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MULTIPLE ORIGINS OF EUSOCIALITY AMONG SPONGE-DWELLING SHRIMPS (*SYNALPHEUS*)

J. EMMETT DUFFY,[1] CHERYL L. MORRISON, AND RUBÉN RÍOS
College of William and Mary, School of Marine Science, Gloucester Point, Virginia 23062-1346
[1]*E-mail: jeduffy@vims.edu*

Abstract.—As the most extreme expression of apparent altruism in nature, eusociality has long posed a central paradox for behavioral and evolutionary ecology. Because eusociality has arisen rarely among animals, understanding the selective pressures important in early stages of its evolution remains elusive. Employing a historical approach to this problem, we used morphology and DNA sequences to reconstruct the phylogeny of 13 species of sponge-dwelling shrimps (*Synalpheus*) with colony organization ranging from asocial pair-bonding through eusociality. We then used phylogenetically independent contrasts to test whether sociality was associated with evidence of enhanced competitive ability, as suggested by hypotheses invoking an advantage of cooperation in crowded habitats. The molecular, morphological, and combined data each strongly supported three independent origins of monogynous, multigenerational (eusocial) colony organization within this genus. Phylogenetically independent contrasts confirmed that highly social taxa, with strong reproductive skew, have significantly higher relative abundance within the host sponge than do less social taxa, a result that was robust to uncertainty in tree topology and varying models of character change. A similar tendency for highly social species to share their sponge with fewer congener species was suggestive, but not significant. Because unoccupied habitat appears to be limiting for many sponge-dwelling shrimp species, these data are consistent with hypotheses that cooperative social groups enjoy a competitive advantage over less organized groups or individuals, where independent establishment is difficult, and that enemy pressure is of central importance in the evolution of animal sociality.

Key words.—Competition, eusociality, phylogenetically independent contrasts, phylogeny, snapping shrimps, social evolution, *Synalpheus.*

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Eusociality is characterized by cooperative colonies in which most members sacrifice individual reproduction. Because it represents the most striking apparent counterexample to Darwinian self-interest in nature, eusociality has long perplexed and fascinated biologists, and explaining its origin and maintenance remains one of the most enduring problems of evolutionary ecology (Darwin 1859; Hamilton 1964; Wilson 1971; Andersson 1984; Seger 1991). What conditions, either intrinsic or extrinsic to the organism, can explain the evolution of the extreme reproductive skew and cooperative behavior that characterizes the familiar social insects? This question can be approached from several perspectives. Fundamentally, it involves explaining why individual animals (i.e., workers) should behave altruistically, sacrificing personal reproductive opportunities to help other individuals (i.e., queens). At this level, inclusive fitness theory (Hamilton 1964) applied to data on individual behavior has been of central importance in explaining many aspects of the biology of social insects (Crozier and Pamilo 1996) and vertebrates (Emlen 1991).

A complementary approach to deciphering the evolution of social organization employs historical analysis. Explaining the origin, as opposed to the maintenance, of eusociality in a particular group is an explicitly historical question and is most rigorously addressed in a phylogenetic context. In principle, for example, the necessary and sufficient conditions for the origin of eusociality could be determined by comparing eusocial taxa with their closest noneusocial sister taxa (e.g., Carpenter 1989, 1991; Crespi 1996). For major social insect taxa such as the ants and termites, however, this approach is frustrated by the ancient origins of eusociality (Hölldobler and Wilson 1990; Choe and Crespi 1997) and the paucity of robust phylogenies for the groups in which eusociality is believed to have arisen (Crespi 1996; Choe and Crespi 1997). Crespi (1996, p. 263) concluded, in fact, that application of comparative statistical tests to illuminate the origins of eusociality would be ''premature or misleading for almost all groups of social insects'' because of four considerations: ''(a) the present lack of well-resolved phylogenies for most clades containing eusocial forms; (b) the uncertainties concerning whether or not all cases of eusociality have the same set of causes; (c) the likelihood that one or two variables alone cannot accurately predict the origin or loss of eusociality, due to the absence of ceteris paribus among morphologically and ecologically divergent lineages; and (d) the rarity of transitions to and from eusociality.''

The recent finding of eusocial-like colonies in the sponge-dwelling marine shrimp *Synalpheus regalis* (Duffy 1996a) and in several of its congeners (Duffy 1998; Duffy and Macdonald 1999) offers an intriguing opportunity to address the organismal and ecological correlates of advanced sociality in a taxon both phylogenetically and ecologically distinct from the familiar social insects. The genus *Synalpheus* (Decapoda: Alpheidae) is one of the most species-rich genera of tropical crustaceans and represents a dominant component of coral reef cryptofaunas throughout the world (Bruce 1976; Chace 1989). Most of its more than 100 described species form specific, obligate associations with sessile invertebrates, particularly sponges and crinoids, and feed either on host tissue (Ruetzler 1976; Erdman and Blake 1987; Ríos and Duffy 1999) or microalgae and detritus (e.g., Duffy 1998). In the tropical West Atlantic, the genus is represented primarily by the morphologically distinctive ''gambarelloides group'' (Coutière 1909; Dardeau 1984), a collection of 21 described and several undescribed species of sponge-dwellers, which are mostly endemic to this region. The species richness of

the gambarelloides group is accompanied by considerable diversity in body size, host specificity (Dardeau 1984; Duffy 1992, 1996b,c), and social structure (Duffy 1996a, 1998; Duffy and Macdonald 1999). In terms of social organization, the gambarelloides-group species range from asocial pair-bonding through subsociality and communality to eusociality. Thus, *Synalpheus* offers promising opportunities for comparative analysis of social evolution.

Explanations for the origin and maintenance of eusociality have been sought among a wide variety of genetic, ecological, and behavioral factors (Wilson 1975; Andersson 1984; Alexander et al. 1991; Crespi 1996). Intense pressures from enemies, including competition for valuable nest resources and predation, long have been recognized as primary environmental factors selecting for cooperation in social insects (Lin and Michener 1972; Alexander 1974; Wilson 1975; Evans 1977), and recent findings of eusociality outside the Hymenoptera and termites tend to support this view (Alexander et al. 1991; Stern and Foster 1996; Crespi and Mound 1997). Cooperative social groups often acquire and defend contested resources more effectively than individuals or less organized groups do (Lin and Michener 1972; Alexander 1974; Evans 1977; Emlen 1991). In several taxa, notably ants and termites, highly cooperative social life has resulted in both marked ecological dominance and much higher population densities than in noneusocial relatives (Oster and Wilson 1978; Wilson 1990). Recent attempts to explain the taxonomically scattered origins of eusociality outside the Hymenoptera have focused more specifically on the coincidence of strong enemy pressure with the use of enclosed nest sites that are valuable, defensible, and that foster kin interactions (Alexander et al. 1991; Crespi 1994; Thorne 1997). Queller and Strassmann (1998) have termed this suite of characteristics the ''fortress defender'' mode of eusociality as opposed to the ''life insurer'' mode common in Hymenoptera. Fortress defense appears to provide a reasonable model for the evolution of eusociality in *Synalpheus* as well. For many species of *Synalpheus,* virtually all suitable host sponges are occupied in the field and potential competitors show strong aggression toward one another (Duffy 1992, 1996a,c,d), suggesting that competition for nesting sites (sponges) is intense, as it is for many social insects.

Because the categorization of animal sociality remains a subject of debate (Gadagkar 1994; Crespi and Yanega 1995; Sherman et al. 1995; Costa and Fitzgerald 1996; Wcislo 1997), it is worth emphasizing how we use terms and what we seek to explain. The traditional definition of eusociality (Wilson 1971) entails prolonged cohabitation of multiple generations, substantial reproductive skew, and cooperative care of young. Each of these criteria has been demonstrated or inferred for the social snapping shrimp *S. regalis* (Duffy 1996a), and colony organization suggests a similarly developed social grade in several of its congeners (Duffy 1998; Duffy and Macdonald 1999). Even relatively restrictive definitions based on irreversible caste differentiation (Crespi and Yanega 1995) may apply to *S. filidigitus* insofar as the lone reproductive female in colonies of this species generally sheds her massive major chela and replaces it with a second, minor-form chela, rendering her morphologically distinct from all other individuals in the colony (Duffy and Macdonald 1999).

