOI sequences (strict consensus of 3 MP trees); (B) weighted-parsimony analysis of partial 16S rRNA sequences (strict consensus of 9 MP trees); and (C) unweighted-parsimony analysis of 54 morphological characters (strict consensus of 188 MP trees). Numbers above branches are bootstrap proportions; numbers below branches (A, B) are decay indices. Bold branches designate eusocial taxa as defined in the text. Vertical bars to the right of terminal taxa represent morphologically defined species complexes and correspond to the same complexes on each tree. PN, Caribbean Panamá; BE, Belize; Atl., Atlantic; Pac., Pacific. Statistics for each tree are shown in Table 1.

supported, whereas bootstrap resampling and decay indices showed good support for many terminal clades (Fig. 1A). The morphologically defined "*brooksi*," "*longicarpus*," and "*rathbunae*" species complexes were recovered by both weighted (Fig. 1A) and unweighted (data not shown) parsimony analyses of the COI data, although only the "*rathbunae*" group was strongly supported. The *rathbunae* species-group contains four taxa designated as eusocial on the basis of having usually monogynous colonies numbering in the 10s–100s. Although the *brooksi* species-group was only marginally supported, the COI data strongly support a relationship between its component eusocial species *S. chacei* and two undescribed taxa, *S.* "*chacei* A" and *S.* "*bousfieldi* blade." The final eusocial taxon, *S.* "*paraneptunus* small," falls within the strongly supported taxon *S. paraneptunus* and is nested among geographically separated samples thereof, suggesting that these populations are all conspecific. Thus, the COI data are consistent with our earlier finding (Duffy et al., 2000) of three independent origins of eusociality within the gambarelloides group. The four putative transisthmian taxon-pairs are each recovered as sister taxon-pairs by COI.

3.2.2. 16S rRNA

Weighted parsimony analysis of 16S data for 50 *Synalpheus* taxa resulted in nine equally parsimonious trees (Fig. 1B, Table 1). Analysis of the same data with unweighted parsimony resulted in five equally parsimonious trees, with a consensus tree that had similar topology to the weighted analysis, so Bremer support values calculated for the unweighted analysis are shown on the weighted tree (Fig. 1B). Although the gambarelloides group was monophyletic in the 16S analyses, and in a ML analysis (data not shown), this node was not supported by bootstrap analysis. The 16S data provided weak support for the transisthmian pair *S. dominicensis* and *S. bannerorum* as the sister taxon of the gambarelloides group (see combined analyses below). The three morphologically defined species complexes found in the COI analysis were also recovered by 16S, and were more strongly supported. The 16S data also supported three origins of eusociality. All four transithsmian pairs were strongly supported by bootstrap analysis.

3.2.3. Morphology

Unweighted parsimony analysis of 40 taxa, with *A. cylindricus* designated as outgroup, produced 188 equally parsimonious trees with generally poor resolution and bootstrap support (Fig. 1C). The most notable feature of the morphological analysis was its support for monophyly of the gambarelloides group at 72%. According to Coutière (1909), the eight remaining *Synalpheus* taxa fall into two species-groups: *S. fritzmuelleri* and *S. bannerorum/dominicensis* are part of the neomeris group, and *S. brevicarpus* and *S. diguetilminus* fall within the brevicarpus group. Only one of these groups, the neomeris group, received bootstrap support (85%, Fig. 1C). Among the four geminate pairs of species, only the *S. bannerorum/dominicensis* pair was supported (79%, Fig. 1C).

3.2.4. Congruence between datasets

ILD tests for congruence between the two molecular data partitions, and between the molecular and morphological datasets, were not significant ($P_{ILD} = 0.12$ and $P_{ILD} = 0.26$, respectively). We were somewhat skeptical of the utility of these ILD tests, however, given that different models of sequence evolution were optimal for the two genes, and the necessity of using different evolutionary models for our molecular and morphological data (Dowton and Austin, 2002). Nevertheless, it appeared that the phylogenetic signals in the three datasets are not strongly conflicting. Moreover, our strongest phylogenetic hypothesis resulted from the combined analysis of molecular and morphological data (Fig. 3), strengthening the justification for combining the data in this instance.

3.2.5. Simultaneous analysis of molecular data

Simultaneous analysis of the COI and 16S data using 6P parsimony produced three equally parsimonious trees (Fig. 2A, Table 1). Although the deeper internal nodes were generally unresolved in this analysis, most clades recovered by analyses of COI or 16S separately were more strongly supported in the combined analysis. This included, for example, the *longicarpus* complex (bootstrap = 80%) and the *brooksi* complex (88%, Fig. 2A). The gambarelloides group, however, was not monophyletic in the combined molecular analysis, as *S. dominicensis* and *S. bannerorum* were nested within this clade. In contrast, ML analysis of the combined COI and 16S data recovered a monophyletic gambarelloides species group, but lacked bootstrap support (Fig. 2B). The resulting ML phylogram reveals a potential reason for the poor resolution of basal relationships, namely that many terminal branches were long whereas internal branches were generally quite short, resulting in few synapomorphies along the internal branches. The consensus of trees generated in Bayesian analyses of 1-million MCMC generations (consensus of 9801 sampled trees, disregarding 200 burn-in trees; Fig. 2C) recovered the same species complexes and was similar in topology to both the 6P parsimony consensus tree (Fig. 2A) and the ML tree (Fig. 2B). The topology of the three analyses (Fig. 2) varied slightly regarding the relationships among species-complexes, and in the placement of several taxa, namely *S. androsi*, *S. macclendoni*, *S. gambarelloides*, and *S. brevifrons* (note low clade credibility values for placement of these species-complexes and taxa; Fig. 2C). Bayesian clade credibility values were generally higher than bootstrap support in 6P parsimony and ML

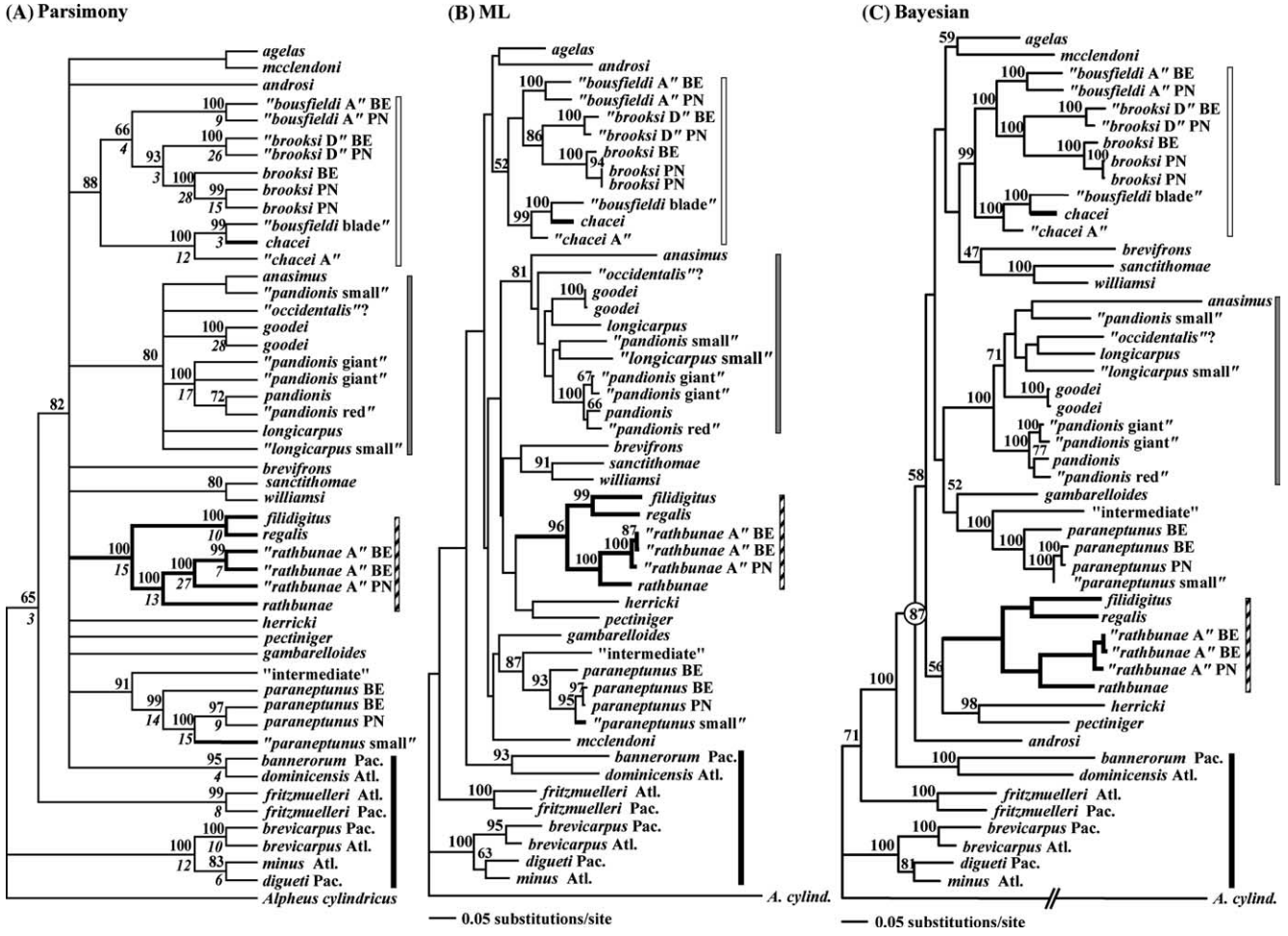


Fig. 2. Phylogenetic hypotheses for West Atlantic *Synalpheus* species in the gambarelloides group based on combined COI and 16S data. (A) Weighted parsimony, strict consensus of 3 MP trees; (B) maximum likelihood tree with best-fit model for combined 16S and COI data, $-\ln = 15275.92034$; (C) consensus phylogram produced from 9801 sampled trees in Bayesian analysis (1 million generations). Numbers above branches are bootstrap proportions (A, B) or Bayesian clade credibility values (C); numbers below branches in (A) are decay indices. Abbreviations and symbols as in Fig. 1.