In any case, our purpose is not to prolong debate over defining eusociality, but rather to document the repeated evolution within a single genus of a remarkable form of colony organization characterized by strong reproductive skew, apparent matrilineal kin groups, and cooperative behavior in the few species that have been studied alive. Wcislo (1997) emphasized that the definition of sociality used in any given context should reflect the characteristics that are appropriate to the question. Our question is: Does competition for limited habitat select for the large (tens to hundreds of individuals), strongly reproductively skewed aggregations typical of several *Synalpheus* species? Our analysis is based on the working hypothesis that large colony size and reproductive skew are correlates of cooperative behavior that enhances effectiveness of gaining and holding the host resource (Brown 1974, 1987; Koenig and Pitelka 1981; Emlen 1982, 1991; Andersson 1984; Alexander et al. 1991). We use the term eusociality as a label for this suite of characteristics to emphasize its strong similarity to the phenomenon in many insect taxa that have historically been labeled eusocial (Wilson 1971; Crozier and Pamilo 1996). Establishing a robust hypothesis of relationships among the social *Synalpheus* species and of the number of independent origins of eusocial colony organization is the first step in seeking to explain those origins using comparative methods.

Here we present a phylogenetic analysis of 13 of the estimated 30 species (21 currently recognized plus several undescribed taxa) within the gambarelloides group of *Synalpheus,* based on morphology and sequence data from the mitochondrial large-subunit (16S) ribosomal RNA gene and the mitochondrial cytochrome *c* oxidase subunit I (COI) gene. We use the resulting phylogenetic hypothesis in a preliminary exploration of the ecological correlates of social organization in shrimp. Specifically, we address the hypothesis that social level is related to competition for limited nesting sites by comparing ecological correlates of competitive ability as a function of social level, using phylogenetically independent contrasts (Felsenstein 1985b).

Materials and Methods

Taxa and Specimens

For phylogenetic analysis, we sampled 13 species within the gambarelloides species-group of *Synalpheus* and two outgroup taxa, *S. fritzmuelleri* and *Alpheus cylindricus* (Table 1). The selection of taxa was intended to include: (1) as wide a range as possible of the morphology, life history, and social phenotypes found within the ingroup; and (2) all three of the gambarelloides species previously described as eusocial, that is, *S. regalis* (Duffy 1996a,d), *S. filidigitus* (Duffy and Macdonald 1999), and *S. chacei* (Duffy 1998), as well as species that we judged, based on morphology, to be their closest relatives. *Synalpheus regalis* and *S. filidigitus* are part of a complex of morphologically similar species that includes the undescribed species we refer to as *S.* ''*rathbunae* A'' (see Duffy 1996c). *Synalpheus chacei* is most similar morphologically to *S. bousfieldi* and *S. brooksi* (Duffy 1998). Finally, we also sampled *S. paraneptunus,* which is generally found

TABLE 1. Taxa included in the phylogenetic analysis, with sources of specimens and social data. Quotes enclose provisional names of undescribed taxa. *N*, number of quantitative samples used to calculate values of the ecological and social variables; colony size, number of individuals cohabiting within a sponge. For some species, only portions of individual sponges were collected so that values shown are minimum estimates; these values are preceded by >. Eusociality index is from Keller and Perrin (1995).

Species	Genbank accession no.	Host sponges*	*N*	Colony size		No. females/colony			Eusociality index (median)
				median	max	median	90th percentile	max	
Synalpheus									
''bousfieldi A''	AF230260, AF230789	Ac, Ad	15	3	7	1	2	3	0
brooksi	AF230263, AF230791	Ac, If, Sv	53	> 42.5	> 221	> 6	> 13	> 18	0.72
''brooksi D''	AF230262, AF230790	Ac, Ha, Lc	8	> 26.5	> 71	> 5	> 8	> 24	0.42
chacei	AF230261, AF230792	Ac, Ad, Ha, Hi, Lc, Na, Osp	62	33	243	1	1	13	0.93
filidigitus	AF230270	Hi, Osp, Xsp	31	24	121	1	1	2	0.92
longicarpus	AF230265	Sv	30	> 14	> 39	> 3	> 7	> 13	0.50
''pandionis red''	AF230266	Sv	6	> 2	> 4	> 1	> 1	> 1	0
paraneptunus	AF230267, AF230793	Hi, Osp, Pp, Xr	15	2	18	1	2	3	0
''paraneptunus small''	AF230268	Osp, Xsp	18	9	23	1	1	3	0.67
pectiniger	AF230259, AF230796	Sv	14	> 4.5	> 19	> 1	> 6	> 6	0.04
''rathbunae A''	AF230269, AF230797	Ac, Hc, Lc, Ls	7	8.5	80	1	3	3	0.71
regalis	AF230271	Hi, Xsp	35	122	356	1	1	1	0.98
williamsi	AF230264, AF230795	Hc	6	2	5	1	2	2	0
fritzmuelleri	AF230798, AF230788	—							
Alpheus cylindricus	AF230272	Sv							

* Ac, *Agelas clathrodes*; Ad, *Agelas dispar*; Ha, *Hymeniacidon amphilecta*; Hc, *Hymeniacidon caerulea*; Hi, *Hyattella intestinalis*; If, *Ircinia felix*; Lc, *Lissodendoryx colombiensis*; Ls, *Lissodendoryx strongylata*; Na, *Niphates amorpha*; Osp, *Oceanapia* sp.; Pp, *Pachypellina podatypa*; Sv, *Spheciospongia vesparium*; Xr, *Xestospongia rosariensis*; Xsp, *Xestospongia* sp.

in heterosexual pairs, and a population tentatively identified as *S. paraneptunus* (''small''), which typically is found in monogynous colonies suggestive of eusociality.

We obtained shrimp by collecting their host sponges or pieces of sponge-encrusted coral rubble using scuba, dissecting the live sponges in the laboratory, and removing the shrimp. With the exception of some very small juveniles, we identified all shrimp, which made up the vast majority of macroscopic animals in most samples. For phylogenetic analysis, shrimp were preserved soon after collection in cold 95% ethanol and stored at −20°C until used. One individual of each taxon was sampled for molecular analysis. Analyses of social and ecological characters used data from shrimp assemblages collected between 1988 and 1998 from sponges in the San Blas Islands of Panama, Carrie Bow Cay in Belize, the Florida Keys, and the Bahamas. Often these samples came from sponges collected for other purposes, rather than from sponges randomly sampled from the environment. This resulted in widely different sample sizes for particular species of sponges and shrimp (Table 1). In all cases, however, the entire assemblage of shrimp was removed from each sampled sponge. Thus, there was no evidence that the samples were biased in terms of the variables considered in this study.

For each shrimp taxon, we tabulated the number of individuals cohabiting within the sponge (hereafter colony size) and the number of reproductive females in the colony, as assessed by presence of brooded embryos or ovaries visible through the dorsal body wall. In the case of some large species of sponges such as *Spheciospongia vesparium* and *Agelas clathrodes*, we collected only a portion of an individual sponge; in such cases (noted in the Results) colony sizes presented are minimum estimates (preceded by > in Table 1). Because the abundance and distribution of host sponges differed greatly, the number of colonies (i.e., number of host sponges) sampled varied from five to 51 for different shrimp species.

Morphology

We identified and scored 23 morphological characters (Table 2, Appendix 1) for phylogenetic analysis, by direct examination of specimens from the set of 13 gambarelloides-group species for which 16S data were also available. Shrimp specimens, stained with methylene blue, were examined under dissecting and compound microscopes. Several specimens of each taxon were examined to assess the degree of variation within species. All taxa were scored by the same two researchers (J. E. Duffy and R. Ríos). Autapomorphic characters were not included in the analysis.

DNA Isolation, Amplification, and Sequencing

We amplified segments of the 16S rRNA gene and the COI gene for this phylogenetic analysis. Total DNA was extracted either from eggs or whole body tissues of single ethanol-preserved specimens, using the QIAmp™ tissue kit (Qiagen, Valencia, CA) or G-NOME™ extraction kit (Bio 101, Vista, CA).

Approximately 500 bp of the mitochondrial 16S rDNA gene was amplified with primers 16Sar (CGCCTGTTTATCAAAAACAT) and 16Sbr (CCGGTCTGAACTCAGAT-

TABLE 2. Morphological character matrix for phylogenetic analysis of *Synalpheus* spp. and outgroups. See Appendix for description of character states.