analyses (Fig. 2). This is especially true at interior nodes of the tree (e.g., nodes relating gambarelloides and nongambarelloides taxa), which received little, if any, support in analyses other than Bayesian. In particular, Bayesian analysis showed good support for the monophyly of the gambarelloides group (clade credibility of 87%; Fig. 2C), with *S. bannerorum/dominicensis* falling outside of this group. Comparisons of nonparametric bootstrap values and Bayesian clade credibilities suggest that clade probabilities over 80% indicate strong branch support (Whittingham et al., 2002). Branch lengths were very similar between ML (Fig. 2B) and Bayesian analyses (Fig. 2C; note same scale).

3.2.6. Simultaneous analysis of molecular and morphological data

Analysis of the three combined data partitions using weighted parsimony produced a single tree, with fair support (65%) for the gambarelloides group and generally stronger bootstrap support for most clades than found in the analyses of any single dataset (Fig. 3). The combined data gave moderately strong support to the *S. bannerorum/dominicensis* geminate pair as the sister taxon to the gambarelloides group, a topology also found, but with little support, in the analysis of 16S data alone and in the ML analysis of the combined molecular data. Interestingly, when *S. bannerorum* and *S. dominicensis* were removed, the gambarelloides clade was well supported in most analyses (bootstrap support of 81, 94, and 97% in 6P parsimony analyses using 16S, 16S/COI, and combined molecular and morphological datasets, respectively).

3.3. Statistical tests of temporal distributions of phylogenetic nodes

The contemporaneous-tips KITSCH tree for gambarelloides taxa based on combined 16S and COI data

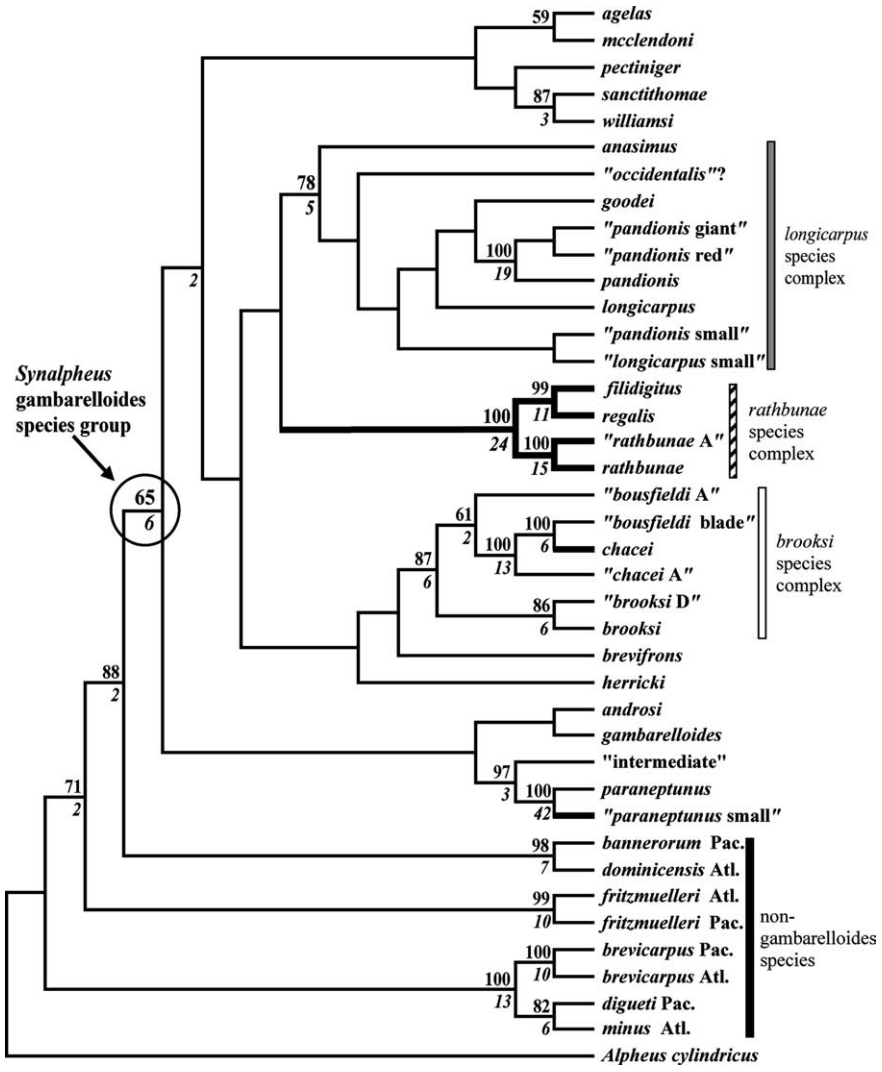


Fig. 3. Phylogenetic hypothesis for West Atlantic *Synalpheus* species in the gambarelloides group based on combined molecular (COI and 16S) and morphological characters using weighted parsimony. The single most parsimonious tree is shown (statistics in Table 1). Numbers above branches are bootstrap values (1000 replicates, 100 random additions/replicate), and numbers below branches refer to decay indices (Bremer support) calculated using the unweighted consensus tree. Abbreviations and symbols as in Fig. 1.

and using *S. brevicarpus* as the outgroup (Appendix D) was similar in topology to that recovered in other analyses of combined molecular data (e.g., Figs. 2 A, B, and 3). However, most of the internal branches of the KITSCH tree were extremely short (Appendix D). Accordingly, the CFD of normalized branching times from this analysis differed strongly from that expected under a null Markovian model (Appendix D). The empirical CFD from the gambarelloides tree is shifted well to the left of the average distribution for 32-taxon trees under the null model, indicating an ancient clustering of branching events. The Kolmogorov–Smirnov test supports this conclusion ($D = 0.70354$, $P < 0.0001$), indicating a very small probability that cladogenesis within the gambarelloides group was temporally random.

4. Discussion

Our phylogenetic analysis of the Caribbean sponge-dwelling *Synalpheus* species more than doubles the number of taxa considered in a previous study (Duffy et al., 2000) and includes most of the described and undescribed taxa currently known from the region. This new analysis, based on combined data from two mitochondrial genes and 54 morphological characters, reveals several main results. First, all of the 15 putative species from which we obtained multiple, geographically separated sequences were strongly supported as conspecific (Figs. 1A and B), with the possible exception of one case that could not exclude paraphyly, justifying our practical species concept and our use of single exemplar populations to represent most species in the overall

analysis. Second, most of the informal species complexes we had recognized on morphological grounds (*brooksi*, *longicarpus*, and *rathbunae* complexes) were also supported by the combined molecular data (Fig. 2), suggesting again that traditional morphological characters accurately diagnosed natural groups of taxa. At the same time, however, our analysis corroborates the difficulties faced historically by taxonomists using morphological characters to understand relationships within *Synalpheus*—the analysis of morphological characters alone yielded an almost completely unresolved tree, the principal feature being support for the gambarelloides clade (Fig. 1C). Importantly, despite the apparently low information content of the morphological data, they appeared to complement the molecular data, as the combined analysis of all three datasets yielded a single, completely resolved tree in which nearly all clades showed stronger bootstrap support than in any single dataset (Fig. 3). In particular, the combined-data analysis supported the monophyly of the gambarelloides clade with 65% bootstrap support (Fig. 3), which was also recovered from the molecular data alone by Bayesian analysis (Fig. 2C), but not by other methods of phylogeny estimation (Figs. 2A and B). Higher support values from Bayesian analyses, compared with nonparametric bootstrap values, have been observed by other researchers as well (e.g., Whittingham et al., 2002; Wilcox et al., 2002). Although nonparametric bootstrap values are not directly comparable with Bayesian clade credibility values, simulations have suggested that Bayesian support values are better estimates of phylogenetic accuracy than nonparametric bootstraps (Wilcox et al., 2002).

A conspicuous feature of all of our analyses was the poor support for most internal nodes in the tree. Whereas relationships among many of the terminal taxa were moderately to well-supported, as were the basal branches outside the gambarelloides group, basal relationships within the gambarelloides group were uniformly poorly supported (Fig. 3). This situation could presumably be improved by adding additional characters, particularly appropriate nuclear gene sequences. However, the conspicuously short internal and long terminal branches within the gambarelloides group (Figs. 2B and C) suggest that the poor resolution may reflect a rapid but ancient radiation. Comparison of estimated branching times within the gambarelloides group with those in a null model, following the method of Wollenberg et al. (1996), strongly supported this interpretation.