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
A. cylindricus	2	0	0	1	0	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
S. fritzmuelleri	0	0	1	0	0	0	0	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0
S. "bousfieldi A"	1	1	0	2	1	2	2	2	1	1	2	2	2	0	1	1	1	0	1	1	1	0	0
S. brooksi	1	1	0	2	1	2	2	2	1	1	2	2	2	0	1	1	1	0	1	0	1	0	0
S. "brooksi D"	1	1	0	2	1	2	2	2	1	1	2	2	2	0	1	1	1	0	1	1	1	0	0
S. chacei	1	1	0	2	1	2	2	2	1	1	2	2	2	0	1	1	1	0	1	1	1	0	0
S. filidigitus	1	1	1	2	1	2	2	1	1	1	2	1	0	1	1	1	1	0	1	1	1	1	1
S. longicarpus	1	1	0	1	1	1	2	2	1	1	2	2	2	0	0	1	1	1	0	0	1	1	1
S. "paraneptunus small"	1	1	0	1	1	1	1	0	2	0	2	0	1	1	1	1	0	0	1	1	0	0	1
S. "pandionis red"	1	1	0	2	1	1	2	2	1	1	2	2	2	0	0	1	1	1	0	0	1	1	1
S. paraneptunus	1	1	0	1	1	1	1	0	1	0	2	0	1	0	0	1	0	0	0	1	0	0	1
S. pectiniger	1	1	0	2	1	1	1	2	1	1	2	2	2	0	0	1	1	1	0	0	1	0	0
S. "rathbunae A"	1	1	1	2	1	2	2	1	1	1	0	1	0	1	0	1	1	1	1	0	1	1	1
S. regalis	1	1	1	2	1	2	2	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1
S. williamsi	1	1	1	1	1	1	1	0	1	0	1	1	1	0	0	1	1	0	0	0	0	0	1

CACGT) from Palumbi et al. (1991) with 1.25 units Amplitaq® (Perkin Elmer, Foster City, CA), manufacturer's buffer, 0.25 mM of each dNTP, 1 μM of each primer, and 35 cycles of 94°C (20 s), 48°C (15 s), and 72°C (90 s). Products were purified by agarose gel electrophoresis, excised under UV light, and extracted from agarose using the QiaQuik™ (Qiagen) gel extraction protocol. Flourescent dye-terminated cycle sequencing reactions employed a 45°C annealing temperature, and each reaction included one of the primers used for amplification, BigDye™ (Perkin Elmer), and 30 ng of the amplification product in a 2.5-μl volume. Unincorporated primers and dye-terminators were removed using G50-fine Sephadex columns. Purified products were desiccated and resuspended in 1.8 μl of formamide and blue-dextran, and 1.5 μl was electrophoresed on an ABI 377 automated DNA sequencer (Applied Biosystems, Foster City, CA). All sequences were deposited in GenBank (Table 1).

Approximately 650 bp of the mitochondrial COI gene was amplified, initially with primers COI-a (AGTATAAGCGTCTGGGTAGTC) and COI-f (CCTGCAGGAGGAGGAGATCC; Palumbi et al. 1991). Additionally, the following alpheid-specific primers were used for amplification and/or sequencing of some taxa: COI-1 (CATTTAGGCCTAAGAAGTG TTG), COI-1e (CRTTWAGWCCTAAGGAGTGTTG), COI-4 (CCATTCTATACCAACACCTAT), and COI-s (TTCCTATTYACMATAGGAGG). A typical 100 μL reaction included: Pyrostase PCR buffer (Molecular Genetic Resources, Inc., Tampa, FL) at 1× final concentration; 4 mM $MgCl_2$; 0.2 mM of each dNTP; 1 μM of each primer; 1–4 μL template DNA; 2.5 units Amplitaq® DNA polymerase (Perkin Elmer). The following temperature profile was used for PCR on a model 480 thermal cycler (Perkin Elmer): 5 min at 94°C, followed by 40 cycles of 94°C (60 s), 50–55°C (90 s), 72°C (90 s), ending with 10 min at 72°C. Products were purified using the QIAquick™ PCR purification kit (Qiagen), then were either directly sequenced or gel purified by either agarose (JETsorb, Genomed, Research Triangle Park, NC) or acrylamide (see Maniatis et al. 1989) gel techniques. Purified DNA was sequenced using standard protocols, primers listed above, and fluorescent-dye terminators (ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit, Perkin Elmer) followed by electrophoresis on an ABI 373A Automated Sequencing System. Both heavy and light strands were sequenced for confirmation.

Phylogenetic Analysis

Sequences for light and heavy strands were reconciled using Sequence Navigator (Perkin Elmer). COI sequences were aligned with Clustal W (Thompson et al. 1994), using default settings. 16S sequences were aligned using the CLUSTAL algorithm in GeneJockey (Taylor 1993) and MALIGN (Wheeler and Gladstein 1994). Parameter settings in MALIGN were varied to assess sensitivity of groupings to variation in alignment assumptions that included cost ratios of base-mismatch to insertion-deletion of 1:1, 1:2, 1:3, 1:4, and 1:5; there were few indels, and these variations resulted in little change in the alignment. Maximum-parsimony analyses were conducted using PAUP* version 4.0b2 (Swofford 1999). Heuristic searches used 1000 random taxon addition

sequences and Tree-bisection-reconnection branch swapping. The degree of internal support for the resulting trees was estimated in two ways. First, the proportion of 1000 bootstrapped (Felsenstein 1985a) pseudoreplicates that supported each node was calculated using the heuristic search algorithm of PAUP*. Second we calculated Bremer's (1988) support index, that is, the number of additional steps required to collapse a clade, for each node in the tree. To explore the interactions of and potential incongruence among the three data partitions (morphology, 16S, COI), we analyzed each separately prior to combining them in an analysis of all the available data.

Analysis of Social Evolution

We used two complementary approaches to define social phenotypes in our analysis. First, we attempted to categorize shrimp species using the definition of eusociality popularized by Wilson (1971), which we refer to hereafter as the ''discrete'' definition. Using quantitative data from field samples, we scored a taxon as putatively eusocial if: (1) colonies typically contained at least two generations (breeding females and juveniles) living together; (2) the number of cohabiting individuals was too large to be attributed to one cohort (i.e., was larger than the resident female's clutch size); this criterion excluded subsocial species (sensu Michener 1969; Wilson 1971) in which a single cohort of offspring remains with its mother for an extended period before dispersing; and (3) at least 90% of the colonies we examined contained only a single breeding female. The last criterion is admittedly arbitrary and was intended to make our discrete definition conservative. In most cases classifying taxa by these criteria was unambiguous (Table 1). To test whether inclusion of the character ''eusociality'' (defined as a discrete character) influenced our phylogenetic reconstruction (de Queiroz 1996), we conducted phylogenetic analyses with and without this character included; tree topology was identical in the two cases. The most parsimonious distribution of social states on the resultant tree provided an hypothesis of the number of independent origins of eusociality in *Synalpheus.* We assessed the overall level of support for the hypothesis of multiple origins by comparing lengths of the most parsimonious trees estimated with and without the constraint of monophyly of all eusocial taxa. As a more quantitative estimate of the relative amount of character support for the constrained and unconstrained trees, we compared the number of changes in each character on the two trees using Templeton's (1983) test as implemented in PAUP*.

Our second approach to defining social states recognizes that many aspects of social organization vary continuously rather than discretely (Sherman et al. 1995). Because of this continuous variation, applying criteria such as number 3 in the previous paragraph inevitably results in classifying differently some species with fairly similar colony organization. To avoid this problem, we also classified shrimp social phenotypes using the Eusociality index (E) of Keller and Perrin (1995):

$$E = \left(\sum_{i=1}^{n} |f_i - g_i|\right) \Big/ 2, \qquad (1)$$

where f_i and g_i represent an individual's proportional contributions of energy (work) and genes, respectively, to the next generation and n is the number of individuals in the colony (i.e., within a given individual sponge). This index has the desirable quality of accounting for both reproductive skew and colony size, both of which vary considerably among *Synalpheus* species. Moreover, the index does not require data on lifetime reproductive success, such that it can be calculated validly from data collected over short time spans such as the point estimates available from our collections. Several assumptions were necessary to calculate E for *Synalpheus.* First, because we lack data on the number of breeding males, we assumed that the number of breeding males in each colony was equal to the number of breeding females in the colony. This assumption is supported for *S. regalis* by allozyme data suggesting that only a single male breeds in each colony (Duffy 1996a) and for several less social species by the typical occurrence of adult males and females living in pairs (J. E. Duffy, pers. obs.). Second, because in *Synalpheus* neither gender nor sexual maturity can be identified externally in individuals other than ovigerous females (Felder 1982; Dardeau 1984), we included in our calculations all individuals in the colony, rather than adults per se, which would necessarily be estimated subjectively. Third, we made the parsimonious assumption that all individuals in the colony contributed equally to colony work (f). Finally we assumed that all breeders (i.e., the ovigerous females and an equal number of presumed breeding males) contributed equally to production of offspring (g); this latter assumption was necessary because many of the females from our earliest collections were damaged so it was not possible to count their eggs reliably. These assumptions may skew the value of the index somewhat, such that our values may not be directly comparable with those calculated for other animals (Keller and Perrin 1995), but we believe that they are unlikely to introduce systematic error that would bias comparison among species of *Synalpheus.* Moreover, despite the assumptions, the E index provides a much better resolved picture of the range in social variation within *Synalpheus* than does the discrete characterization into eusocial and noneusocial taxa.