The null-model approach also has been used to identify rapid ancient radiations, or "ancient species flocks," in the cranes (Wollenberg et al., 1996), *Sebastes* rockfishes and Antarctic icefishes (Johns and Avise, 1998), and North American *Dendroica* warblers (Lovette and Bermingham, 1999). The same approach has identified rapid recent radiations in columbine flowers (Wollenberg et al., 1996) and African cichlid fishes (Johns and Avise, 1998). In all these cases, resolution and/or support for the nodes in question were poor, suggesting a real phenomenon resulting from rapid radiation, rather than a simple paucity of appropriate data.

The finding of a rapid, ancient radiation among *Synalpheus* shrimps begs the question of what factors may have stimulated such a burst of speciation. To explore this issue, we obtained a molecular clock estimate for *Synalpheus* using the transisthmian geminate pairs in our analysis. Knowlton and Weigt (1998) found that genetic distances differed considerably among 15 transisthmian geminate pairs in the related shrimp genus *Alpheus* but were consistent between COI and allozyme estimates, with some evidence that habitat preferences influenced the timing of their divergence. Accordingly, they chose the geminate pair with the smallest sequence divergence to estimate the molecular clock, reasoning that this divergence should be closest to the final closure of the Panamanian isthmus, at ~3 Mya (Coates and Obando, 1996). Three of the four geminate pairs of *Synalpheus* we examined had quite similar divergence estimates (K2P distances), ranging from 8.5 to 10.4% for the combined 16S/COI data. Calibrating a clock using the smallest of these values, for the *S. brevicarpus* pair, yields an estimate of 1.4% divergence My^{-1} for the combined COI and 16S data, which is similar to divergence estimates from geminate *Alpheus* species for mtDNA COI, 1.5% divergence My^{-1} (data reanalyzed from Knowlton and Weigt, 1998; Knowlton et al., 1993), and fishes, which generally have slightly lower divergence rate estimates (Collins, 1996b), at about 1.2–1.3%/MY (Bermingham et al., 1997). The distribution of all pairwise sequence divergences among gambarelloides-group taxa, calibrated with our *Synalpheus* clock estimate of 1.4%/MY, shows a clearly bimodal (perhaps even trimodal) distribution, with most values clustering fairly tightly around a node at ~6 Mya, and a smaller peak at ~4 Mya (Fig. 4). The concentration of most values around a single node further supports the conclusion of a rapid radiation giving rise to most extant taxa. Interestingly, nearly all of our pairwise divergences fall within the range of 3–9 Mya, corresponding quite closely with Knowlton and Weigt's (1998) range of estimated divergence times for 15 pairs of transisthmian geminates in the related genus *Alpheus*.

Although the Panamanian seaway's final closure produced an obvious and widely appreciated vicariant event, the associated oceanographic and environmental changes, which included birth of the warm Gulf-Stream current, and increased surface salinities and spread of carbonate shoals and reefs in the Caribbean, had even more pervasive impacts on evolution and faunal composition in the Caribbean region (Collins, 1996a; Cronin

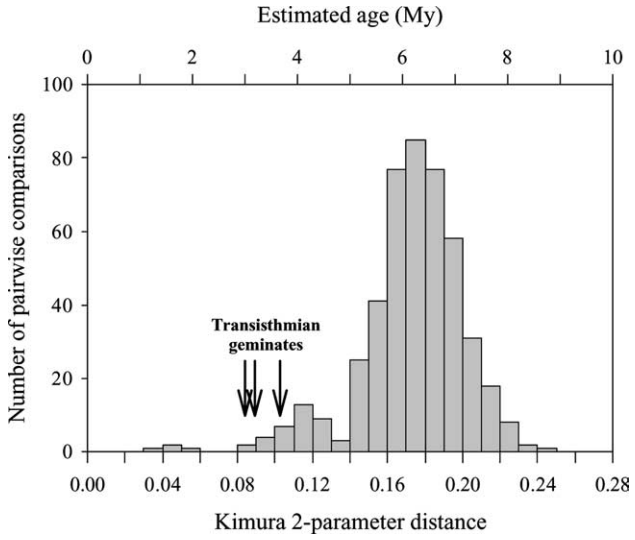


Fig. 4. Distribution of pairwise sequence divergences among taxa in the gambarelloides group, based on Kimura's two-parameter distances calculated from combined COI and 16S data. Divergence values for three transisthmian geminate pairs of *Synalpheus* are indicated, as is an approximate time scale, based on the assumption that the youngest geminate divergence occurred at final closure of the isthmus ~3 Mya.

and Dowsett, 1996). These effects began several Mya before final closure, as the proto-isthmus rose and first disrupted circulation between the oceans, and coincided with radiations of several Caribbean marine taxa, some with striking similarities to the pattern we found in *Synalpheus* (Cheetham and Jackson, 1996; Collins, 1996a). For example, fossil data show that Caribbean foraminifera radiated "a few million years before complete seaway closure around 3.5 Mya, after which time there apparently was little speciation or extinction" (Collins, 1996a). Similarly, after a long period of relative stasis, the bryozoan genera *Stylopoma* and *Metrarabdotos* produced 10 and 11 new species, respectively, between 8 and 6 Mya (Cheetham and Jackson, 1996). The scale of environmental change, and evolutionary responses, in the region during this time is further supported by the rapid radiation of East Pacific crabs in the genus *Petrolisthes*, which occurred "after the Cretaceous but prior to the Miocene" (Stillman and Reeb, 2001), and by radiations of Caribbean terrestrial *Anolis* lizards, *Eleutherodactylus* frogs, and terrestrial crabs in the late Miocene and Pliocene (Hedges, 1989; Jackman et al., 1997; Schubart et al., 1998). Paleontological data suggest that a decline in nutrient concentrations in the West Atlantic was a major factor, together with increasing temperatures, fostering faunal turnover, and radiation of reef-dwelling organisms during this period (Allmon, 2001).

In addition to the main cluster of divergences at ~6 Mya in gambarelloides *Synalpheus*, there is a second, smaller mode at 3–4 Mya (Fig. 4). Similarly, the two bryozoan genera studied by Cheetham and Jackson (1996) each showed a second, minor wave of originations beginning between 5 and 3 Mya. This is around the time at which stable isotope, sea level, and fossil data indicate severe restriction of the Panamanian seaway (reviewed in Collins, 1996a) and the beginning of a major faunal turnover among Caribbean corals that culminated at the end of the Pliocene, ~2 Mya (Budd et al., 1996). Most of the values in this minor mode for *Synalpheus* correspond to divergences among species within the morphologically recognized *brooksi* complex and, especially, the *longicarpus* complex. The latter complex in particular consists of morphologically cryptic species that have yet to be described and that were poorly resolved in the phylogenetic analyses. The contemporaneous-tips KITSCH tree (Appendix D) also shows evidence of a rapid radiation of the *longicarpus* complex, in the form of a series of very short internal branches. This evidence of rapid radiation(s) in the sponge-dwelling *Synalpheus* help to explain both the poor resolution in our phylogenetic analysis, and the taxonomic difficulty for which the group is famous (Banner and Banner, 1975; Chace, 1972; Coutière, 1909; Dardeau, 1984).

Molecular clocks have wide confidence intervals (Hillis et al., 1996) and are potentially quite variable among taxa, even closely related ones (Collins, 1996b; see Li et al., 1987; Martin and Palumbi, 1993 for reviews). Our calibration was more rigorous than many, however, in using divergence values from two (admittedly linked) gene segments in three geminate pairs from the same genus as the ingroup. The similarity in divergence values among the three geminate pairs of *Synalpheus* increases confidence in the reliability of these dates and, even if they are somewhat skewed, sets a younger limit on the age of the radiation, insofar as it occurred before the geminates diverged approximately 3 Mya (Fig. 4).

Although we were unable to reconstruct detailed relationships among sponge-dwelling *Synalpheus* due to their rapid radiation, the tree resulting from analysis of the combined data has several important features. First, the gambarelloides clade is monophyletic in this analysis, albeit with low bootstrap support (65%). Interestingly, Bremer support for the gambarelloides clade (6 steps) is equal to or higher than several other clades in the tree that have considerably higher bootstrap support. According to the combined-data analysis, the sister taxon of the gambarelloides clade is the pair of transisthmian putative geminates *S. bannerorum* and *S. dominicensis*, which join with the gambarelloides group with a bootstrap value of 88%. Morphologically, these two taxa are quite distinct from the relatively uniform gambarelloides group, and we hypothesize that their relatively long branches (Fig. 2B) may help explain why they fall within the gambarelloides group in some analyses (Figs. 1A and 2A). In fact, removal of these

taxa from phylogenetic analyses greatly increases support for a monophyletic gambarelloides species-group. A second result of these analyses is confirmation of our previous finding (Duffy et al., 2000), which was based on only about a third of these taxa, that there are three independent origins of eusociality in the gambarelloides group. These are in *S. chacei*, *S.* "*paraneptunus* small," and the ancestor of *S. filidigitus* through *S. rathbunae* (Fig. 3).