Tsuji and Tsuji (1998) recently drew attention to some potential drawbacks in the use of common indices of reproductive skew, including Keller and Perrin's (1995) E index. The central problem is that indices of reproductive skew depend on average reproductive success. Specifically, taxa with lower expected numbers of offspring tend to have higher skew values due simply to random sampling error in offspring number. For this reason comparisons of taxa with different expected numbers of offspring will be biased toward finding higher skew in societies of less fecund individuals. Thus, such indices are not appropriate for comparing degree of sociality in taxa with widely different expected fecundities. This issue should not pose a problem for our application of E in *Synalpheus* because we were forced to assume (see above) that individual fecundity is equal for all females of all taxa considered. Thus, variation in E among taxa in our study results solely from variation in number of active breeders.

Ecological Correlates of Social Organization

We assessed potential correlations between social organization and host-resource dominance in *Synalpheus*, using phylogenetically independent contrasts (Felsenstein 1985b). We used Keller and Perrin's (1995) eusociality index (*E*) to quantify social level. Correlates of host-resource dominance included the number of congener species cohabiting in the sponge with the focal species and the proportion of total *Synalpheus* individuals within the sponge made up by the focal species. The rationale for using these variables is that superior competitors should be better able to exclude other species from a limiting resource. More highly social species are hypothesized to be more effective, compared with individuals or loosely organized groups, at excluding or evicting intruders because cooperation among group members provides an advantage in strength and/or vigilance. We used median rather than mean values of the variables in the analyses because the former are less sensitive to outliers and departures from a normal distribution. We focused on congeners as potential competitors because *Synalpheus* species typically comprised the vast majority, and often all, of the macroscopic animals within a sponge (J. E. Duffy, unpubl. data) and because intense interspecific aggression (Duffy 1996a) and low overlap in host use (Duffy 1996c,d) suggest that interspecific competition for space is common among these shrimp.

To account for the statistical nonindependence of related species in our analysis, we employed Felsenstein's (1985b) method of phylogenetically independent contrasts, as implemented in the computer package CAIC (Comparative Analysis by Independent Contrasts, Purvis and Rambaut 1995). Felsenstein's method uses both the topology and branch lengths of a phylogenetic tree. We estimated the latter using the number of unambiguous character changes on each branch of the maximally parsimonious (MP) tree. Our lack of COI data for several of the taxa, however, means that the potential number of changes on those branches will necessarily be less than in the remainder of the combined-data tree. For this analysis, therefore, we estimated branch lengths using only the 16S and morphological data, which were available for the same set of 13 ingroup taxa. We then computed contrasts for several different topologies (see Results) to assess sensitivity of our analysis to uncertainty in tree topology: (1) the single MP tree from the combined 16S and morphology data (from which branch lengths were estimated); (2) the two MP trees obtained from the combination of all three datasets; and (3) the six MP trees obtained from the morphological data alone, which showed some incongruence with the combined data tree (see Results).

The scaling of expected evolutionary change by branch lengths in Felsenstein's (1985b) method is based on assumption of a Brownian motion model of evolutionary change in which change in a character occurs at a constant average rate through time. At the other extreme, it is possible that character change occurs solely at speciation events, which is equivalent to assuming that all branch lengths are equal for the purposes of the comparative analysis. To test the sensitivity of our results to the model of character change, we conducted analyses under both ''gradual'' (branch lengths derived from number of unambiguous changes along a branch) and ''speciational'' (branch lengths equal) models. For the analyses based on the morphology trees we used only the speciational model because the exclusion of autapomorphies in the morphology dataset precluded accurate estimates of branch lengths.

The method of phylogenetically independent contrasts employs regression and correlation to test for associations between variables after accounting statistically for correlations resulting from phylogenetic relatedness. In addition to the primary consideration of statistical independence of observations, these tests rely on the usual assumptions of parametric statistics. For example, standardization of each contrast by its expected variance (i.e., the sum of branch lengths between the compared taxa) is meant to equalize weighting of each observation. Because the mode of evolution of the characters in question is uncertain, however, it is necessary to test the appropriateness of estimated branch lengths before proceeding with analysis of the contrasts (Garland et al. 1992). Recent studies have shown that failure to check, and when necessary transform, estimated branch lengths can lead to highly inflated Type I error rates when calculating independent contrasts (Díaz-Uriarte and Garland 1996, 1998). We tested the adequacy of branch length standardization following Garland et al. (1992), by regressing the absolute value of the standardized contrasts against their standard deviations (i.e., the square root of their summed branch lengths), and testing for significant association. Where this relationship was significant and negative, we transformed branch lengths by $\log_{10}$ and tested again for a significant relationship. We also tested whether the variables used in our contrasts (the eusociality index, the number of cohabiting congener species, and the proportion of total individuals in a sample made up by the focal species) were distributed appropriately for parametric statistics by testing for association between the absolute value of the standardized contrast and the estimated value of the character at the node where the contrast was estimated (i.e., the raw value of the contrast; see Purvis and Rambaut 1995). Once our data had been transformed to meet the assumptions of parametric statistics, we used simple linear regression to test for significant association between the eusociality index (*E*) and each of the ecological variables. Regressions were forced through the origin, reflecting the necessity that the mean of the contrasts equals zero (Garland et al. 1992), and significance of the relationship was tested with $n - 4$ degrees of freedom, where n is the number of taxa. The degrees of freedom reflect the number of independent contrasts ($n - 1$), with one degree subtracted for estimating the slope of the relationship and one more degree subtracted for estimating the appropriate branch length transformation for each of the two contrasted variables (Díaz-Uriarte and Garland 1996, 1998).

RESULTS

Phylogenetic Analysis: Individual Datasets

Parsimony analysis of 23 morphological characters, scored in the 13 ingroup and two outgroup taxa, produced six MP trees (Fig. 1A; length = 53, CI = 0.62, RI = 0.78) with strong support for the monophyly of the gambarelloides