Despite the poor resolution of basal relationships within the gambarelloides species-group, many important sister-taxon relationships are now well supported. This should allow for rigorous studies using comparative statistical tests involving the origin and maintenance of eusociality within a tightly defined phylogenetic group, something which has been difficult in eusocial insects due to both the ancient origins of eusociality and the paucity of robust phylogenies for groups in which eusociality has arisen.

Acknowledgments

C.L.M. and J.E.D. thank Nancy Knowlton for introducing us to alpheids in Panamá and sponsoring early explorations that launched this research. We are grateful to the NSF (DEB 92-01566, DEB 98-15785, and IBN-0131931, all to J.E.D.) and the Smithsonian Institution's Caribbean Coral Reef Ecosystem (CCRE) program for their generous support of this research. Dave Carlon, Mike Carpenter, Peter Dworschak, Javier Jara, Brian Kensley, Nancy Knowlton, Tripp Macdonald, Peter Marko, Joe Pawlik, Paul Renaud, and Klaus Ruetzler facilitated field work in various parts of the world or contributed specimens. We are grateful to all. We thank Mark Siddall for help with the 16S alignment, Scott Marion for technical support, and John Graves and Jan McDowell for sharing lab facilities and advice. This is CCRE contribution XXX and VIMS contribution XXX.

Appendix A. Taxa sampled for DNA and morphological analysis

Taxon	Collection locality	CODE	Host[a]	16S	COI
Synalpheus					
agelas	FL Keys, Molasses reef	agelFL01	Ac	new	new
anasimus	Porvenir, Panama	anasPA01	brown unid.	new	new
androsi	Carrie Bow Cay Ridge, Belize	andrBE01	Hi	new	new
"*bousfieldi* A"	Aquadargana, San Blas, Panama	bouaPA01	Ac	AF230260	new
"*bousfieldi* A"	Carrie Bow Cay Spur, Belize	bouaBE01	Ad	new	new
"*bousfieldi* B"	Curlew Ridge, Belize	boubBE01	Hc	new	new
brevifrons	Carrie Bow Cay Ridge, Belize	brefBE01	gray unid.	new	new
"*brooksi* A"	Sand Bores, Belize	brooBE02	Sv	new	new
"*brooksi* A"	Tiantupo, San Blas, Panama	brooPA07	Sv	new	new
"*brooksi* A"	Tiantupo, San Blas, Panama	brooPA01	Ac	AF230263	new
"*brooksi* D"	Limones, Panama	brodPA02	Lc	–	AF230790
"*brooksi* D"	Limones, Panama	brodPA01	Lc	AF230262	—
"*brooksi* D"	Carrie Bow Cay Ridge, Belize	brodBE01	Ha	new	new
"*brooksi* D"		brodBE02			new
chacei	Carrie Bow Cay Ridge, Belize	chacBE01	Hi	AF230261	new
chacei	Carrie Bow Cay Ridge, Belize	chacBE02	Ls	—	AF230792
"*chacei* A"	Curlew Ridge, Belize	chanBE01	Hc	new	new
"*pandionis* giant"	Limones, Panama	longPA01	Sv	AF230265	new
"*pandionis* giant"	Limones, Panama	lnpnPA02	Lc	new	new
filidigitus	Carrie Bow Cay Ridge, Belize	filiBE01	Xsp	AF230270	—
filidigitus	Carrie Bow Cay Ridge, Belize	filiBE02	Osp	—	new
gambarelloides	Croatia	gambCR01	Isp	new	new
goodei	Sand Bores, Belize	goodBE02	Pp	new	new
goodei	Sand Bores, Belize	goodBE04	Pp	new	new
"*occidentalis*" unid.	Isla Perico, Panama	dispPP01	gray unid.	new	new
"*occidentalis*" unid.		dispPP02		—	new
herricki	Carrie Bow Cay Ridge, Belize	herrBE01	Hi	new	new
"*intermediate*"	Sand Bores, Belize	kensBE01	yellow unid.	new	new
longicarpus	Tiantupo, San Blas, Panama	longPA03	Sv	new	new
"*longicarpus* small"	Sand Bores, Belize	lonsBE01	Pp	new	new
macclendoni	Sail Rock, San Blas, Panama	maccPA01	Ap	new	new
pandionis		pandBE02		new	new
"*pandionis* small"	Twin Cays, Belize	lnpnBE01	Lc	new	new
"*pandionis red*"	Aquadargana, San Blas, Panama	panrPA01	Sv	AF230266	new
paraneptunus	Portobelo, Panama	paraPA01		new	new
paraneptunus		paraPA02		—	new

Appendix A. (*continued*)

Taxon	Collection locality	CODE	Host[a]	16S	COI
paraneptunus	Carrie Bow Cay Ridge, Belize	paraBE01		—	new
paraneptunus	Carrie Bow Cay Slope, Belize	paraBE02	Osp	new	AF230793
paraneptunus		paraBE03	Pp	AF230267	new
"*paraneptunus* small"	White Banks, FL Keys	parsFL01	Xsp	AF230268	new
pectiniger	Tiantupo, San Blas, Panama	pectPA01	Sv	AF230259	—
pectiniger	Three Sisters, FL Keys	pectFL01	Sv	—	AF230796
rathbunae	Guigalatupo, San Blas, Panama	rathPA01	Xsp	new	new
"*rathbunae* A"	Carrie Bow Cay Ridge, Belize	rataBE07	Lsp	new	new
"*rathbunae* A"	Sand Bores, Belize	rataBE08	Hc	new	new
"*rathbunae* A"	Wichubhuala, San Blas, Panama	rataPA01	Hc	AF230269	new
"*rathbunae* A"	Pickles reef, FL Keys	rataFL01	blue unid.	—	AF230797
regalis	Carrie Bow Cay Ridge, Belize	regaBE01	Xsp	AF230271	—
regalis	Carrie Bow Cay Ridge, Belize	regaBE02	Xsp	—	AF230794
sanctithomae	Carrie Bow Cay Ridge, Belize	sancBE01	unid.	new	new
williamsi	Carrie Bow Cay Ridge, Belize	willBE02	He	AF230264	AF230795
fritzmuelleri		fritPA03	None	—	new
fritzmuelleri	Korbiski, San Blas, Panama	fritPA06	None	AF230798	AF230788
fritzmuelleri	Isla Bartolome, Panama	fritPP01	None	new	new
fritzmuelleri		fritPP01	None	—	new
minus	Korbiski, San Blas, Panama	minuPA02	None	new	new
minus		minuPA04	None	—	new
digueti	Isla Bartolome, Panama	diguPP01	None	new	new
digueti		diguPP03	None	—	new
brevicarpus		brevPA03	None	—	new
brevicarpus	San Blas, Panama	brevPA06	None	new	new
brevicarpus	Isla Bartolome, Panama	brepPP01	None	new	new
brevicarpus		brepPP04	None	—	new
brevicarpus		brepPP05	None	—	new
bannerorum	Isla Bartolome, Panama	bannPP01	None	new	new
dominicensis	Sail Rock, San Bias, Panama	domiPA01	None	new	new
Alpheus					
cylindricus	Panama	24-2e	Sv	AF230272	new

[a] Ac, *Agelas clathrodes*; Ad, *Agelas dispar*; Ap, *Acropora palmata* (coral); Ha, *Hymeniacidon amphiletcta*; Hc, *Hymeniacidon caerulea*; Hi, *Hyatella intestinalis*; Isp, *Ircinia* sp; Lc, *Lissodendoryx colombiensis*; Ls, *Lissodendoryx strongylata*; Osp, *Oceanapia* sp.; Pp, *Pachypellina podatypa*; Sv, *Spheciospongia vesparium*; Xsp, *Xestospongia* sp.; unid., unidentified.

Appendix B

List of informative morphological characters used in phylogenetic analysis of *Synalpheus*, with description of the states. Character numbers correspond to those in Appendix C.

(1) Carapace texture: 1, glabrous; 2, sparsely setose.

(2) Pterygostomian corner: 1, acute; 2, obtuse.

(3) Posterior margin of carapace with cardiac notch: 1, distinct; 2, diminished.

(4) Rostrum, compared to orbital teeth: 1, clearly narrower; 2, about as wide; 3, wider.

(5) Rostrum, compared to orbital teeth: 1, noticeably shorter; 2, about as long; 3, clearly longer.

(6) Rostrum: 1, distally upturned; 2, not upturned.

(7) Rostrum margins in dorsal view: 1, straight; 2, concave; 3, convex.

(8) Orbitorostral process: 1, absent; 2, present.

(9) Ocular hoods, shape in dorsal view: 1, sharply acute; 2, acute; 3, obtuse; 4, squarely rounded; 5, bluntly triangular.

(10) Adrostral sinus: 1, deep; 2, shallow.

(11) Ocular processes: 1, absent; 2, present, but not elongated; 3, produced.

(12) Ocellary beak: 1, rodlike; 2, not rodlike.

(13) Stylocerite: 1, slender; 2, stocky.

(14) Stylocerite, mesial margin: 1, slightly concave; 2, straight; 3, convex.

(15) Stylocerite: 1, acute; 2, blunt.

(16) Stylocerite, length compared to distal margin of first segment of antenna 1: 1, clearly exceeding; 2, about the same; 3, distinctly shorter.

(17) Mesio-ventral tooth on first segment of antenular peduncle: 1, present; 2, absent.

(18) Ventral basal processes on antenna 1:1, none; 2, one; 3, two.

(19) Spine on dorso-lateral corner of basicerite: 1, absent; 2, present.

(20) Ventrolateral spine of basicerite compared to tip of Stylocerite: 1, clearly overreaching; 2, not overreaching.

(21) Scaphocerite blade: 1, present; 2, reduced; 3, absent.

(22) Lateral margin of scaphocerite: 1, straight; 2, slightly concave.

(23) Scaphocerite spine compared to antennular peduncle: 1, not overreaching; 2, clearly overreaching.

(24) Mesial projection at base of scaphocerite: 1, absent; 2, present.

(25) Fingers of major first pereiopod compared to half length of palm: 1, clearly not longer; 2, clearly longer.

(26) Pollex of major first pereiopod compared to dactyl: 1, about as long; 2, reduced; 3, longer.

(27) Protuberance on outer face of pollex of major chela: 1, absent; 2, present.

(28) Kind of projection on superior distal margin of palm of major chela: 1, prominent blunt tubercle; 2, prominent tubercle with acute spine; 3, tapering acute spine.

(29) Extensor margin of merus of major first pereiopod: 1, straight or slightly convex; 2, strongly convex.

(30) Extensor margin of merus of major first pereiopod: 1, with distinct distal spine; 2, with flat distal angular projection; 3, ending in acute angle; 4, ending in right angle; 5, ending in obtuse angle.

(31) Palm of minor first chela: 1, clearly less than two times longer than high; 2, about two times longer than high; 3, more than twice as longer as high.

(32) Number of teeth on dactyl of minor first chela: 1, one; 2, one with subdistal accessory bump; 3, two or three, subequal in length.

(33) Arrangement of dactyl teeth on minor first chela in relation to dactyl axis: 1, perpendicular; 2, parallel.

(34) Transverse dorsal setal combs on dactyl of minor first chela: 1, absent; 2, very conspicuous; 3, much reduced.

(35) Number of teeth on pollex of minor first chela: 1, one; 2, one with subdistal accessory bump; 3, two subequal in length.

(36) Extensor margin of merus of minor first pereiopod: 1, straight; 2, convex.

(37) Extensor margin of merus of minor first pereiopod: 1, with distinct distal spine; 2, with flat distal angular projection; 3, ending in acute angle; 4, ending in right angle; 5, ending in obtuse angle.

(38) Segments on carpus of second pereiopod: 1, five; 2, four.

(39) Second pereiopod, carpus/merus length relation: 1, >1; 2, $\leqslant 1$.

(40) Third pereiopod: 1, slender; 2, stout.

(41) Relative size of ungues on dactyl of third pereiopod: 1, subequal; 2, clearly unequal.

(42) Unguis, widest at base: 1, extensor 2, flexor.

(43) Movable spines on flexor margin of merus of third pereiopod: 1, absent; 2, present.

(44) Mesial lamella on coxa of third pereiopod: 1, absent; 2, present.

(45) First abdominal pleura of male with posterior corner: 1, weakly produced, or rounded; 2, strongly produced posteriorly; 3, acutely produced ventrally; 4, distinctly produced ventrally and anteriorly, hook-like.

(46) Second abdominal pleura of male: 1, rounded to obtuse; 2, produced posteriorly into acute projection; 3, produced both anteriorly and posteriorly into acute projections.

(47) Terminal setae on endopod of male first pleopod: 1, five or less; 2, six or more.

(48) Second pleopod of male with marginal setae on exopod originating: 1, close to base; 2, near midpoint.

(49) Appendix interna on second to fifth male pleopods: 1, present; 2, absent.

(50) The space between distal spines of telson compared to one-third of its distal margin: 1, greater; 2, equal or less.

(51) Convex lobe on distal margin of telson: 1, present; 2, absent.

(52) Posterior corners of telson: 1, obtuse; 2, rectangular; 3, acute.

(53) Postanal setal brush: 1, absent; 2, present.

(54) Number of fixed teeth on outer margin of uropodal exopod: 1, one; 2, more than one.

Appendix C. Matrix of morphological character states for *Synalpheus* taxa and the outgroup, *Alpheus cylindricus*