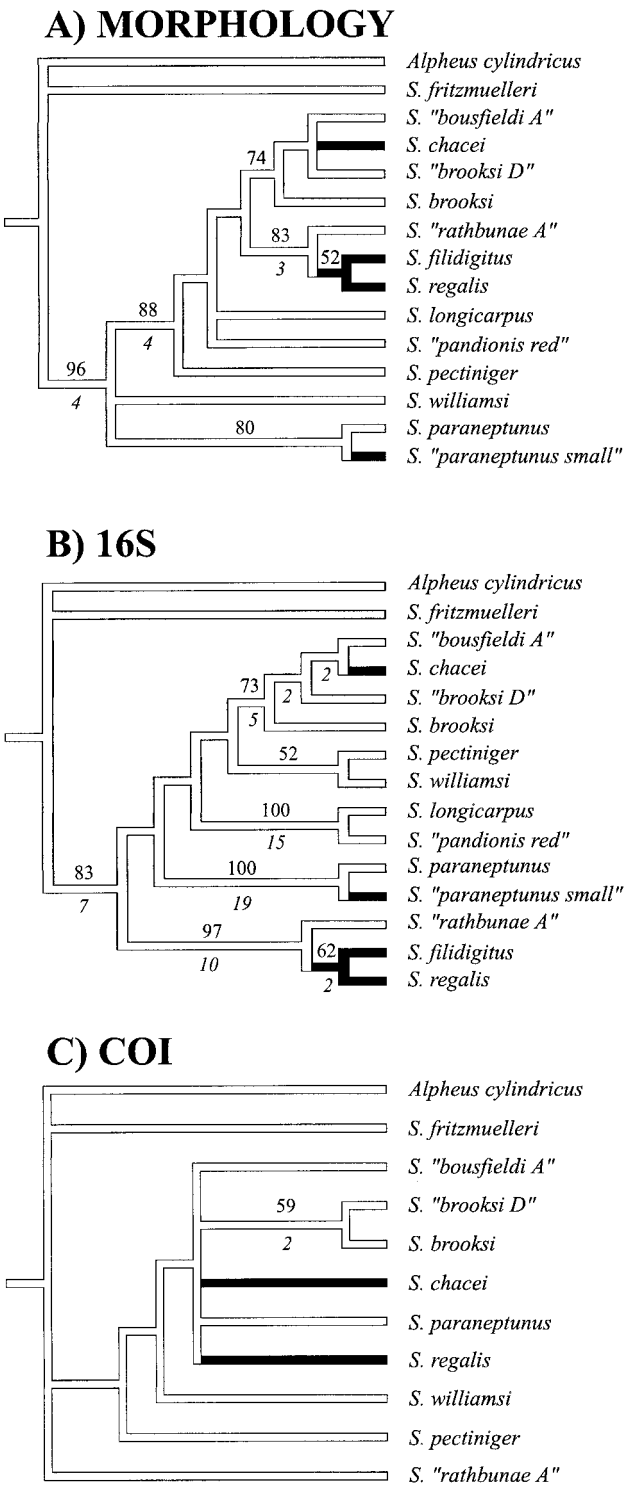

FIG. 1. Phylogenetic hypotheses for selected species of *Synalpheus* derived from each of the three datasets analyzed separately. (A) Morphology (consensus of six trees). (B) 16S rDNA. (C) COI (consensus of two trees). The number above each branch represents the percentage of 1000 bootstrap pseudoreplicates supporting that branch; number below the branch is Bremer's (1988) support index. Values are not shown for branches with less than 50% bootstrap support or Bremer support of fewer than two steps. Black terminal branches show taxa that meet the discrete criteria of eusociality (monogynous, multigenerational colonies) discussed in the text.

group and for several less-inclusive clades. Notably, there was good bootstrap support for the clades (*paraneptunus*, ''*paraneptunus* small''), (''*rathbunae* A,'' *filidigitus, regalis*), and (''*bousfieldi* A,'' ''*brooksi* D,'' *chacei*, ''*brooksi*''), each of which contains both eusocial and noneusocial taxa. Because none of the other taxa studied are eusocial, this analysis suggests that eusocial colony organization has arisen independently in each of these clades.

We obtained partial sequences (538 positions, 150 parsimony-informative sites) from the 16S gene for the 13 ingroup and two outgroup taxa. Parsimony analysis of these data, with all characters equally weighted, produced a single tree (Fig. 1B; length = 571, CI = 0.62, RI = 0.48) with strong support for the monophyly of the gambarelloides group and for the same three clades, each containing both eusocial and noneusocial taxa, supported by the morphological data.

We obtained partial sequences (639 positions, 169 parsimony-informative sites) from the COI gene for nine of the 13 gambarelloides-group species and the two outgroup taxa, for which morphological and 16S data were also available. Parsimony analysis of these data, with all characters equally weighted, produced two MP trees (Fig. 1C, length = 975, CI = 0.62, RI = 0.31) with bootstrap support greater than 50% only for the morphologically cryptic sister-species pair (''*brooksi, brooksi* D''). The generally low resolution of the COI tree is consistent with the faster rate of substitution in COI (at least at third positions) compared with 16S. Pairwise sequence divergences among the ingroup taxa were higher for COI (13.2–19.9%) than for 16S (1.8–16.4%).

Phylogenetic Analysis: Congruence and Combination

Inspection of the trees recovered from the three datasets (Fig. 1) suggested some incongruence between the morphology and 16S trees, particularly in the positions of the (''*rathbunae* A'' (*filidigitus, regalis*)) clade, which is basal in the 16S tree (Fig. 1B), but relatively derived in the morphological tree (Fig. 1A). We attempted to test the statistical significance of this incongruence but an ILD test (implemented as the partition homogeneity test in PAUP*) was unable to reach completion, apparently because of the lack of resolution in the morphology trees. Despite this apparent incongruence, both morphology and 16S datasets agree on monophyly of the gambarelloides group and on the distribution of eusociality among three well-supported clades.

Finally, we analyzed all data together in a combined analysis. Despite apparent incongruence between the 16S and morphology datasets, we believe that simultaneous analysis of all data provides the best hypothesis of relationships in this case, for the empirically based reasons summarized by Remsen and DeSalle (1998). The combined data produced two trees (Fig. 2), the consensus of which was identical to the 16S tree except that relationships among *S. chacei, S.* ''*bousfieldi* A,'' *S. brooksi,* and *S.* ''*brooksi* D'' were unresolved in the combined tree. Justification for the combined approach comes from the generally higher bootstrap and Bremer support for individual clades in the combined tree compared with the topologically similar 16S tree. Specifically, the combined tree offered substantially stronger support for monophyly of the ingroup (bootstrap = 95%) than the 16S

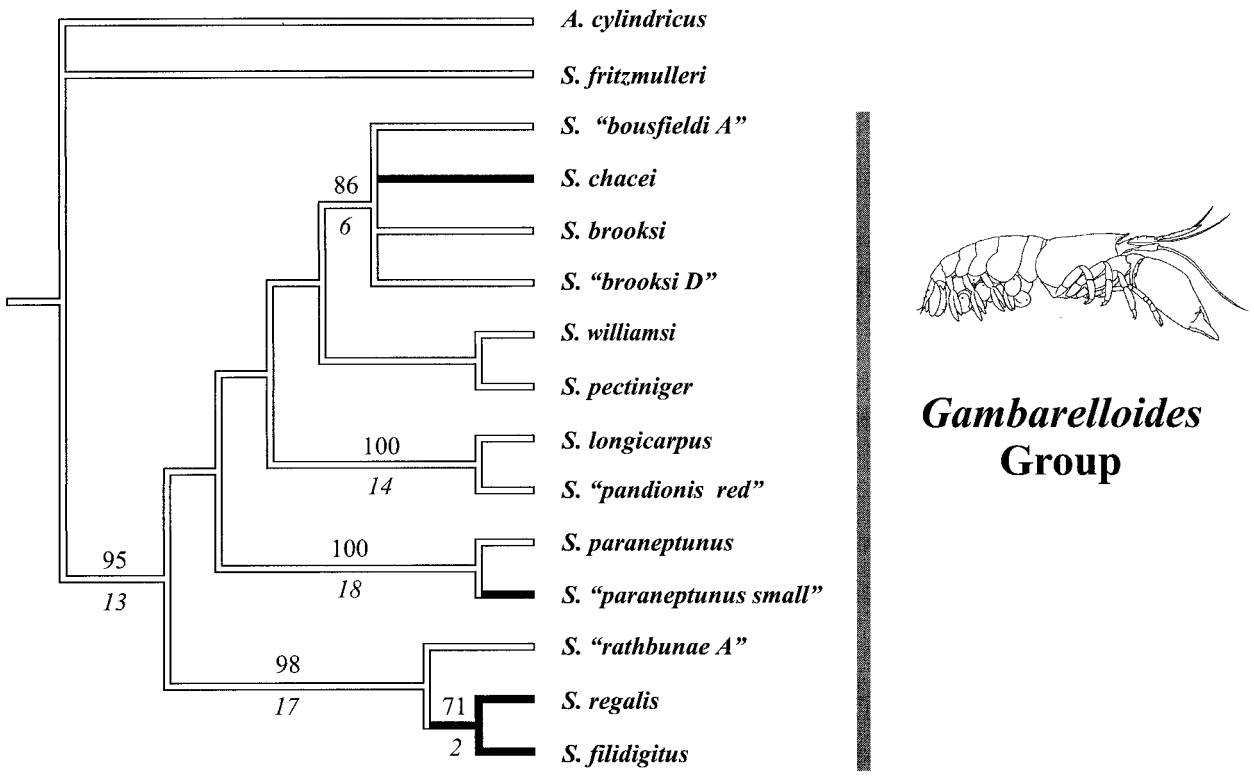

FIG. 2. Consensus of two most parsimonious trees resulting from the analysis of the combined morphological and molecular data. Symbols as in Figure 1.

data alone and greater support for the clades (*chacei, bousfieldi* A, *brooksi,* and ''*brooksi* D'') and (*regalis, filidigitus*) than any of the single datasets. Conversely, one fairly well-supported (bootstrap $> 70\%$) branch in the morphology tree was not supported in the combined tree. Increased branch support in combined-data trees has similarly been found by Remsen and DeSalle (1998) and Cannatella et al. (1998).