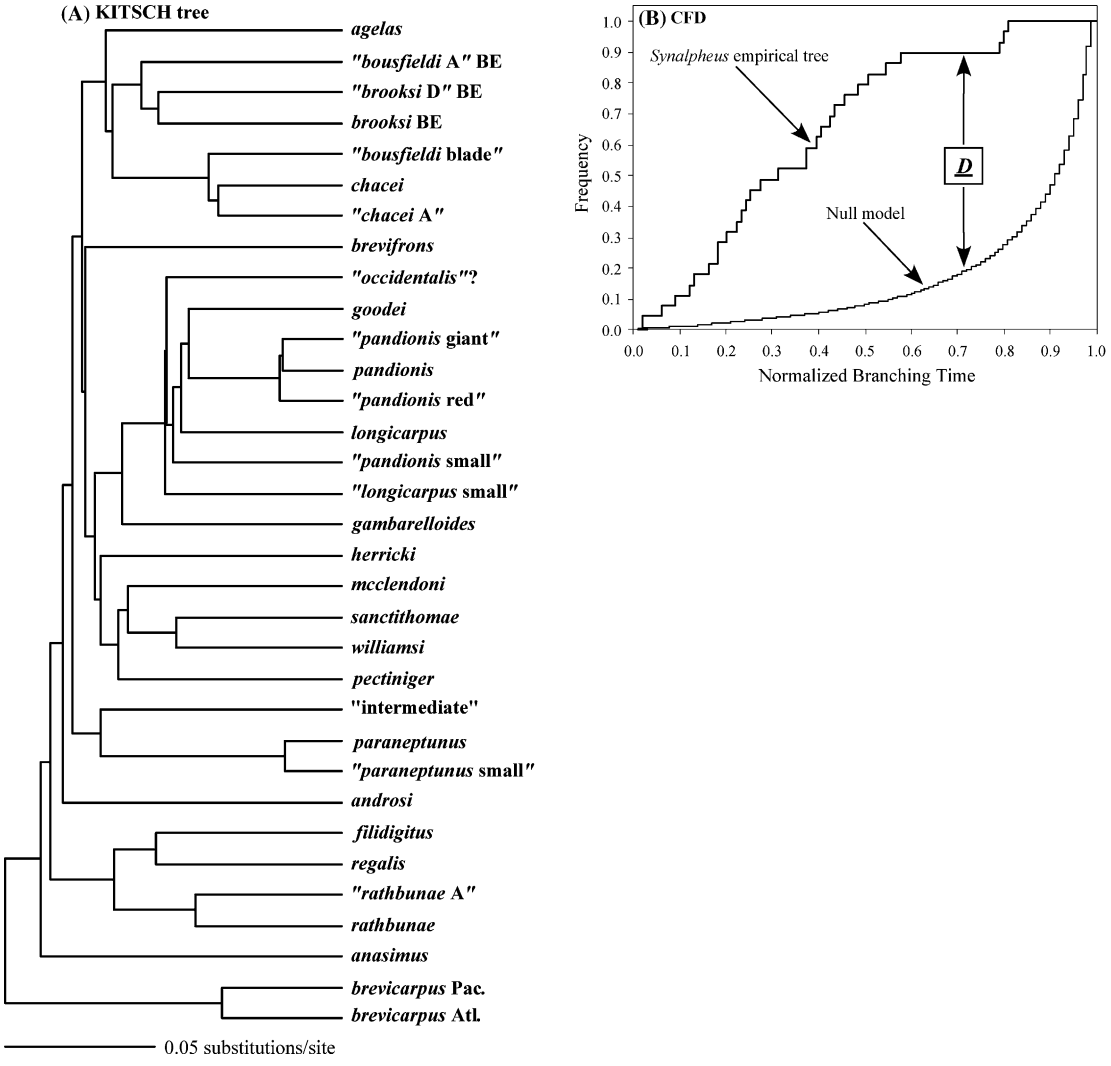
Taxon	Characters																										
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Alpheus cylindricus	1	2	1	1	3	2	1	1	3	2	2	2	2	3	1	3	1	1	1	2	2	2	1	2	1	2	1
Synalpheus agelas	2	1	1	1	2	1	(2 3)	1	2	1	2	2	1	1	1	3	2	3	1	1	2	2	1	2	1	2	1
S. anasimus	1	1	1	1	(2 3)	1	1	1	2	1	2	2	2	1	1	2	2	3	1	1	2	2	2	2	1	1	1
S. androsi	1	1	1	2	2	1	(1 2)	1	2	1	2	2	2	(1 2)	1	2	2	3	1	1	3	2	2	2	1	2	1
S. bannerorum	2	1	1	1	3	2	1	2	1	1	1	1	1	2	1	1	1	3	2	1	1	2	2	2	1	1	1
S. "*bousfieldi* A"	1	1	1	1	3	1	2	1	5	1	2	2	1	1	1	2	2	3	1	1	3	1	1	2	1	1	1
S. "*bousfieldi* blade"	2	1	1	1	3	1	2	1	5	1	2	2	1	1	1	2	2	3	1	1	2	2	1	2	1	1	1
S. brevicarpus ATL	1	1	1	2	2	1	1	1	2	1	2	2	1	2	1	1	1	2	1	2	1	2	2	2	1	1	2
S. brevicarpus PAC	1	1	1	1	2	1	2	1	5	1	2	2	1	1	1	1	1	2	2	2	1	2	1	2	1	2	2
S. brevifrons	1	1	1	1	2	2	1	1	3	2	2	2	1	1	1	2	2	3	1	1	3	1	2	2	1	1	1
S. brooksi	1	2	1	1	2	1	(2 3)	1	2	1	1	1	2	1	2	3	2	3	1	1	3	1	1	2	1	1	1
S. "*brooksi* D"	1	1	1	1	2	1	1	1	2	1	2	1	2	1	2	3	2	3	1	1	3	2	1	2	1	1	1
S. chacei	1	2	1	1	2	1	2	1	5	1	1	2	2	(1 3)	2	3	2	3	1	1	3	2	1	2	1	1	1
S. "*chacei* A"	2	1	1	1	2	1	3	1	5	1	2	2	2	(2 3)	1	3	2	3	1	1	3	2	1	2	1	1	1
S. digueti	1	1	1	2	2	2	2	1	5	1	2	2	1	1	1	1	1	2	1	2	1	2	1	2	2	1	1
S. dominicensis	1	1	1	1	3	2	1	2	1	1	1	1	1	1	1	1	1	3	2	2	1	1	2	1	(1 2)	1	1
S. filidigitus	1	2	1	1	2	1	1	1	2	1	1	1	1	1	1	2	2	1	2	1	3	1	2	2	1	2	1
S. fritzmuelleri ATL	2	1	1	1	3	2	1	2	1	1	2	2	1	1	1	1	1	1	2	2	1	2	2	2	1	1	1
S. fritzmuelleri PAC	1	1	1	1	2	3	1	2	1	1	2	2	1	2	1	1	1	1	2	2	1	2	2	1	1	1	1
S. gambarelloides	1	1	1	1	2	1	1	1	2	1	2	2	1	1	1	2	2	3	1	1	2	2	1	2	1	2	2
S. goodei	1	1	1	1	2	1	1	1	2	1	2	2	1	1	1	2	2	3	2	1	2	2	2	2	1	1	1
S. herricki	2	1	1	3	2	1	3	1	5	1	3	2	1	3	1	3	2	3	1	1	3	2	1	2	1	1	1
S. "*intermediate*"	1	1	1	1	2	1	1	1	2	1	2	2	1	1	1	2	2	3	2	1	3	2	2	2	1	1	1
S. longicarpus	1	1	2	1	2	1	1	1	2	1	2	1	1	(2 3)	1	2	2	3	1	2	2	1	2	2	1	2	1
S. "*longicarpus* small"	1	1	1	1	3	1	1	1	2	1	2	1	1	1	1	2	2	3	1	1	2	2	2	2	1	2	2
S. mcclendoni	1	1	1	1	2	2	(2 3)	1	2	1	2	2	1	1	2	1	1	3	1	1	1	1	2	2	2	3	1
S. minus	2	1	1	2	2	1	2	1	2	1	2	2	1	1	1	1	1	2	1	2	1	2	2	2	1	1	1
S. occidentalis?	1	1	1	1	(1 2)	1	1	1	2	1	2	2	1	2	1	2	2	3	1	1	3	2	1	2	1	2	1
S. paraneptunus	2	2	1	1	2	1	(2 3)	1	2	1	2	1	1	1	1	1	2	3	1	1	2	2	2	2	1	2	2
S. "*paraneptunus* small"	2	2	1	1	3	1	1	1	2	1	2	1	1	1	1	1	2	3	1	1	(2 3)	2	2	2	1	1	2
S. pandionis	1	1	1	1	2	1	1	1	4	1	3	2	1	1	1	3	2	3	1	1	1	2	1	2	1	2	2
S. "*pandionis* giant"	1	1	1	1	2	1	1	1	2	1	3	2	1	3	1	3	2	3	1	1	2	2	1	2	1	2	2
S. "*pandionis* red"	1	1	1	1	2	2	1	2	4	1	2	2	1	(1 3)	1	3	2	3	1	1	3	2	1	2	1	2	2
S. "*pandionis* small"	1	1	1	1	2	2	1	2	4	1	2	1	1	1	1	2	2	3	1	1	3	2	1	2	1	2	1
S. pectiniger	1	1	2	2	2	1	2	1	2	1	2	2	1	(1 3)	1	2	2	3	1	1	3	2	1	2	1	2	1
S. rathbunae	1	2	1	1	2	1	1	1	2	1	1	2	1	1	1	2	2	3	2	1	3	(1 2)	2	2	1	1	1
S. "*rathbunae* A"	2	1	1	1	2	1	1	1	5	1	2	1	1	1	1	3	2	3	2	1	3	1	2	2	1	2	2
S. regalis	2	2	1	1	2	1	1	1	4	1	1	2	2	1	1	3	2	3	2	1	3	2	1	2	1	1	1
S. sanctithomae	1	1	1	1	2	1	2	1	5	1	2	1	1	2	2	2	1	3	1	1	1	2	1	2	1	2	2
S. williamsi	2	(1 2)	1	1	2	1	1	1	2	1	2	1	1	1	1	2	2	3	2	1	2	2	2	2	1	2	2

Appendix C. (*continued*)

Taxon	Characters																										
	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
Alpheus cylindricus	—	1	2	1	1	—	1	1	2	2	1	1	1	2	1	1	1	1	1	2	1	1	1	2	1	1	1
Synalpheus agelas	1	2	2	1	3	1	2	2	2	5	2	2	1	2	2	1	2	4	1	1	1	1	1	1	3	1	2
S. anasimus	3	2	5	1	3	2	2	3	2	3	1	2	1	2	2	1	2	3	1	1	1	1	2	1	1	1	(1 2)
S. androsi	1	2	2	1	2	2	2	2	1	5	1	1	1	2	1	1	2	3	1	1	2	1	1	1	1	1	1
S. bannerorum	3	2	1	1	2	1	1	2	1	1	1	1	2	2	2	2	1	2	1	1	2	1	1	1	3	1	1
S. "*bousfieldi* A"	3	2	5	1	3	2	2	3	2	5	1	2	1	1	—	1	2	3	1	2	2	1	2	1	1	1	1
S. "*bousfieldi* blade"	2	2	5	1	3	2	2	3	2	5	1	2	1	2	1	1	2	3	1	1	2	1	2	1	1	1	1
S. brevicarpus ATL	3	2	2	1	2	1	1	2	2	2	1	1	1	2	1	1	1	2	1	2	1	1	1	1	3	1	1
S. brevicarpus PAC	2	1	2	1	2	1	1	2	2	2	1	1	2	2	1	1	1	2	1	2	1	1	1	1	3	1	1
S. brevifrons	1	2	5	1	3	2	2	3	2	5	1	2	1	1	—	1	2	4	3	1	2	1	1	1	1	1	1
S. brooksi	3	2	5	1	3	2	2	3	2	4	1	2	1	2	2	1	2	4	1	1	2	1	2	1	2	1	1
S. "*brooksi* D"	3	2	5	1	3	2	2	3	2	4	1	2	1	2	2	1	2	4	1	1	2	1	2	1	1	1	1
S. chacei	1	2	5	1	3	2	2	3	2	3	1	2	1	1	—	1	2	3	1	2	2	1	2	1	1	1	1
S. "*chacei* A"	1	2	5	1	3	2	2	3	2	5	1	1	1	1	—	1	2	3	1	1	2	1	2	1	1	1	1
S. digueti	3	1	1	1	2	1	1	2	2	2	1	1	1	2	1	1	1	2	1	1	1	1	1	1	3	1	1
S. dominicensis	3	1	1	1	2	1	1	2	2	1	1	1	1	2	2	2	1	2	1	1	2	1	1	1	3	1	1
S. filidigitus	2	2	5	1	2	1	2	1	2	5	2	2	1	2	2	1	2	3	1	1	2	1	2	2	1	1	2
S. fritzmuelleri ATL	1	2	1	2	2	1	1	2	1	1	1	1	1	2	2	1	1	1	1	1	1	2	1	1	3	1	1
S. fritzmuelleri PAC	4	1	1	1	2	1	1	3	1	3	1	1	2	2	2	1	1	3	1	2	1	2	1	1	3	1	1
S. gambarelloides	3	1	2	2	2	1	2	2	2	5	1	1	1	1	—	1	2	3	1	2	2	1	1	1	2	1	1
S. goodei	2	2	2	2	3	1	2	2	2	3	1	1	1	1	—	1	2	3	1	2	1	1	2	1	1	2	2
S. herricki	3	2	2	1	3	2	2	3	2	5	1	1	1	1	—	1	2	3	1	2	2	1	2	1	2	1	1
S. "*intermediate*"	2	2	2	1	3	1	2	2	2	2	1	1	1	2	2	1	2	1	1	2	1	1	1	1	2	1	2
S. longicarpus	2	1	2	3	3	2	2	2	2	3	1	2	1	2	1	1	2	3	2	1	1	1	2	1	2	1	2
S. "*longicarpus* small"	2	1	2	3	2	2	2	2	2	5	1	2	1	1	—	1	2	4	1	2	1	1	2	1	1	2	2
S. mcclendoni	2	2	2	1	1	—	2	1	2	3	1	2	1	2	2	1	2	4	1	2	1	1	1	1	3	1	1
S. minus	3	2	1	1	2	1	1	2	2	2	1	1	1	2	1	1	1	1	1	1	2	1	1	1	3	1	1
S. occidentalis?	2	1	2	3	3	2	2	3	2	5	1	2	1	1	—	1	2	3	1	2	1	1	2	1	1	1	2
S. paraneptunus	3	2	2	1	3	1	3	3	2	2	2	2	2	2	1	1	2	1	1	1	2	1	1	1	3	1	2
S. "*paraneptunus* small"	3	2	2	1	3	1	3	3	2	5	2	2	2	2	1	1	2	1	1	1	2	1	1	2	2	1	2
S. pandionis	2	2	2	3	3	2	2	2	2	3	1	2	2	2	1	1	2	3	2	2	1	1	2	1	1	2	2
S. "*pandionis* giant"	2	2	2	3	3	2	2	3	2	5	1	1	1	2	2	1	2	4	2	2	1	1	2	1	2	2	2
S. "*pandionis* red"	2	2	2	1	3	2	2	3	2	5	1	2	1	2	2	1	2	4	2	2	1	1	2	2	2	1	2
S. "*pandionis* small"	2	2	2	2	2	2	2	2	2	4	1	1	2	2	2	1	2	4	2	2	1	1	2	2	2	1	2
S. pectiniger	3	1	2	1	3	2	2	3	2	5	1	1	1	2	2	1	2	4	2	1	1	1	2	2	1	1	2
S. rathbunae	1	2	5	2	3	1	2	1	2	3	2	2	1	2	2	1	2	3	2	2	1	1	2	2	1	1	2
S. "*rathbunae* A"	2	2	2	1	1	—	2	1	2	5	2	2	1	2	2	1	2	4	2	1	2	1	2	2	1	1	2
S. regalis	1	2	5	1	2	1	2	1	2	3	2	2	1	2	2	1	2	3	2	2	2	1	2	2	1	1	2
S. sanctithomae	2	2	2	1	2	2	2	2	2	4	1	2	1	2	1	1	2	4	1	1	2	1	1	1	3	1	1
S. williamsi	2	2	2	1	2	1	2	2	2	2	1	1	1	1	—	1	2	4	1	1	1	1	1	1	2	1	2

Appendix D

Statistical test of departure from temporally random cladogenesis in the gambarelloides species-group. (A) Contemporaneous-tips KITSCH tree for species in the gambarelloides group. (B) Cumulative frequency distributions of normalized branching times inferred from combined analysis of COI and 16S data, compared to a null model of Markovian bifurcation and extinction for 32 taxa (after Wollenberg et al., 1996).



References

Allmon, W.D., 2001. Nutrients, temperature, disturbance, and evolution: a model for the late Cenozoic marine record of the western Atlantic. Palaeogeogr., Palaeoclimatol., Palaeoecol. 166, 9–26.

Banner, D.M., Banner, A.H., 1975. The alpheid shrimp of Australia. Part 2: the genus *Synalpheus*. Rec. Aust. Mus. 29, 267–389.

Bermingham, E., McCafferty, S.S., Martin, A.P., 1997. Fish biogeography and molecular clocks: perspectives from the Panamanian Isthmus. In: Kocher, T.D., Stepien, C.A. (Eds.), Molecular Systematics of Fishes. Academic Press, San Diego, pp. 113–128.

Bremer, K., 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evolution 42, 795–803.

Bremer, K., 1994. Branch support and tree stability. Cladistics 10, 295–304.

Bruce, A.J., 1976. Shrimps and prawns of coral reefs, with special reference to commensalism. In: Jones, O.A., Endean, R. (Eds.), Biology and Geology of Coral Reefs. Biology 2, vol. III. Academic Press, New York, pp. 37–94.

Budd, A.F., Johnson, K.G., Stemann, T.A., 1996. Plio-pleistocene turnover and extinctions in the Caribbean reef-coral fauna. In: Jackson, J.B.C., Budd, A.F., Coates, A.G. (Eds.), Evolution and Environment in Tropical America. The University of Chicago Press, Chicago, pp. 168–204.

Bull, J.J., Huelsenbeck, J.P., Cunningham, C.W., Swofford, D.L., Waddell, P.J., 1993. Partitioning and combining data in phylogenetic analysis. Syst. Biol. 50, 67–86.

Chace Jr., F.A., 1972. The shrimps of the Smithsonian–Bredin Caribbean expeditions with a summary of the West Indian shallow-water species (Crustacea: Decapoda: Natantia). Smithsonian Contr. Zool. 98, 179 pp.

Chace Jr., F.A., 1989. The caridean shrimps (Crustacea: Decapoda) of the *Albatross* Philippine expedition, 1907–1910, Part 5: family Alpheidae. Smithsonian Contr. Zool 46, 1–99.

Cheetham, A.H., Jackson, J.B.C., 1996. Speciation, extinction, and the decline of arborescent growth in Neogene and Quaternary Cheilostome Bryozoa of tropical America. In: Jackson, J.B.C., Budd, A.F., Coates, A.G. (Eds.), Evolution and Environment in Tropical America. The University of Chicago Press, Chicago, pp. 205–233.

Christofferson, M.L., 1979. Campagne de la Calypso au large des côtes Atlantiques de l'Amérique du sud (1961–1962). I. Decapod Crustacea: Alpheoida. Résult. Scient. Camp. Calypso 11, 297–377.

Coates, A.G., Obando, J.A., 1996. The geologic evolution of the Central American Isthmus. In: Jackson, J.B.C., Budd, A.F., Coates, A.G. (Eds.), Evolution and Environment in Tropical America. The University of Chicago Press, Chicago, pp. 21–56.

Collins, L.S., 1996a. Environmental changes in Caribbean shallow waters relative to the closing of the Central American Seaway. In: Jackson, J.B.C., Budd, A.F., Coates, A.G. (Eds.), Evolution and Environment in Tropical America. The University of Chicago Press, Chicago, pp. 130–167.

Collins, T., 1996b. Molecular comparisons of transisthmian species pairs: rates and patterns of evolution. In: Jackson, J.B.C., Budd, A.F., Coates, A.G. (Eds.), Evolution and Environment in Tropical America. The University of Chicago Press, Chicago, pp. 303–334.

Coutière, H., 1909. The American species of snapping shrimps of the genus *Synalpheus*. Proc. United States Natl. Museum 36, 1–93.

Cronin, T.M., Dowsett, H.J., 1996. Biotic and oceanographic response to the Pliocene closing of the Central American Isthmus. In: Jackson, J.B.C., Budd, A.F., Coates, A.G. (Eds.), Evolution and Environment in Tropical America. The University of Chicago Press, Chicago, pp. 76–104.

Cunningham, C.W., 1997. Can three incongruence tests predict when data should be combined? Mol. Biol. Evol. 14, 733–740.

Dardeau, M.R., 1984. *Synalpheus* shrimps (Crustacea: Decapoda: Alpheidae). I. The gambarelloides group, with a description of a new species. Mem. Hourglass Cruises 7 (Part 2), 1–125.

De Queiroz, A., 1993. For consensus (sometimes). Syst. Biol. 42, 368–372.

Donoghue, M.J., Olmstead, R.G., Smith, J.F., Palmer, J.D., 1992. Phylogenetic relationships of *Dipscales* based on *rbcL* sequences. Ann. Mo. Bot. Gard. 79, 333–345.

Dowton, M., Austin, A.D., 2002. Increased congruence does not necessarily indicate increased phylogenetic accuracy—the behavior of the incongruence length difference test in mixed-model analyses. Syst. Biol. 51 (1), 19–31.

Duffy, J.E., 1996a. Eusociality in a coral-reef shrimp. Nature 381, 512–514.

Duffy, J.E., 1996b. Resource-associated population subdivision in a symbiotic coral-reef shrimp. Evolution 50, 360–373.

Duffy, J.E., 1996c. Specialization, species boundaries, and the radiation of sponge-dwelling alpheid shrimp. Biol. J. Linn. Soc. 58, 307–324.

Duffy, J.E., 1996d. *Synalpheus regalis*, new species, a sponge-dwelling shrimp from the Belize Barrier Reef, with comments on host specificity in *Synalpheus*. J. Crust. Biol. 16, 564–573.

Duffy, J.E., 1998. On the frequency of eusociality in snapping shrimps (Decapoda: Alpheidae), with description of a second eusocial species. Bull. Mar. Sci. 63, 387–400.

Duffy, J.E., Macdonald, K.S., 1999. Colony structure of the social snapping shrimp, *Synalpheus filidigitus*, in Belize. J. Crust. Biol. 19, 283–292.

Duffy, J.E., Morrison, C.L., Rios, R., 2000. Multiple origins of eusociality among sponge-dwelling shrimps (*Synalpheus*). Evolution 54 (2), 503–516.

Duffy, J.E., Morrison, C.L., Macdonald, K.S., 2002. Colony defense and behavioral differentiation in the eusocial shrimp *Synalpheus regalis*. Behav. Ecol. Sociobiol. 51, 488–495.