Evolution of Social Organization

Median colony size in the 13 ingroup *Synalpheus* taxa studied ranged from two in *S. williamsi,* in which a single heterosexual pair typically occupied a sponge, to 122 in the eusocial (Duffy 1996a) species *S. regalis* (Table 1). The largest colony of any species was also found in *S. regalis* (356 individuals within a sponge). Between these extremes the remaining species spanned a continuous range in colony organization (Table 1) from small family groups containing a single breeding pair and a few juveniles (e.g., *S.* ''*bousfieldi* A,'' *S.* ''*pandionis* red''), through larger gregarious groups with several females breeding (e.g., *S. brooksi, S.* ''*brooksi* D,'' *S. longicarpus*), to large, usually monogynous colonies (*S. chacei, S. filidigitus*). There was a corresponding range in values of the eusociality index (*E*) among species (Table 1).

When eusociality is defined as a discrete state based on application of Wilson's (1971) definition (see Methods), optimization of social organization on the combined-data tree (Fig. 2) reveals that monogynous, eusocial taxa are distributed among three well-supported clades, each of which also contains noneusocial taxa. All other taxa, including outgroups, are noneusocial. Thus, using the discrete definition, there are three independent origins of eusocial colony structure within the gambarelloides group. In support of multiple origins of eusociality, constraining all eusocial taxa to be monophyletic resulted in a combined-data tree that was 62 steps longer than the unconstrained tree. Similarly, Templeton's (1983) test comparing character support for the MP trees with and without the constraint of monophyletic eusociality rejected the null hypothesis of eusocial monophyly ($P < 0.001$).

The conclusion of three separate origins of eusociality is somewhat less clear when eusociality is treated as a continuous variable (Fig. 3). This reveals that sister taxa of different social states, as defined by the discrete criteria, are sometimes similar in the value of the E index (e.g., *S.* ''*rathbunae* A,'' *S. regalis, S. filidigitus*; Fig. 3), that is, there is some conservatism in social level. Nonetheless, it seems safe to conclude that eusociality has arisen independently at least twice, given that the four taxa interposed between the clades containing (*regalis, filidigitus*) and *chacei* have relatively low *E* indices (Fig. 3).

Ecological Correlates of Social Organization

Comparing estimates of host dominance among species revealed that highly social taxa tended to share their host sponges with fewer congener species and made up a larger proportion of the total shrimp assemblage within the sponge than did less social taxa. This pattern is evident among the three clades containing eusocial species as classified using

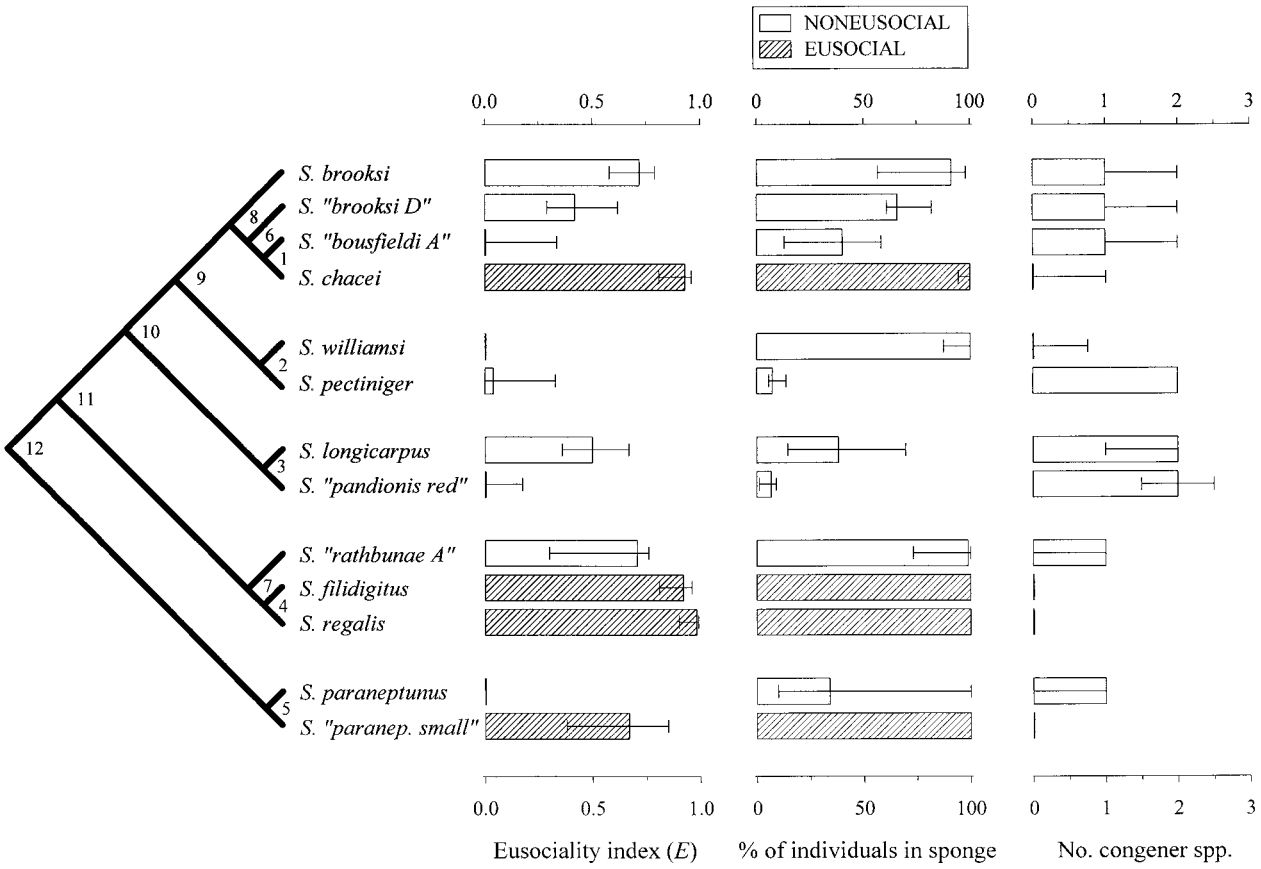

FIG. 3. Social level (eusociality index, *E*, of Keller and Perrin 1995) and potential correlates of ecological dominance in the 13 ingroup *Synalpheus* taxa, plotted on the single maximum-parsimony tree obtained for the combined 16S and morphological data (this tree was used to estimate branch lengths for the independent contrasts shown in Fig. 4). Bar represents the median, with error bars showing first and third quartiles. Hatched bars represent taxa meeting the discrete criteria of eusociality discussed in text. Numbered nodes in the tree refer to the specific contrasts shown in Figure 4.

the discrete definition (Fig. 3), as well as when sociality is defined as a continuous variable (*E*) and phylogenetic relatedness is controlled using phylogenetically independent contrasts (Fig. 4). In the latter case there is a significant correlation between contrasts in the eusociality index and in the proportion of total individual shrimp in the sponge made up by the focal species, under all tree topologies and under both gradual and speciational models of change. For example, in the single MP tree obtained from the combined 16S and morphology data, the contrast correlation between *E* and the percentage of individuals in the sponge was $r^2 = 0.57$ ($P < 0.01$) under the gradual model ($\log_{10}$-transformed branch lengths) and $r^2 = 0.48$ ($P < 0.01$) under the speciational model. For the two trees obtained from the three combined datasets and the six trees derived from morphology data results were similar (combined: $r^2 > 0.47$ and $P < 0.02$ in all cases; morphology: $r^2 > 0.50$ and $P < 0.02$ in all cases).

A similar tendency for highly social taxa to share their host sponge with fewer congener species was not significant. For the 16S/morphology tree, under the gradual model of change ($\log_{10}$-transformed branch lengths), $r^2 = 0.24$ ($P < 0.10$), and under the speciational model, $r^2 = 0.22$ ($P = 0.11$). Results were similar for the other two topologies.