Farris, J.S., Källersjö, M., Kluge, A.J., Bult, C., 1994b. Testing significance of incongruence. Cladistics 10, 315–319.

Felsenstein, J., 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. J. Mol. Evol. 17, 368–376.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Felsenstein, J., 1993. Phylogeny Inference Package (PHYLIP), ver. 5.3c. Department of Genetics, University of Washington, Seattle.

Fishbein, M., Hibsch-Jetter, C., Soltis, D.E., Hufford, L., 2001. Phylogeny of Saxifragales (Angiosperms, Eudicots): analysis of a rapid, ancient radiation. Syst. Biol. 50, 817–847.

Fu, J., 2000. Toward the phylogeny of the family Lacertidae—why 4708 base pairs of mtDNA sequences cannot draw the picture. Biol. J. Linn. Soc. 71, 203–217.

Givnish, T.J., Sytsma, K.J. (Eds.), 1997. Molecular Evolution and Adaptive Radiation. Cambridge University Press, Cambridge, UK.

Hedges, S.B., 1989. An island radiation: allozyme evolution in Jamaican frogs of the genus *Eleutherodactylus* (Leptodactylidae). Carib. J. Sci. 25, 123–147.

Hillis, D.M., Mable, B.K., Moritz, C., 1996. Applications of molecular systematics: the state of the field and a look to the future. In: Hillis, D.M., Moritz, C., Mable, B.K. (Eds.), Molecular Systematics, second ed. Sinauer Associates, Inc, Sunderland, MA, pp. 515–543.

Huelsenbeck, J.P., Bull, J.J., Cunningham, C.C., 1996. Combining data in phylogenetic analysis. Trends Ecol. Evol. 11, 152–157.

Huelsenbeck, J.P., Larget, B., Miller, R.E., Ronquist, F., 2002. Potential applications and pitfalls of Bayesian inference of phylogeny. Syst. Biol. 51, 673–688.

Huelsenbeck, J.P., Ronquist, F., 2000. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Jackman, T., Losos, J.B., Larson, A., de Queiroz, K., 1997. Phylogenetic studies of convergent adaptive radiations in Caribbean *Anolis* lizards. In: Givnish, T.J., Systsma, K.J. (Eds.), Molecular Evolution and Adaptive Radiation. Cambridge University Press, Cambridge, UK, pp. 535–557.

Johns, G.C., Avise, J.C., 1998. Tests for ancient species flocks based on molecular phylogenetic appraisals of *Sebastes* rockfishes and other marine fishes. Evolution 52, 1135–1146.

Jordal, B.H., Normark, B.B., Farrell, B.D., 2000. Evolutionary radiation of an inbreeding haplodiploid beetle lineage (Curculionidae, Scolytinae). Biol. J. Linn. Soc. 71, 483–499.

Kimura, M., 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16, 111–120.

Knowlton, N., Weigt, L.A., 1998. New dates and new rates for divergence across the Isthmus of Panama. Proc. R. Soc. Lond. B 265, 2257–2263.

Knowlton, N., Weigt, L.A., Solorzano, L.A., Mills, D.K., Bermingham, E., 1993. Divergence in proteins, mitochondrial DNA, and reproductive compatibility across the Isthmus of Panama. Science 260, 1629–1632.

Lanave, C., Preparata, G., Saccone, C., Serio, G., 1984. A new method for calculating evolutionary substitution rates. J. Mol. Evol. 20, 86–93.

Larget, B., Simon, D.L., 1999. Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. Mol. Biol. Evol. 16, 750–759.

Larson, A., 1994. The comparison of morphological and molecular data in phylogenetic systematics. In: Schierwater, B., Streit, B., Wagner, G.P., DeSalle, R. (Eds.), Molecular Ecology and Evolution: Approaches and Applications. Birkhäuser, Berlin, pp. 371–390.

Li, W.-H., Tanimura, M., Sharp, P.M., 1987. An evaluation of the molecular clock hypothesis using mammalian DNA sequences. J. Mol. Evol. 5, 313–330.

Lovette, I.J., Bermingham, E., 1999. Explosive speciation in the New World Dendroica warblers. Proc. R. Soc. Lond. B 266, 1629–1636.

Mardulyn, P., Whitfield, J.B., 1999. Phylogenetic signal in the COI, 16S, and 28S genes for inferring relationships among genera of Microgastrinae (Hymenoptera; Braconidae): evidence of a high diversification rate in this group of parasitoids. Mol. Phylogenet. Evol. 12, 282–294.

Martin, A.P., Palumbi, S.R., 1993. Body size, metabolic rate, generation time, and the molecular clock. Proc. Natl. Acad. Sci. USA 90, 4087–4091.

Palumbi, S.R., Martin, A.P., Romano, S., McMillian, W.O., Stice, L., Grabowski, G., 1991. The Simple Fool's Guide to PCR. Special Publishing Department, Zoology, University of Hawaii, Honolulu.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Posada, D., Crandall, K.A., 2001. Selecting the best-fit model of nucleotide substitution. Syst. Biol. 50, 580–601.

Rannala, B., Yang, Z., 1996. Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. J. Mol. Evol. 43, 304–311.

Reed, J.K., Gore, R.H., Scotto, L.E., Wilson, K.A., 1982. Community composition, areal and trophic relationships of decapods associated with shallow- and deep-water *Oculina varicosa* coral reefs. Bull. Mar. Sci. 32, 761–786.

Ríos, R., Duffy, J.E., 1999. Description of *Synalpheus williamsi*, a new species of sponge-dwelling shrimp (Crustacea: Decapoda: Alpheidae), with remarks on its first larval stage. Proc. Biol. Soc. Wash. 112, 541–552.

Rodriguez, F.J., Oliver, J., Martin, L., Medina, J.R., 1990. The general stochastic model of nucleotide substitution. J. Theor. Biol. 142, 485–501.

Schubart, C.D., Diesel, R., Hedges, S.B., 1998. Rapid evolution to terrestrial life in Jamaican crabs. Nature 393, 363–365.

Schluter, D., 2000. The Ecology of Adaptive Radiation. Oxford University Press, Oxford, UK.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., Flook, P., 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Ent. Soc. Am. 87 (6), 651–701.

Snelgrove, P.V.R., Lewis, J.B., 1989. Response of a coral-associated crustacean community to eutrophication. Mar. Biol. 101, 249–257.

Sokal, R.R., Rohlf, F.J., 1995. Biometry. Freeman, New York.

Sorenson, M.D., 1999. TreeRot. Boston University, Boston, MA.

Stanger-Hall, K., Cunningham, C.W., 1998. Support for a monophyletic Lemuriformes: overcoming incongruence between data partitions. Mol. Biol. Evol. 15, 1572–1577.

Stillman, J.H., Reeb, C.A., 2001. Molecular phylogeny of Eastern Pacific porcelain crabs, genera Petrolisthes and Pachycheles, based on the mtDNA 16S rDNA sequence: phylogeographic and systematic implications. Mol. Phylogenet. Evol. 19, 236–245.

Swofford, D.L., 2002. PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4. Sinauer Associates, Sunderland, MA.

Tamura, K., Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10, 512–526.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.

Von Dohlen, C.D., Moran, N.A., 2000. Molecular data support a rapid radiation of aphids in the Cretaceous and multiple origins of host alternation. Biol. J. Linn. Soc. 71, 689–717.

Wheeler, W.C., Cartwright, P., Hayashi, C.Y., 1993. Arthropod phylogeny: a combined approach. Cladistics 9, 1–39.

Wheeler, W.C., Gladstein, D., 1992. MALIGN. American Museum of Natural History, New York.

Wheeler, W.C., Gladstein, D., 1994. MALIGN: a multiple sequence alignment program. J. Hered. 85, 417.

Whittingham, L.A., Slikas, B., Winkler, D.W., Sheldon, F.H., 2002. Phylogeny of the tree swallow genus, *Tachycinecta* (Aves: Hirundinidae), by Bayesian analysis of mitochondrial DNA sequences. Mol. Phylogenet. Evol. 22, 430–441.

Wicksten, M.K., 1994. Taxonomic remarks on two species of the genus *Synalpheus* from the tropical Eastern Pacific. Bull. Mus. Natl. Hist. Nat. Paris 4^{e} sér. 16 (1), 209–216.

Wilcox, T.P., Zwickl, D.J., Heath, T.A., Hillis, D.M., 2002. Phylogenetic relationships of the dwarf boas and a comparison of Bayesian and bootstrap measures of phylogenetic support. Mol. Phylogenet. Evol. 25, 361–371.

Williams, S.T., Knowlton, N., 2001. Mitochondrial pseudogenes are pervasive and often insidious in the snapping shrimp genus *Alpheus*. Mol. Biol. Evol. 18, 1484–1493.

Williams, P.L., Fitch, W.M., 1990. Phylogeny determination using the dynamically weighted parsimony method. Methods Enzymol. 183, 615–626.

Wollenberg, K., Arnold, J., Avise, J.C., 1996. Recognizing the forest for the trees: testing temporal patterns of cladogenesis using a null model of stochastic diversification. Mol. Biol. Evol. 13 (6), 833–849.

Yoder, A.D., Irwin, J.A., Payseur, B.A., 2001. Failure of the ILD to determine data combinability for slow loris phylogeny. Syst. Biol. 50, 408–424.