DISCUSSION

Our results support the conclusion that eusocial colony organization, as recognized by the traditional definition of monogynous, multigenerational colonies (Wilson 1971), has originated three times independently within the gambarelloides species group of *Synalpheus*, and that the latter group is monophyletic. Despite poor resolution in the COI tree and apparent incongruence between 16S and morphological datasets, the combined-data tree showed generally stronger branch support than any individual dataset. Most importantly, the co-occurrence of eusocial and noneusocial taxa in each of three well-defined clades, implying three separate origins of eusociality, was supported by 16S alone, morphology alone, and the combined data from all three datasets. Multiple origins of eusociality are also supported by the large number of extra steps required to make eusocial taxa monophyletic on the combined-data tree and by the highly significant Templeton test, which rejected the null hypothesis of eusocial monophyly based on these data. Treatment of eusociality as a discrete phenomenon, however, glosses over the gradation in social organization among taxa (Sherman et al. 1995). When we scored social organization as a continuous char-

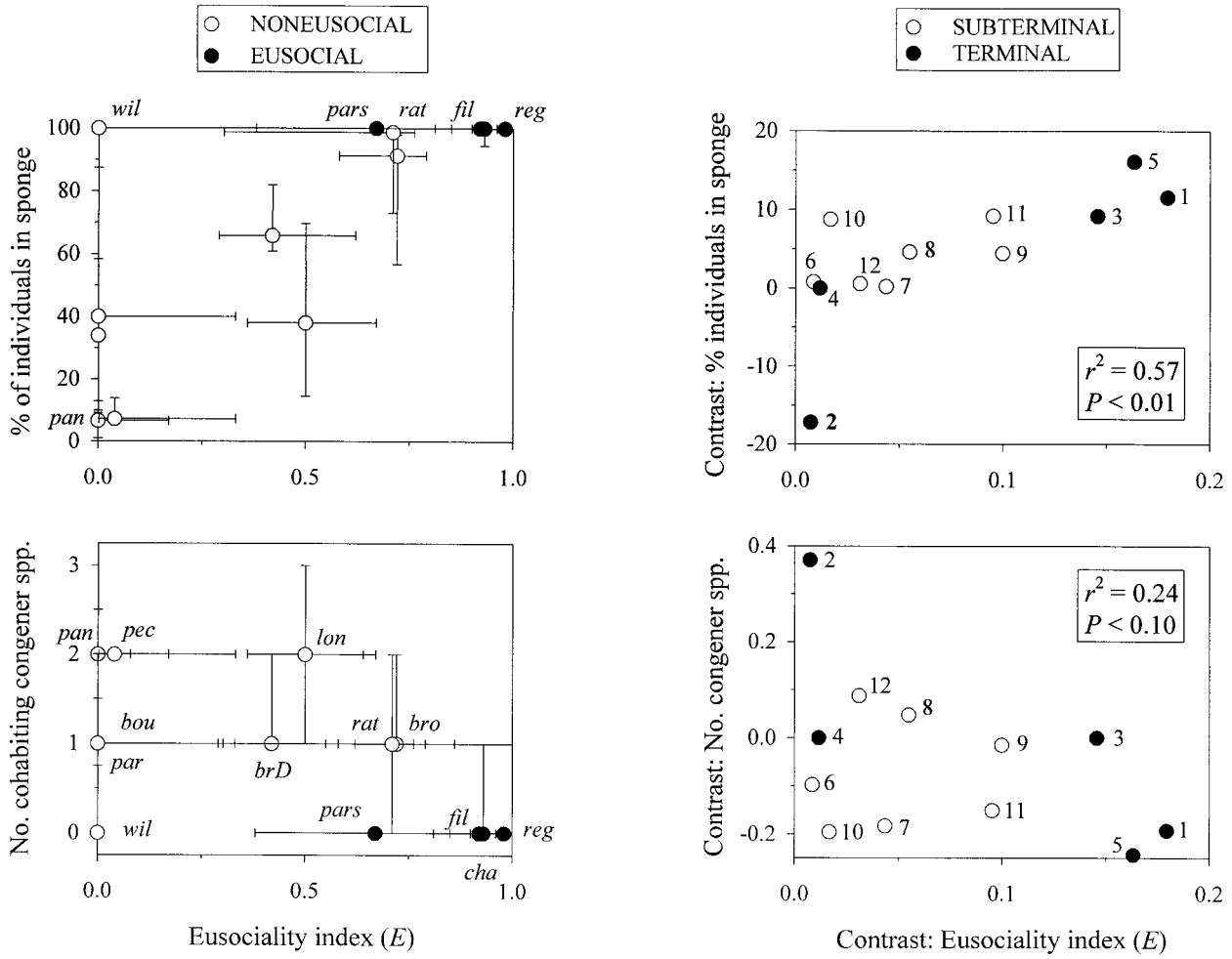

FIG. 4. Correlates of ecological dominance plotted against the eusociality index (*E*) for the 13 ingroup species of *Synalpheus*. Left: Values for individual species (median, with error bars showing first and third quartiles); symbols are identified by the first three letters of the species name, except for *S.* ''*brooksi* D'' (brD) and *S.* ''*paraneptunus* small'' (pars). Eusocial species, as defined by the discrete criteria discussed in text, are indicated. Right: Values for phylogenetically independent contrasts. Symbols are identified by the number of the node where the contrast was computed, as shown in Figure 3. Contrasts between terminal taxa (extant sister species) are indicated. Statistics shown are from contrasts estimated using the 16S/morphology tree (Fig. 3) with log-transformed branch lengths and $n - 4$ degrees of freedom, where n is the number of taxa.

acter, we found a wide range in values within *Synalpheus*, but a broadly similar picture of the phylogenetic distribution of eusociality (Figs. 3, 4). A few taxa were scored differently under the discrete and continuous concepts of eusociality. For example, *S.* ''*rathbunae* A'' ranked high on the Eusociality index, despite being scored as a noneusocial species under the discrete definition because it does not meet the arbitrary criterion that 90% of colonies have only a single breeding female (Table 1). The situation is similar for *S. brooksi*. Thus, sociality in this group varies along a continuum between asocial pair-bonding and large-colony, monogynous taxa. Nevertheless, the presence of large (hundreds of individuals), consistently monogynous colonies in *S. chacei* and the *S. filidigitus/regalis* group, which are not from sister clades, seems a striking example of parallelism in social evolution. This conclusion is noteworthy in that eusociality has arisen only a handful of times in the animal kingdom (Crozier and Pamilo 1996; Choe and Crespi 1997).

Historical analysis of any character, such as the ecological and social traits we studied, depends fundamentally on estimation of ancestral states. Yet reconstruction of ancestral states not only requires a strongly supported tree, but is also sensitive to violation of several, often implicit assumptions. A primary one of these assumptions is that gains and losses are equally probable (Cunningham et al. 1998). This assumption could have important consequences for our conclusions (Wcislo and Danforth 1997). If eusociality is more easily lost than gained, as might be expected from its complexity and rarity among animals, optimizing this character on the tree under the assumption of equiprobable gains and losses will bias toward the finding of multiple origins, when multiple losses in descendants of a single eusocial ancestor are more likely (e.g., Olmland 1997). We believe, however, that our conclusion of multiple origins of eusociality in *Synalpheus* is robust for two reasons. First, large monogynous colonies such as those characteristic of eusocial *Synalpheus* are unknown among shrimps outside this genus. Thus, it seems unlikely that the ancestor of the genus or of the gam-

barelloides clade was eusocial. Second, our sample of taxa represents only about half of the species in the gambarelloides group, nearly all other species of which appear to be low on the scale of eusociality. We anticipate that inclusion of these additional, noneusocial taxa in a more complete phylogenetic analysis (in progress) will provide even stronger support for multiple origins of eusociality in *Synalpheus.*

Besides the single origin each in ants and termites, eusociality is thought to have arisen once in beetles (Kent and Simpson 1992), once or twice in gall-forming thrips (Crespi 1992; Crespi et al. 1998), twice in the mole-rats (Jarvis and Bennett 1993), and perhaps 11 times among the wasps and bees (Crespi 1996; Crozier and Pamilo 1996). Eusocial-like caste systems also occur in some clonal aphids (Stern and Foster 1996). The multiple origins of eusociality in the Hymenoptera, and particularly the range in social systems found within lower-level taxa such as the halictid bees, have made the Hymenoptera especially valuable for comparative approaches to understanding the evolution of social systems (e.g., Michener 1969; Wilson 1971; Carpenter 1989, 1991; Choe and Crespi 1997). The multiple origins of advanced sociality in *Synalpheus* could provide similar opportunities. Our phylogenetic analysis of *Synalpheus* addresses each of the concerns raised by Crespi (1996) regarding comparative analysis of the origins of eusociality (see the introduction). First, we have employed three datasets to construct a generally well-supported hypothesis of relationships among selected species of *Synalpheus,* including all known putatively eusocial species and their morphologically closest relatives. Second, despite their social diversity, the species of *Synalpheus* in the gambarelloides-group are generally quite similar in ecology. All are obligate inhabitants of sponges in tropical reef-associated habitats, and many are in fact sympatric (Duffy 1992, 1996c). Therefore, the factors driving the transition between social systems should be less confounded by other differences in biology and environment and thus more easily revealed in *Synalpheus* than in the larger social insect taxa, in which the nearest noneusocial sister taxa are separated by relatively ancient divergences. Third, the multiple origins within the gambarelloides group offer opportunities for replicated sister-group comparisons within a single genus in which eusociality arose comparatively recently.

We used the comparative approach to investigate the potential role of competition for the host resource in fostering the evolution of social life in *Synalpheus.* We found consistent differences in ecology between highly social *Synalpheus* taxa and their less social relatives: Shrimp taxa that scored high on the eusociality index (*E*) were significantly more abundant within their host sponges than were less social taxa (Fig. 4). Highly social taxa also tended to share their hosts with fewer congener species, although this pattern was marginally nonsignificant. A conspicuous exception to this pattern was *S. williamsi* (Fig. 4), which was generally the sole occupant of its host sponge despite a value of zero for the eusociality index. For reasons that remain unclear, *S. williamsi*'s host sponge *Hymeniacidon* cf. *caerulea* is frequently unoccupied by any shrimp in the field, suggesting that the usual absence of congeners in sponges occupied by *S. williamsi* is likely unrelated to competition.

Like any analysis using reconstructed ancestral character states, our use of phylogenetically independent contrasts to reach these conclusions depends on assumptions about how characters change between speciation events. Uncertainty about the tempo and mode of character evolution can result in low confidence in the reconstructed ancestral states of social and ecological characters, even beyond the statistical uncertainty inherent in historical analysis of any single character (Frumhoff and Reeve 1994; Schluter et al. 1997; Cunningham et al. 1998). We attempted to circumvent these problems by examining terminal contrasts (those between extant sister taxa) separately as a subset of contrasts that requires no assumptions about ancestral states. Because contrasts between extant sister taxa result from more recent evolutionary divergences than do deeper nodes, and because their character states are directly measured rather than estimated, we consider them more reliable estimates of evolutionary association between variables. There are only five such terminal contrasts in the ingroup, however, such that hypothesis testing with this data subset has very low power (two degrees of freedom using the recommendations of Díaz-Uriarte and Garland 1996, 1998) and all correlations using only terminal contrasts were nonsignificant at $\alpha = 0.05$. Nevertheless, the trends in the terminal contrasts are at least as pronounced as those shown by the complete dataset (Fig. 4), and correlation coefficients were comparable or greater when only terminal contrasts were considered. Thus, we tentatively conclude that there is a significant correlation between social level and dominance of the host sponge in sponge-dwelling shrimps.

Our results are consistent with a long history of research supporting competition and enemy pressure as primary selective pressures favoring the evolution of sociality and reproductive skew in social insects (Lin and Michener 1972; West-Eberhard 1975; Evans 1977; Strassmann and Queller 1989; Alexander et al. 1991; Crespi 1994; Stern and Foster 1996; Brockmann 1997; Shellman-Reeve 1997; Thorne 1997; Queller and Strassmann 1998) and cooperatively breeding vertebrates (Emlen 1982, 1991; Brown 1987). In many vertebrates and some insects, cooperative breeding appears to be favored by habitat saturation, that is, the extreme scarcity of suitable, unoccupied territory. In such situations cooperation among individuals, generally close relatives, often yields an advantage to the group in acquiring or holding the limiting resource and an advantage to nonbreeding helpers through inclusive fitness benefits accruing from greater offspring production by related breeders (Emlen 1982, 1991, 1994; Taborsky 1984; Reeve 1991; Brockmann 1997). Similarly, for many species of *Synalpheus,* unoccupied sponges are virtually absent in the field (Duffy 1992, 1996c,d), suggesting that host sponges are a limiting resource. Moreover, resource limitation appears more pronounced among eusocial shrimp taxa, which tend to be more abundant and occupy a greater percentage of individual hosts than their less social sister taxa. Specifically, the eusocial species *S. regalis, S. filidigitus, S. chacei,* and *S.* ''*paraneptunus* small'' all occupied nearly 100% of sampled host individuals (Duffy 1992 [*S. chacei* was listed as *S. bousfieldi*], 1996c,d; J. E. Duffy, pers. obs.), whereas their sister taxa *S.* ''*rathbunae* A'' (*S.* ''*bousfieldi* A,'' *S. brooksi, S.* ''*brooksi* D''), and *S. paraneptunus* generally occupy smaller proportions of the available sponges. Our hypothesis, based on those developed for

social vertebrates and insects discussed above, was that cooperation among group members should make highly social species (those with high *E* indices) more effective at excluding and evicting potential competitors. Although detailed behavioral data are lacking, observations of cooperative defense among closely related colony mates in *S. regalis* (Duffy 1996a) show that cooperation can indeed be effective in territory defense in shrimps, as it is in some social vertebrates and insects. The advantages of such cooperation in enhanced survival or productivity of family-based colonies might explain the correlations we found between social level and host-resource dominance (Fig. 4). Clearly, behavioral studies are needed to confirm this in *Synalpheus.*

We have provided evidence that large social groups with strong reproductive skew have arisen independently in multiple lineages within the single genus *Synalpheus.* We also found that sociality is significantly related to estimates of competitive ability. If eusociality is indeed favored by its competitive advantage, then the patterns in host dominance we documented (Fig. 4) are more likely to be effects than causes of social organization. Thus, they may shed light on the ecological mechanisms by which sociality was favored. In contrast, the intriguing question of what extrinsic environmental factors led to more intense competition in some lineages of *Synalpheus* than in other, superficially similar lineages remains unanswered.

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Corresponding Editor: D. Wheeler

Appendix

List of morphological characters used in phylogenetic analysis of *Synalpheus*, with descriptions of states. Characters are numbered as in Table 2. Presumed autapomorphies are not included.

(1) Antenna 1, stylocerite length, relative to distal margin of segment1: (0) clearly exceeding; (1) equal to or shorter; (2) lacking spine.

(2) Antenna 1, basal segment, processes on ventral surface: (0) none; (1) two.

(3) Antenna 2, basicerite dorsal margin: (0) spine absent; (1) spine present.

(4) Antenna 2, scaohocerite blade development: (0) present; (1) reduced (narrower than spine) or variable; (2) consistently absent.

(5) Antenna 2, scaphocerite base, projection: (0) absent; (1) present.

(6) Major pereiopod 1 merus, distal extensor margin: (0) distinctly spinous; (1) with angular projection; (2) sloping smoothly into articulation.

(7) Minor periopod 1 merus, distal extensor margin: (0) distinctly spinous; (1) with angular projection; (2) sloping smoothly into articulation.

(8) Minor chela dactyl, shape: (0) opposing surface excavate; (1) opposing surface obliquely concave; (2) blade-like, not excavate.

(9) Minor chela dactyl teeth, longitudinal setal combs: (0) absent; (1) present.

(10) Minor chela dactyl teeth, orientation: (0) perpendicular to dactyl axis; (1) parallel to dactyl axis.

(11) Minor chela dactyl, number of teeth: (0) one terminal tooth (1) terminal tooth accompanied by obscure bump proximally; (2) two to three distinct teeth, subequal in length.

(12) Minor chela pollex, shape: (0) opposing surface excavate; (1) opposing surface obliquely concave; (2) blade-like, not excavate.

(13) Minor chela pollex, number of teeth: (0) one terminal tooth; (1) terminal tooth accompanied by obscure bump proximally; (2) two distinct teeth, subequal in length.

(14) Pereiopod 2 carpus, number of segments: (0) five; (1) four.

(15) Pereiopod 2 carpus length: (0) greater than merus length; (1) less than of equal to merus length.

(16) Pereiopod 3 coxa, mesial margin: (0) without lamella; (1) with lamella.

(17) Abdominal pleuron 1 (male), posteroventral margin: (0) weakly produced or rounded; (1) distinctly hooklike.

(18) Abdominal pleuron 2 (male), posteroventral margin: (0) rounded to obtuse; (1) angulate to acute.

(19) Male pleopod 1, number of terminal setae: (0) many (more than six); (1) few (less than five).

(20) Male pleopod 2, exopod, origin of marginal setae: (0) base of exopod; (1) near midpoint of exopod.

(21) Telson, posterior margin, space between medial spines: (0) greater than one-third total margin length; (1) less than one-third margin length.

(22) Telson, posterior margin, convex lobe between mesial spines: (0) present; (1) absent.

(23) Uropod outer ramus, number of fixed teeth on outer margin: (0) one; (1) more than one.